ARO 48TH ANNUAL MidWinter Meeting

Renaissance Seaworld ● Orlando, Florida

Abstract Book

Friday, February 21, 2025

PIHL Satellite Symposium

1:00 p.m. - 5:00 p.m.

Ocean Ballroom 1 – 4

Investigational medicines for hearing loss prevention and hearing restoration" symposium is organized by the Defense Health Agency (DHA) Hearing Center of Excellence (HCE) Pharmaceutical Interventions for Hearing Loss (PIHL) committee. This symposium will provide a comprehensive overview of steps within the discovery pipeline for inner ear medicines, resources available to support development of biologics and pharmaceuticals, and updates on the development of select agents. All presenters will emphasize education on best practices and information to be aware of regardless of whether your work is housed in an academic or industry setting. Tim Bölke, MD (Chief Executive and Chief Medical Officer, Managing Director, Acousia Therapeutics) Jonas Dyhrfjeld-Johnsen, PhD (Chief Development Officer and amp; Managing Director, Acousia Therapeutics) Amanda Henton, PhD (Chief Scientific Officer, Turner Scientific) Ralph Holme, PhD (Director of Research, Royal National Institute for the Deaf) Jonathon Kil, MD (Chief Executive and Chief Medical Officer, Sound Pharmaceuticals) Gaëlle Naert, PhD (Chief Scientific and Operations Officer, Cilcare) The Hearing Center for Excellence gratefully acknowledges symposium support from Acousia Therapeutics, the Acoustical Society of America, CBSET, Cilcare, and Turner Scientific, with additional support from Creighton University Bellucci Translational Hearing Center, the University of Texas at Dallas School of Behavioral and Brain Research, and the University of Texas at Dallas Clinical and Translational Research Center.

Presenters:

Colleen Le Prell*, University of Texas at Dallas*
Tim Boelke*, Acousia Therapeutics*

Jonas Dyhrfjeld-Johnsen*, Acousia Therapeutics*

Amanda Henton*, Turner Scientific*

Ralph Holme*, RNID*

Jonathan Kil*, Sound Pharmaceuticals, Inc.*

Gaëlle Naert*, CILcare*

Ménière's Disease Satellite Symposium

2:00 p.m. - 8:00 p.m.

Ocean Ballroom 9 - 12

Patient Perspective
Heather Davies*, Vestibular Disorders Association*

History of Ménière's Disease
Robin Bigelow

Treatment: State of the Art Clinical Care; Clinical Unmet Needs
Habib Rizk*, Medical University of South Carolina*

Migraine and Ménière's Disease
Jeffrey Sharon*, UCSF*

Imaging/MRI
Amy Juliano*, Massachusetts Eye and Ear; Harvard Medical School*

Panel Discussion
William Slattery*, The House Institute*

Grant Funding Sources
Amy Poremba*, NIH-NIDCD*

Molecular Basis
Andreas Eckhard*, Massachusetts Eye and Ear*

Genetics
Jose Lopez-Escamez*, The University of Sydney*

Immunology and Ménière's Disease
Andrea Vambutas*, Zucker School of Medicine at Hofstra-Northwell*

Social and Psychological Triggers
Joanna Wolfson*, NYU Langone Health*

Moderators:

John Oghalai*, University of Southern California*
Divya Chari*, Massachusetts Eye and Ear*

gEAR Session: Beginner Workshop - Introduction to gEAR

7:00 p.m. - 9:00 p.m.

Ocean Ballroom 1 – 4

Presenters:

Joshua Orvis*, Institute for Genome Sciences*

Daniel Lesperance*, Institute for Genome Sciences, University of Maryland, Baltimore*

Session Overview: In this beginner-friendly session, we'll guide you through the essentials of navigating and using the platform's features to simplify gene expression analysis. Topics include creating an account, using the user dashboard, performing gene searches, and customizing expression displays. You'll also explore dataset collections, gene lists, and the Dataset Explorer and Comparison Tool for deeper insights. Hands-on experience with our Single Cell Workbench, tailored for single-cell RNA-Seq analysis, is also included. This workshop is perfect for newcomers to gene expression analysis or anyone seeking a user-friendly platform to streamline workflows!

gEAR Session: Advanced Workshop

7:00 p.m. - 9:00 p.m.

Ocean Ballroom 5 – 8

Presenters:

Ricky Adkins*, Institute for Genome Sciences*
Joseph Receveur*, University of Maryland, Baltimore*

Session Overview: Designed for those familiar with the basics of the gEAR Portal, this advanced workshop provides an in-depth look at the platform's customizable features. Participants will learn to upload and organize their datasets, create curated displays, and develop custom gene lists. We'll cover building dataset collections, utilizing transfer learning (projections), and adapting the portal to specific research needs. This session includes time for hands-on practice, where you can

experiment with different features under expert guidance. Plus, test your skills in our “gEAR-lympics” competition—a fun, task-based challenge!

Saturday, February 22, 2025

Presidential Symposium: Innovations, Challenges, and Personalization in Hearing Aids - Part 1

Chair: Sunil Puria*, Harvard Medical School*

8:00 a.m. - 10:00 a.m.

Ocean Ballroom

Listening Through Hearing Aids With Thomas Edison

Mara Mills[1]

[1]*New York University*

Individual Abstract: In addition to his famous work on light bulbs and moving pictures, Thomas Edison invented well over 200 acoustic devices: the phonograph, dictating machines, acoustic clocks, talking dolls, and more. Despite his long list of acoustic patents, according to most accounts Edison never tried to develop a hearing aid. This is particularly surprising because Edison described himself (alternately) as deaf, partially deaf, half-deaf, and hard of hearing. It is even said that Edison never used an electrical hearing aid, preferring to work undisturbed by sound or conversation—yet in his archives there is abundant evidence to the contrary. Edison's preserved correspondence with engineers at Western Electric and Bell Laboratories, and with colleagues like Hugo Gernsback, documents the many models of hearing aid he indeed planned and tested. His correspondence also reveals the abundant lay expertise and inventiveness of deaf and hard of hearing people who had fewer resources than him, and whose names and ideas have been lost to history. People wrote to him not only to request advice, or to ask him to build devices on their behalf, but to send in their own plans for new or improved amplifiers. In this talk, I'll draw on the collections at the Edison National Historical Park to survey the new electronic listening that became possible in the early twentieth century, with Edison and other hard of hearing people serving as early adopters of vacuum tube-powered technology.

Hearing Aids: What Works Well and What Can Be Improved

Brian Moore[1]

[1]*University of Cambridge*

Individual Abstract: "This presentation is based on research findings and on my personal experiences with several different hearing aids over the past 25 years. An important factor affecting the performance of hearing aids is the type of acoustic coupling to the ear. The fitting can be "closed" (sealing the ear canal), but this can lead to the occlusion effect; the user's own voice sounds too loud or too boomy. Alternatively, the fitting can be open (the eartip has a vent). This alleviates the occlusion effect but has undesirable effects.

A basic requirement is to improve audibility via amplification over a wide frequency range. While many hearing aids do this well, the highest frequency at which useful gain can be achieved is often

about 5 kHz, which is lower than optimal. Also, when an open fitting is used, little or no gain can be achieved at low frequencies (except for the Earlens device) and the gain at high frequencies may be limited by acoustic feedback (howling). While feedback cancellation systems have improved markedly, they can still introduce artifacts and impair sound quality, especially for music. Finally, an open fitting leads to comb-filtering effects (the direct sound passing though the vent interacts with the delayed sound through the aid), which introduces spectral ripples and reduces sound quality.

A second requirement is to compensate for loudness recruitment via frequency-dependent amplitude compression. Hearing aids do this moderately effectively, but they often fail to restore the audibility of soft sounds, especially at high frequencies, and the amount of compression is often limited (and less than indicated by the manufacturers' fitting software), leading to loudness discomfort (and sometimes reduced speech intelligibility) at high sound levels. Also, compression systems introduce cross-modulation, impairing sound quality.

Most hearing aids incorporate noise-reduction systems. These can operate on the output of a single microphone or use multiple microphones to create a directional characteristic ("beam"), usually pointing to the front. However, it is sometimes necessary to look away from the target talker (e.g. when viewing a poster) and this can greatly impair speech intelligibility when a beamformer is used. Some hearing aids incorporate deep neural networks to enhance the most prominent talker (or talkers) and suppress other sounds, regardless of the direction of the selected talker. These can be effective with a closed fitting, but much of the benefit is lost with an open fitting, because of leakage of background sounds through the vent."

Acoustic Scene-Aware and Auditory Model-Based Compensation Strategies

Torsten Dau[1]

[1]*Technical University of Denmark*

Individual Abstract: Despite significant advances in our understanding of human hearing and assistive hearing technologies, the benefits of these advancements vary widely across individuals. There is ongoing debate regarding the most effective approach to compensate for the perceptual consequences of hearing loss. A common strategy involves frequency- and level-dependent amplification, guided by biological principles such as fast-acting compression, to restore 'normal' auditory function, particularly given that cochlear impairment is the most frequent cause of hearing loss. Alternative signal processing strategies focus on supporting 'central' processing functions, including cognition, memory, and auditory attention - areas particularly relevant in age-related hearing loss. More recently, 'black box' statistical machine learning approaches have been explored for hearing aid optimization. These methods are not always rooted in auditory processing principles and may instead be guided by listener attention, intention, or acoustic scene-aware criteria, such as direct-to-reverberant ratio or signal-to-noise ratio-driven compression settings. Other machine learning approaches draw on auditory models inspired by peripheral and higher-level processing, designed to address real-world auditory challenges. While these approaches show promise, achieving effective nonlinear system compensation in real-life, real-time scenarios

remains a significant challenge. This presentation explores the opportunities and limitations of current strategies for individualized hearing aid compensation.

Presidential Symposium: Innovations, Challenges, and Personalization in Hearing Aids - Part 2

Chair: Sunil Puria*, Harvard Medical School*

10:30 a.m. - 12:00 p.m.

Ocean Ballroom

Personalized Hearing Loss Compensation for the Next-Generation Hearables and Hearing Aids

Sarah Verhulst[1]

[1]*Ghent University*

Individual Abstract: Recent advances in precision hearing diagnostics and machine-learning techniques for audio signal processing have brought fully personalized audio solutions for early-onset and standard hearing impairments within reach. However, this innovation presents several challenges. Embedding biophysical models of hearing impairment into closed-loop systems for algorithm design remains computationally demanding. Additionally, neural network (NN)-based audio processing introduces unique sound-quality issues distinct from those encountered in standard hearing aids. In this presentation, I will discuss how novel diagnostic methods can be employed to individualize the parameters of biophysical and NN-based models of human auditory signal processing. I will also demonstrate how these models, such as CoNNear, can be integrated into differentiable closed-loop systems to enable end-to-end signal processing for hearing aids and hearables. Furthermore, I will present examples showcasing the restoration quality of these systems and explore strategies for optimizing them for real-time processing and superior sound quality. These translational steps are essential for integrating these advanced systems into next-generation embedded platforms for hearables and hearing aids, paving the way for a new era of personalized auditory technology.

The Potential and Limitations of Applying DNN Based Algorithms for Speech Enhancement in Hearing Aids

Stefan Launer[1]

[1]*Sonova AG*

Individual Abstract: Actively participating in social life and here specifically in conversations in challenging listening conditions, the cocktail party, has ranked as the top problem for people with hearing loss over decades. In hearing aids many solutions have been introduced to improve speech intelligibility in a variety of challenging listening conditions. The most successful solution to date is to apply multi-microphone technology, so called beam-former, enhancing sounds from specific directions. By combining microphones in different ways, targets from different directions, either, front, side, rear, can be enhanced while interfering sounds from other directions can be reduced. These types of algorithms have shown significant improvements in clinical studies and provide

significant benefit to people with hearing loss in daily life communication. However, these techniques also have some drawbacks, one of which being that in very dynamic conversation situations they might not be capable of following the target sound fast enough. In recent years techniques from machine learning or artificial intelligence have been extensively explored regarding their potential and limitations for speech enhancement in many applications, hearing aids being one of them. Many different types of DNN based algorithms have been explored and tested. Hearing aids pose specific challenges to DNN-based speech enhancement algorithms in that hearing aids are very constrained regarding computation power and memory capacity due to the small volume of the devices. Furthermore, hearing aid signal processing requires "real-time" processing, the delay through the hearing aid should be less than 15 ms. Finally, hearing aids have to work in a vast number of different conversation situations taking place in different acoustic scenes. This requires very sophisticated training procedures to assure generalization of the algorithm will still be computationally efficient.
This talk will discuss the key challenges for speech enhancement in hearing aids and explore the potential and limitations of existing and new algorithms such modern DNN-based algorithms. We will present a solution employing a well-known U-shape network architecture, discuss the approach to training and implementation in hearing aids. Finally, we will present results from various clinical studies which clearly show how DNN-based algorithms outperform existing algorithms.

A Future of Hearing Aids
Jaipreet Virdi[1]

[1]*University of Delaware*

Individual Abstract: Every new technology," philosopher George Estreich writes, "is accompanied by a persuasive story, one that minimizes downsizes and promises enormous benefits…Too often that narrative frames disability as a cost." This presentation chronicles how the design of hearing aids has transformed through the 20th and 21st centuries, from fashionable devices offering a multitude of styles for the deaf user, to digital aids favoring a sleek, industrial aesthetic reminiscent of futuristic designs. Analyzing commercial and prototype models of hearing aids, Dr. Virdi shows how the emphasis on technology creating a "post-deaf" reality is problematic, as it diminishes the value of disabled perspective. By examining historical and contemporary perspectives of deaf innovations and the marketing of hearing devices, Dr. Virdi invites alternative approaches for thinking about the future of hearing aids, particularly a future where hearing aid design moves beyond discretion to open possibilities for imagining deaf worlds to radically disintegrate ableist stereotypes.

Poster Session I

1:00 p.m. - 2:30 p.m.

Peninsula Ballroom

SA1. Investigating the Relationship Between the Ventral Attention Network, Vigilance, and Fatigue Utilizing Listening Effort, Neurophysiological, and Neurostimulation Methods: An Electroencephalogram (EEG) and Transcranial Alternating Current Stimulation (tACS) Study

Corrin Stines-Ringling*[1], Edward Golob[1], Alyssa Randez[1], Juan Fernandez[1], Ricardo Castañeda[1], Jeffrey Mock[1]

[1]*University of Texas at San Antonio*

Category: Auditory Cortex and Thalamus: Human Studies

Background: Prior research has implicated a cortical network in the right hemisphere for maintaining attention on vigilance tasks, termed the ventral attention network (VAN). Vigilance tasks typically last 10s of minutes, induce a high mental workload, increase mental fatigue, and influence mood. Coordination between the two key areas of the VAN, the temporoparietal junction (TPJ) and inferior frontal gyrus (IFG) likely involves cortical oscillations, which can be non-invasively measured with EEG, but this has not been tested.
Methods: Experiment 1 utilized EEG to identify oscillations between the TPJ and IFG in addition to an ideal oscillation frequency. Experiment 2 utilized transcranial AC stimulation at the identified EEG frequency to determine if performance can be enhanced during an auditory spatial attention task by increasing the oscillation amplitude. Experiment 3 is currently testing the effects of transcranial AC stimulation of the VAN using ideal oscillation frequencies to explore whether this intervention also reduces mental workload and cognitive fatigue.
Results: In experiment 1 participants (n=10) performed a spatial attention task for 38 min. Current density modeling of cortical oscillations between the TPJ and IFG showed that 5 Hz oscillation amplitudes decreased with time-on-task (p LESS THAN .01). In Experiment 2, participants (n=34) performed the same spatial attention task and were randomly divided into stimulation (5 Hz) and sham groups. Results showed that reaction times were faster in the stimulation vs. sham group (p LESS THAN .01), with no significant group differences in accuracy. Experiment 3 uses an auditory listening effort task to induce cognitive fatigue, as well as EEG monitoring and several batteries designed to assess workload, fatigue, and mood, to test if 5 Hz transcranial AC stimulation reduces changes in workload, fatigue, and mood relative to sham. The specificity of 5 Hz will also be further tested against other oscillation frequencies. Data collection has started as of July 10th for Experiment 3 and will be completed at the end of the Fall 2024 Semester.
Conclusions: Collectively, these findings suggest that oscillations in the theta band (4-8 Hz) help coordinate activity within the VAN, and may be causally related to behavior, and perhaps the conscious experience, of attentional vigilance. Applications include the use of transcranial stimulation to mitigate fatigue-related impairments which are common in a variety of neurological, psychological, and physical disorders.

SA2. Envelope Representations Substantially Enhance the Predictive Power of Spectrotemporal Receptive Models in the Human Auditory Cortex

Guoyang Liao*[1], Dana Boebinger[2], Jenelle Feather[3], Christopher Garcia[4], Kirill Nourski[4], Matthew Howard III[4], Thomas Wychowski[2], Webster Pilcher[2], Sam Norman-Haignere[2]

[1]*University of Rochester,* [2]*University of Rochester Medical Center,* [3]*Flatiron Institute,* [4]*The University of Iowa*

Category: Auditory Cortex and Thalamus: Human Studies

Background: Neural responses throughout the auditory pathway show tuning for modulations in a time-frequency representation of sound, but how these spectrotemporal modulations are encoded in the human auditory cortex remains poorly understood. Classical, linear spectrotemporal receptive field (STRF) models are simple to understand and fit but have limited predictive power, particularly for complex natural sounds in non-primary regions of the human auditory cortex.
Methods: We measured responses to a diverse set of natural sounds using spatiotemporally precise intracranial recordings from human neurosurgical patients. We then attempted to predict the response of each electrode using a linear STRF or a two-stage model that first computed the spectrotemporal envelope from a bank of STRFs, and then linearly mapped these envelopes to the neural response. We also compared the prediction accuracy of these spectrotemporal models with multi-layer deep neural network (DNN) models trained to perform challenging tasks such as speech recognition.
Results: We find that the two-stage envelope model nearly doubles the predictive power of STRF models in non-primary auditory cortex. Moreover, the two-stage STRF model performed nearly as well as complex, multi-layer DNN models, while retaining much of the simplicity and interpretability of classic, linear STRF models.
Conclusions: These findings reveal how spectrotemporal modulations are represented in the human auditory cortex and demonstrate how to substantially enhance the predictive power of a workhorse auditory model.

SA3. Neural Processing the Global Properties of Natural Auditory Scenes

Margaret McMullin*[1], Nathan Higgins[2], Rodica Constantine[1], Joel Snyder[1]

[1]*University of Nevada, Las Vegas,* [2]*University of South Florida*

Category: Auditory Cortex and Thalamus: Human Studies

Background: Conscious perception of an auditory scene is often assumed to rely on the identification and segregation of multiple objects making sounds around the same time. However, it is possible that a more global process may also occur when we evaluate auditory scenes. Studies in the visual domain have identified global properties (e.g., openness, naturalness) that aid in our rapid recognition of scenes – even without identifying each individual object within it. Recent behavioral work from our lab has extended these findings by providing preliminary evidence for global processing of complex, real-world auditory scenes.
Methods: The aim of the present study is to expand these behavioral findings by investigating the neural processing of natural auditory scenes. 45 normal hearing participants listened to 200 scenes (4 sec each) and completed separate object (e.g., “Did you hear a dog bark?”) and setting (e.g., “Could this be a café?”) identification tasks during EEG recording.

Results: Non-parametric cluster-based analyses were conducted to compare performance on the setting and object identifications tasks. Preliminary results indicate similar early ERP responses (N1, P2) for both tasks. A sustained potential was also elicited by both tasks but is more negative for the object identification task.
An additional set of cluster analyses will be conducted to compare setting and object identification task performance to the average global property ratings of the scenes (from McMullin et al., 2024). The scenes were categorized into a "high" and "low" group based on their scores on Factors 1 and 2 from the exploratory factor analysis conducted on the average global property ratings of the scenes from McMullin et al. (2024). We expect to find distinct patterns of activity for scene- and object-related processing at the nine frontocentral electrodes which often reflect activity associated with auditory cortex activation. We will also conduct a time-frequency analysis to assess changes in power across frequencies and measure inter-trial phase coherence across trials for the same comparisons made in the cluster analysis.

Conclusions: The results of this study will deepen our understanding of how object-level and scene-level information relates to the global properties of auditory scenes, as well as the neural activity associated with identifying objects and setting information. Although EEG will not allow us to identify the location of pathways for scene and/or object processing, it may reveal amplitude differences between the setting and object identification tasks in regions distinct to the auditory ventral stream, which could merit future fMRI studies on natural auditory scene perception.

SA4. The Impact of Musical Expertise on Disentangled and Contextual Neural Encoding of Music Revealed by Generative Music Models

Yinghao Li[1], Gavin Mischler*[1], Stephan Bickel[2], Ashesh Mehta[2], Nima Mesgarani[1]

[1]*Columbia University,* [2]*The Feinstein Institute for Medical Research*

Category: Auditory Cortex and Thalamus: Human Studies

Background: Music perception involves the intricate processing of both individual notes and their contextual development throughout an entire piece. How the auditory cortex constructs a coherent musical
narrative by encoding disentangled and contextual information remains unclear.

Methods: Here, we investigate the neural basis of music perception using noninvasive and invasive electrophysiological recordings combined with advanced generative music models.
Results: Our study reveals that musical training enhances the brain's capacity to process complex musical structures within the broader context. Comparative analyses reveal that musicians exhibit more precise and disentangled neural encoding of music's various dimensions than non-musicians. Furthermore, invasive recordings highlight the spatial organization of musical context encoding across different brain regions, elucidating the hierarchical nature of musical processing.
Conclusions: These findings underscore the significant influence of musical expertise on neural encoding and
contribute to a deeper understanding of music perception in the human brain.

SA5. Two Waves of Acetylcholine Encode Distinct Aspects of Sensation, Action, and Related Expectations

Chung-Wei Chiang*[1], Hemant Kumar Srivastava[1], Zakir Mridha[1], Siddhartha Joshi[2], Jan Willem de Gee[3], Marina Rodriguez Alonso[1], Hong Jiang[1], Matthew McGinley[1]

[1]*Baylor College of Medicine,* [2]*Stanford University,* [3]*University of Amsterdam*

Category: Auditory Cortex and Thalamus: Structure & Function

Background: The functions of acetylcholine in auditory cortex (AC) are diverse and poorly understood. For example, cholinergic inputs to AC fluctuate with pupil-indexed brain state (Reimer et al., 2016) but also respond to sounds (Guo et al., 2019) and goal-directed movements (Zou et al., 2024).

Methods: Here, we have monitored cortical acetylcholine (ACh) levels using the GRAB-ACh sensor in two attention paradigms in head-fixed mice. In both paradigms we find that ACh exhibits two response waves, separated by seconds.

Results: The first paradigm is a pre-pulse inhibition (PPI) noise burst protocol to study attentional blink. Head-fixed mice in 40 dB background noise were presented with a 'pre-pulse' noise burst drawn from three loudnesses (44-52 dB) followed 80 ms later by a 'probe' burst (90 dB), or pre-pulses or probe bursts alone. Responses to all sounds exhibited two waves of ACh. The magnitude of the first wave was proportional to overall acoustic energy in the stimulus, being smallest for the weak pre-pulse and largest for the pre-pulse + probe condition. The second wave showed a reversed pattern regarding probe versus pre-pulse + probe, suggesting a more processed form of response, which mirrored the pattern of response observed in the pupil size, and exhibited PPI. Laminar probe recordings in inferior colliculus showed the PPI effect in the phasic response but had no reverberant or 'second wave' of activity, suggesting that longer lasting processing occurs downstream, perhaps within basal forebrain (Guo et al., 2019).

In the second paradigm, we imaged the same sensor in auditory cortex during our recently developed sustained attention value task (de Gee et al., 2024). In this task, mice were trained to detect the unpredictable emergence of temporal coherence in an otherwise random cloud of tones. Reward size alternated between high and low values in blocks of trials. We found that in this attention task, there was also two waves of ACh, but after licks. The first wave of cortical ACh was stronger in hit trials compared to false alarms, but the difference could simply be accounted for by the number of licks (Zou et al., 2024), and was larger during high-reward blocks. The magnitude of the second wave, however, was higher in the low reward blocks, and differed between hit and false alarm trials, long after licking had ceased.

Conclusions: Taken together, these findings suggest that a first wave of ACh is linked to the intensity of the sensory stimuli or behavioral response, while the second wave reflects more processed forms of information related to attentional processes.

de Gee JW, Mridha Z... McGinley MJ (2024).

Reimer J, McGinley MJ… Tolias AS (2016).

Zou J, de Gee JW, … McGinley M, and Hires SA (2024).

Guo W, Robert B, and Polley DB (2019).

SA6. Cell-Type Specific Synaptic Zinc-Signaling in the Auditory Cortex Contributes to the Recovery of Perceptual Hearing After Noise Trauma

Cassandra Linnertz*[1], Aidan Soose[1], Manoj Kumar[1], Thanos Tzounoupoulos[2]

[1]*University of Pittsburgh,* [2]*Pittsburgh Hearing Research Center, University of Pittsburgh*

Category: Auditory Cortex and Thalamus: Structure & Function

Background: Noise trauma reduces auditory sensory inputs relayed from the cochlea to the primary auditory cortex (A1). To compensate for reduced peripheral sensory input, A1 undergoes homeostatic plasticity. Namely, the sound-evoked activity of A1 excitatory principal neurons (PNs) recovers or even surpasses pre-noise trauma levels and shows increased response gain, the slope of sound level against the neuronal response. Moreover, this plasticity in A1 PNs is associated with the recovery of perceptual sound-detection thresholds. Despite the importance of A1 plasticity after NIHL, the precise synaptic signaling mechanisms underlying the recovery of perceptual hearing remain unknown. Our previous work has established that synaptic zinc signaling contributes to cortical gain modulation, sound frequency discrimination, and cortical adaptation to background sounds with different contrasts. Moreover, our work has established that synaptic zinc is expressed and released from cortical neurons in a cell-type-specific manner. Synaptic zinc is expressed in excitatory principal neurons and inhibitory Parvalbumin (PV) and Somatostatin (SOM)-expressing neurons. However, synaptic zinc does not express in Vasoactive intestinal Peptide (VIP)-expressing neurons. This cell-type-specific expression of synaptic zinc modulates cortical circuits in a synapse-specific manner. Given the critical importance of cortical synaptic zinc signaling in gain modulation and auditory processing, the role of cortical synaptic zinc signaling in perceptual recovery after noise trauma remains unknown. Here, we tested the contribution of cortical cell-type specific synaptic zinc-signaling to the recovery of perceptual hearing after noise trauma.

Methods: To genetically eliminate synaptic zinc from the auditory cortex in a cell-type-specific manner, we employed a novel CRE/DRE dual recombinase transgenic mouse line (ZnT3 Cre/Rox). To assess perceptual hearing recovery after noise trauma (8-16 kHz narrowband sound at 100 dB for 2 hr), we employed an operant Go/ No-Go behavioral assay.

Results: We found that pharmacological or genetic elimination of synaptic zinc across the auditory cortex cell types reduced the recovery of perceptual sound-detection thresholds after noise-trauma, suggesting that synaptic zinc signaling contributes to perceptual recovery after noise-trauma. Next, we eliminated synaptic zinc from auditory cortex neurons in a cell-type-specific manner using the ZnT3 Cre/Rox mouse line. We found that eliminating synaptic zinc expression selectively from PV neurons does not affect the perceptual recovery after noise trauma, suggesting that synaptic zinc signaling from PV neurons does not contribute to perceptual recovery after noise trauma.

Conclusions: These results suggest that cell-type specific synaptic zinc signaling contributes to the recovery of perceptual hearing after noise trauma.

SA7. Convergence of Bilateral Auditory Tectothalamic Pathways

John Kara*[1], Tolulope Adeyelu[2], Charles Lee[2]

[1]*Louisiana State University Health Science Center,* [2]*Louisiana State University*

Category: Auditory Cortex and Thalamus: Structure & Function

Background: The medial geniculate body (MGB) receives diverse and robust ascending and descending projections, making it a critical hub in the central auditory system. Ascending excitatory and inhibitory inputs to the MGB originate from the auditory midbrain (inferior colliculus: IC), which convey and regulate auditory signals. While the ipsilateral auditory tectothalamic pathways are well characterized, the contralateral tectothalamic pathways are largely unexplored.
Methods: Therefore, to explore the cell-type specific organization of the contralateral pathways, we employed a cre-lox mediated, dual-anterograde, viral tracing approach using C57BL/6J, VGlut2-Cre and VGAT-Cre mice. Also, electrophysiological experiments would be used to assess the functional impact on the MGB.
Results: Our data demonstrate a topological alignment of terminal arbors originating from bilateral tectothalamic inputs onto MGB neurons. The relative neuroanatomical weight of excitatory and inhibitory terminals from the contralateral IC suggests a substantial role in influencing MGB responses. These data are supported by ongoing optogenetic electrophysiological experiments to delineate the functional impact of these contralateral projections on the MGB and auditory processing
Conclusions: Overall, our data highlight overlooked roles of the contralateral tectothalamic projections in central auditory processing.

SA8. Interneurons Contribute to the Adaptation to Sound of Corticocollicular Neurons in the Auditory Cortex of Mice

Philip Bender*[1], Mason McCollum[1], Kaitlin Bainer[1], Charles T. Anderson[1]

[1]*West Virginia University*

Category: Auditory Cortex and Thalamus: Structure & Function

Background: Processing sounds in a complex auditory environment is a fundamental ability of the auditory system. Stimulus-specific adaptation is observed throughout the auditory system, where the repetition of a stimulus results in diminishing neuronal responses to that stimulus, but not to novel or deviant stimuli. Adaptation to auditory stimuli is altered in many neuropsychiatric disorders, including autism spectrum disorder and schizophrenia, suggesting that this mechanism plays a key role in the normal function of the auditory system. Many types of neurons within the cortex demonstrate stimulus-specific adaptation to auditory stimuli, including both excitatory and inhibitory neurons. Changes in inhibitory interneuron function can alter the adaptation of excitatory principal neurons and cortical stimulus-specific adaptation to auditory stimuli is propagated to subcortical regions, but how these mechanisms are linked is largely unexplored. Here, we show that auditory cortex somatostatin-expressing (SOM) interneurons contribute to the stimulus-specific adaptation of corticocollicular neurons in an intersound-interval-dependent manner.
Methods: We performed stereotaxic injections of AAVs encoding the inhibitory opsin JAWS into the auditory cortex of Cre-driver mouse lines, to express the opsin in cortical interneurons. We

also used AAVs to express GCaMP8m or GCaMP7b in corticocollicular neurons in the same animals. We then performed calcium imaging of neurons in awake, head-fixed mice during and presented both single tones and stimulus trains of tones at different interstimulus intervals and quantified the effects of optogenetic inactivation of interneurons on GCaMP responses.
Results: We find an intersound-interval-dependent effect of cortical interneuron inactivation on the response of corticocollicular neurons to trains of tones.
Conclusions: Interneurons contribute to the adaptation of auditory corticocollicular neurons in a manner which depends on the rate at which the tones are presented. Understanding the specific contributions of various cortical cell-types and circuits to specific aspects of auditory processing is fundamental to our understanding of (1) how normal auditory processing is performed and maintained, and (2) how deficits in aspects of auditory processing may arise from disruptions of these fundamental systems and contribute to the etiology of neuropsychiatric conditions such as schizophrenia or autism spectrum disorder.

SA9. Characterization of Giant Cells of the Dorsal Cochlear Nucleus

Michael Kasten*[1], Reginald Edwards[1], Kendall Hutson[1], Malcolm Lutz[1], Paul Manis[1]

[1]*University of North Carolina - Chapel Hill*

Category: Brainstem: Structure & Function

Background: The glutamatergic projection neurons of the dorsal cochlear nucleus (DCN) are fusiform and giant cells. Fusiform cells are the principal neuronal output cell, with ~2000 neurons per DCN; giant cells are vastly less common, with an estimated 50-100 neurons per DCN. Both cell types are known to project to the contralateral inferior colliculus. However, while fusiform cells are extensively studied, much less is known about giant cells - and there is even debate about whether they are a distinct cell type from fusiform cells. We sought to determine whether we could record from giant cells of the adult DCN and if they were electrophysiologically and morphologically distinct from fusiform neurons. We were able to obtain quality patch clamp recordings from giant cells in transstrial DCN slices from mice aged p16-572.
Methods: CBA/J and NF107::Ai32 (on an FVB/C57BL6 background) mice were used for experiments. Post-natal 16-572 day-old mice were euthanized and transverse 250 um thick slices through DCN were prepared on a vibratome. Fusiform and giant cells were roughly identified by size, shape and location and whole cell recordings were performed with a potassium-based intracellular solution containing tetramethylrhodamine biocytin or lucifer yellow. For histology, slices were subjected to immunostaining and confocal Z-stack images were imported into Imaris for 3D reconstruction.
Results: The input resistance in giant cells was similar to age-matched fusiform cells. A subset of giant cells demonstrated a large Ih-like voltage sag. Giant cells tended to fire at a higher rate than fusiform cells and exhibited extremely rapid action potential repolarization, with dV/dt approximating that seen during action potential depolarization. Still, categorizing separating giant cells and fusiform cells solely on the basis of electrophysiological properties is not always possible. Morphologically, giant cells demonstrated more initial dendrites than fusiform cells but with less robust dendritic branching. Unlike previously reported in young rat (Zhang and Oertel, 1993; Rusznak et al., 2013), we found that giant cell dendrites often traversed into the molecular layer but did not demonstrate the same pattern of spines and endings seen in fusiform cells. Furthermore,

as with fusiform cells, with optogenetic activation of auditory nerve fibers, we found evidence of auditory inputs directly onto giant cells similar to those seen in fusiform cells.
Conclusions: We have identified a number of differences in morphological and electrophysiological properties between fusiform and giant cells. While individual giant cells often display properties easily distiniguishable from fusiform cells, such as higher firing rate, faster action potential repolarization and prominent Ih current, these differences are not fully categorical.
Work supported by NIH grant R01DC0019053

SA10. Noise-Induced Hearing Loss Alters Structure and Function of Inhibitory Cells in the Dorsal Cochlear Nucleus

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Category: Brainstem: Structure & Function

Background: Noise-induced hearing loss (NIHL) permanently impairs ~40 million US adults. While it is known that NIHL can damage cochlear hair cells and spiral ganglion synapses, its effects on the ascending auditory system are still being investigated. The first central auditory structure to receive input from the cochlea via the auditory nerve is the cochlear nucleus (CN). The CN has two main divisions: the ventral cochlear nucleus and the dorsal cochlear nucleus (DCN). DCN neurons integrate auditory information with somatosensory, vestibular, and proprioceptive signals for external sound localization and may participate in the inhibition of self-generated sounds (Ryugo et al., 2003; Singla et al., 2017). Structural changes in the axon initial segment (AIS)—a macrodomain responsible for action potential initiation and propagation— leading to increased excitability have been described in a subset of avian CN neurons after hearing loss. While these and other studies provide insight into the structural and functional changes of auditory neurons in the context of sensorineural hearing loss, there is limited information about the effects of NIHL on DCN-interneurons.
Methods: Forty to forty-five-day-old VGAT-ChR2-EYFP mice received 2 hours of 106 or 115dB SPL octave-band noise or a sham noise exposure (0dB). Two weeks later, electrophysiological recordings and histological analysis were performed. Fusiform cells (FC, an excitatory principle cell) and two inhibitory cell types - cartwheel cells (CWC) and tuberculoventral cells (TVC) were recorded in brain slices. For AIS measurements, mice were perfused, brains harvested, cryopreserved, sliced coronally at 40-60µm, immunostained, and imaged on a confocal microscope. Thirty to fifty-micron Z-stack images were imported into Imaris for 3D reconstruction and analysis of AIS length.
Results: Osteopontin (OPN1) (antibody [RRID: AB_2783651]) was identified as a novel marker of FCs. Immunostaining with OPN1 plus the AIS marker ankyrin-G revealed no structural changes in FC AIS. FC firing rate was also not altered after noise exposure. Interestingly, the AIS of noise-exposed CWCs was longer than in sham-exposed mice, primarily in the high-frequency region of the DCN at both noise exposure levels. TVCs could not be unambiguously identified in the VGAT-ChR2-EYFP mice; however, the firing rate of TVCs was elevated following 106 and 115db noise exposure levels relative to sham mice.

Conclusions: CWC AIS length and TVC firing rates were altered two weeks post-noise exposure. Both CWCs and TVCs are the primary local inhibitory neurons that regulate the firing of FCs. Interestingly, FCs were unaltered by noise exposure. These structural and functional changes may affect the DCN sensory responses after NIHL.

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SA11. Pupil-Indexed Brain State Modulates Activity at Multiple Stations in the Auditory Brainstem

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Category: Brainstem: Structure & Function

Background: The state of the waking brain fluctuates from moment to moment due to release of neuromodulators from the reticular activating system. These modulators innervate the auditory brainstem at all stations, from cochlear nucleus to inferior colliculus. How state fluctuations impact activity in the auditory brainstem, however, is largely unknown. Pupil size provides a proxy measure of neuromodulatory brain state and its impacts on auditory thalamocortical function. Pupil size tracks cholinergic and noradrenergic axonal activity in auditory cortex (Reimer et al., 2016), state-dependent rhythmic structure in auditory cortex, the gain and reliability of sound responses in auditory thalamus and cortex, and their impact on auditory decision-making (McGinley et al., 2015). A consistent pattern is that neural and behavioral responses have an inverted-U dependence on pre-stimulus pupil size; best sound representations and performance occur with mid-sized pupil.
Methods: Here, we recorded single-unit activity using Neuropixels probes from two brainstem regions, the dorsal cochlear nucleus (DCN) and central nucleus of the inferior colliculus (ICC), in separate experiments, in head-fixed awake mice on a cylindrical treadmill. We used pupil size and locomotor status as measures of state.
Results: Mirroring prior results in thalamus and cortex, in the ICC the population average showed an inverted-U shaped dependence of the magnitude of responses to tones on pre-stimulus pupil size. Follow-up, unsupervised clustering showed that subpopulations of neurons in ICC had distinct state-dependence patterns. The largest group exhibited the inverted-U shaped dependence on pupil size, while the second major group showed a monotonically decreasing sound response with increasing pupil-indexed arousal. The basic tuning characteristics of ICC neurons did not correlate with their pattern of state-dependency, suggesting that ensembles with distinct state-dependence maintain broad representational capacity across states. In the DCN, we first putatively identified each unit as one of three major cell types based on their responses to tones or noise across a range of intensities: fusiform cells (the main excitatory projection neurons) and two interneurons classes, cartwheel cells (receive predominantly multimodal input) and vertical cells (receive predominantly auditory nerve input). Spontaneous and sound-evoked DCN activity was highly state-dependent with cell-type specificity. For example, spontaneous activity of putative cartwheel cells peaked at mid-arousal levels, while putative vertical cells showed maximum firing rates at low arousal states. Fusiform cells exhibited diverse state dependency.

Conclusions: These results show that neural activity in both the DCN and ICC is strongly influenced by brain state, with individual neurons and neuronal types exhibiting distinct patterns of modulation. Importantly, these state-dependent modulations were not apparent in Auditory Brainstem Response (ABR), suggesting that neural activity measurements at single unit resolution are needed to capture the effects of neuromodulatory brain state.
Reference:

McGinley MJ, David SV, and McCormick DA (2015)

Reimer J, McGinley MJ, …, Tolias AS (2016).

SA12. Complement Component C3 Accumulates in the Cochlea of CBA/CaJ Mice with Noise-Induced Hearing Loss

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Category: Immunology

Background: Noise-induced hearing loss (NIHL) is characterized by dysfunction and pathological changes of the cochlea, including the loss of outer hair cells (OHC). Clearance of damaged OHCs is thought to be carried out by supporting cells and by cochlear macrophages. However, the underlying mechanisms that regulate OHC clearance remain largely unclear. The complement system is part of the innate immune system and plays important roles in removing cellular debris and apoptotic cells throughout the body, however, complement activation in the cochlea remains understudied. Complement component C3 can initiate the alternative complement cascade resulting in the phagocytosis of dead or dying cells. Recent studies suggested a potential role of the complement system in the proper functioning of the auditory system. Additionally, C3 gene expression increases in mouse cochlea during aging. Therefore, we sought to investigate whether C3 protein exists in the cochlea and if it increases after noise damage.
Methods: To understand whether C3 protein is present or elevated in NIHL, we investigated C3 using immunohistochemistry in the cochlea of CBA/CaJ mice that were noise exposed to an octave band noise (8–16 kHz) at 112 dB SPL at 9 weeks of age. Mice were sacrificed and examined at various post-exposure times. Cochleae were whole mount prepared or cryo-sectioned, and immunostained using antibodies against C3, along with additional markers to probe the potential mechanisms by which complement activation is impacting sensory hair cells.
Results: We found that C3 protein colocalized with varies noise-damaged structures in Organ of the Corti in mice after noise exposure, especially in the OHC area. Importantly, the accumulation of C3 aggregates appeared in the OHC layer where OHCs were lost.
Conclusions: Our results suggest that C3 might be involved in clearing damaged OHCs and OHC synapses potentially by supporting cells. We conclude that the complement system plays important roles in the clearance of damaged tissue in the cochlea after noise trauma.

SA13. Impacts of Extended High-Frequency Hearing Loss on Neural Encoding and Perception of Speech

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Category: Brainstem: Structure & Function

Background: Extended high-frequency (EHF) loss (GREATER THAN 8 kHz) is prevalent among young adults. Despite its high prevalence, the physiological and behavioral consequences of EHF loss remain unclear. Clinically, many individuals report challenges in understanding speech despite presenting with a normal audiogram in the standard frequency range (up to 8 kHz). EHF loss may contribute to these "hidden hearing difficulties," often overlooked in clinical settings.

Most existing studies suggest that speech perception difficulties associated with EHF loss are due to subclinical cochlear damage in the standard frequency region. However, no studies have investigated how the auditory nervous system extracts useful information from the EHF range. The purpose of this ongoing experiment is to examine if the auditory nervous system can extract speech information from the EHF range known to be important for listening in noise (i.e., F0 and slow syllabic-rate modulations) and if this ability predicts speech-in-noise performance. Additionally, the present study examines the relationship between EHF threshold and speech-in-noise perception difficulties.

Methods: Frequency following responses (FFRs) were recorded using the single-channel bipolar electrode montage. Recordings were made using three stimuli sets: (1) a broadband male vibrato stimulus with F0 = 380 Hz, (2) the same stimulus high pass filtered (HPF) above 4 kHz, and (3) HPF at 8 kHz, containing only EHF energy. Additionally, behavioral speech recognition thresholds in noise were assessed using the digit triplet test using the four sets of stimuli: 1) Original recorded digit stimulus, 2) Stimulus with EHF energy only (HPF; GREATER THAN 8 kHz), 3) Stimulus with high frequency energy (HPF; GREATER THAN 4 kHz), and 4) Stimulus without EHF energy (low pass filtered; LESS THAN 8 kHz). A linear mixed model was used to assess if EHF thresholds were predictive of FFR F0 strength, controlling for the effect of other variables such as age, gender, and hearing threshold at standard frequencies. Furthermore, we used EHF thresholds and FFR F0 strength to predict speech-in-noise perception.

Results: While the results of this experiment are pending, we predict that EHF thresholds will predict FFR F0 strength to HP stimuli and that both EHF and FFR thresholds will predict speech-in-noise perception for the high-pass filtered stimuli. Comparing F0 strength between individuals with and without EHF loss and comparing it with behavioral speech perception difficulties will provide insight into the speech perception difficulties experienced by individuals with EHF loss.

Conclusions: Previous studies have examined relationships between EHF thresholds and speech perception; however, it is unclear if the results from this study reflect deficits related to systemic cochlear damage or contributions from the EHF range. This study is the first to examine neural processing of speech among individuals with EHF loss and directly test its consequences on speech perception.

SA14. Calcium-Dependent Conductances Shape Firing in Octopus Cells of the Posterior Ventral Cochlear Nucleus: Implications for Temporal Coding

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Category: Brainstem: Structure & Function

Background: Octopus cells (OCs) of the posteroventral cochlear nucleus detect transients and rapid frequency sweeps in auditory nerve fiber (ANF) inputs. OCs convey this temporal information to higher auditory centers through a precisely timed, single spike at the onset of sound. Spiking is limited to tone onset by low-threshold K conductances, which rapidly repolarize spikes and decrease input resistance, preventing sustained responses. Here, we report that the temporal window of onset spiking as well as responses to ongoing stimulation are determined by Ca++- and Ca++-dependent K+ conductances. Our results reveal a fundamental role of calcium-dependent excitability in coincidence detection and phase-locking.

Methods: Whole-cell patch recordings from OCs were obtained from parasagittal or coronal brain slices of the cochlear nucleus of the mouse (P21-42). Spikes were evoked by currents injected into OC soma. Step currents were used to verify onset spiking. To mimic ongoing fluctuations in ANF activity in vivo, we added a train of sinusoidal currents after a just-suprathreshold step current. The step-sine wave stimulus pattern distinguished between onset and ongoing responses. Recordings were made in standard ACSF and after blocking Ca++- and the BK class of Ca++-activated K+ currents with Co++/Ni++ and iberiotoxin (IbTX) respectively.

Results: Onset spikes had heterogenous shapes across the OC population. Rapidly repolarizing spikes, graded spike amplitudes, post-peak shoulders and second components of after-hyperpolarizations, were suggestive of a Ca-dependent modulation of onset spiking. Co++/Ni++ decreased onset spike amplitude and caused faster spike decays, implying Ca++-dependent boosting of onset spikes. On the other hand, IbTX broadened spikes, generated a secondary depolarization and blocked the delayed after-hyperpolarization. BK conductances therefore narrowed the temporal window of onset spiking. Ca++- and BK conductances thus exert a push-pull effect on the temporal window of OC onset spiking.

During a sinusoidal current train that followed a step current, spikes were phase-locked up to 500 Hz, with one spike/sine wave, suggesting that OCs responded to repeated stimulation with high fidelity. In both Co++/Ni++ and IbTX, spike latencies and the probability of spiking changed, and spikes were not evoked by every sinewave in the train. Additionally, IbTX prevented membrane repolarization following the onset spike evoked by the preceding step current, consequently blocking responses at the beginning of the sinusoidal train. Thus Ca++ and BK conductances exert differential temporal effects on OC responses to repeated stimulation. High current strengths and frequencies GREATER THAN 500 Hz abolished ongoing spiking by reducing depolarization during the stimulus train, suggesting that additional voltage-gated conductances establish the frequency-dependence of OC responses.

Conclusions: Our results suggest a hitherto unacknowledged influence of Ca++-dependent excitability in coding auditory transients. Ca++ conductances and BK channels in OCs set the temporal window for onset and ongoing spiking and increase the temporal precision contained in their ANF inputs.

SA15. Distribution Patterns and Role of BK-Type Calcium-Activated Potassium Channels in the Octopus Cell Area of the Posteroventral Cochlear Nucleus

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Category: Brainstem: Structure & Function

Background: The octopus cell area (OCA) of the posterior ventral cochlear nucleus contains onset neurons that extract precise temporal information from auditory stimuli. This requires temporally precise spikes that are maintained by rapid repolarization of the membrane. Previous studies have focused on low threshold activated potassium conductances as the primary basis for this rapid repolarization and spiking maintenance. Using whole-cell patch recording and immunohistochemistry techniques, we reveal that onset spiking is more heterogenous and mechanistically complex than previously reported.

Methods: Whole-cell current clamp recordings from onset neurons in the OCA were obtained from parasagittal or coronal brain slices of the cochlear nucleus of the mouse (P21-42). Current steps were injected into the soma to identify onset cells and characterize their intrinsic properties. Cells were identified as OCA onset neurons by rapid time constants and transient responses to step currents. Relevant pharmacological blockers were applied to the bath to further elucidate intrinsic mechanisms. Immunohistochemistry was performed with antibodies for BK channels, HCN1 channels, and ryanodine receptors. Imaging was performed using a Nikon AXR-NSPARC confocal microscope system.

Results: Our recordings in the OCA revealed three patterns of intrinsic properties, two of which have not been previously reported. One pattern demonstrated large, rapid action potentials, along with slow afterhyperpolarizations (AHP). Applying 100 nM iberiotoxin, a specific blocker of BK channels, to the bath widened action potentials and reduced the AHP amplitude. Another pattern showed smaller and wider action potentials with fast AHPs, as well as spontaneous miniature outward currents (SMOCs). Iberiotoxin blocked a large portion of the AHP and removed SMOCs. SMOCs were also blocked by bath application of cyclopiazonic acid (CPA), indicating a role for calcium-induced calcium release. Patterns of immunolabeling in the OCA using BK-specific antibodies showed patterns consistent with physiological findings. Many cells in the OCA displayed BK channel labeling restricted to the soma and proximal dendrites. In a subset of OCA neurons BK labeling was co-localized with ryanodine receptor labeling. This colocalization is consistent with the coordination between calcium-induced calcium release and BK channels seen in physiological findings.

Conclusions: Here, we describe a role for BK channels in the onset-spiking mechanism of OCA neurons, along with its differential pattern of distribution. Given that the OCA neurons have been associated with a pure onset response as well as an onset-sustained response, variation in the spiking mechanisms observed in vitro may influence the variety of in vivo responses.

SA16. Investigation of Synaptic Excitation and Inhibition Underpinning Mechanisms Involved in Processing Sound Sequences With Behaviorally Relevant Short Intervals in IC Neurons

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Category: Midbrain: Structure & Function

Background: In both humans and animals, social vocalization typically consists of a complex array of sound stimuli characterized by diverse spectral and temporal features. The auditory system is adept at extracting this spectra-temporal information from intricate vocalization. However, it is common practice in auditory research to use simpler sound stimuli with relatively low repetition rate. Previous studies have indicated that auditory acuity can increase at short behaviorally relevant inter-stimulus intervals (ISIs). In this study, we investigated how auditory neurons in the inferior colliculus (IC) of mice process sound sequences with varying spectra-temporal characteristics at different ISIs and the contribution of synaptic excitation and inhibition to this processing.

Methods: Extracellular recordings were performed in the IC of unanesthetized CBA/CaJ mice using glass microelectrodes. After isolating a single neuron, we recorded its spiking responses to sequence of pure tones, each 20 ms in duration. Each sequence included 2,993 combinations of different sound frequencies ranging from 4-40 kHz in 500 Hz increments and sound levels from 0-80 dB SPL in 2 dB step, presented in a pseudo-random order. Each IC neuron was tested with four identical sequences presented at varying ISIs of 250 ms, 100 ms, 50 ms, and 20 ms. This data was used to reconstruct frequency response areas (FRAs) for each IC neuron. In-vivo whole-cell voltage clamp recordings in anesthetized mice were used to obtain synaptic excitatory (at -70 mV) and inhibitory (at 0 mV) currents to investigate mechanisms underlying FRA of IC neurons.

Results: We found that all IC neurons exhibited similar FRAs across different ISIs. Approximately half of the neurons showed a shift in their threshold accompanied by a narrowing of bandwidth when exposed to sound sequency with shorter, behaviorally relevant ISIs. Additionally, a small proportion of neurons failed to respond to the sound sequence with 20 ms ISI. Using in-vivo voltage clamp technique, we measured the amplitude of excitatory and inhibitory currents in IC neurons and constructed corresponding excitatory and inhibitory FRAs. We found that these FRAs overlapped, but the bandwidth of the inhibitory FRA was broader compared to excitatory FRA. This explains why extracellular recordings often reveal sideband inhibition surrounding the excitatory FRA.

Conclusions: The heightened selectivity of IC neurons in mice at behaviorally relevant short intervals underpins the increased auditory acuity observed during these intervals. Our pilot data suggest that the broader inhibitory FRA compared to excitatory FRA may explain the phenomenon of sideband inhibition commonly observed in conventional spike-based FRAs.

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SA17. Calcium Channel Expression and Localization in the Inferior Colliculus: Unraveling the Interplay Between Pharmacological Blockade and Noise-Induced Hearing Loss

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Category: Midbrain: Structure & Function

Background: Our previous imaging studies using manganese (Mn2+) as a surrogate for calcium, and thus a measure of neuronal activity, show changes in Mn2+ uptake in the inferior colliculus (IC) after a single noise exposure. This demonstrates a relationship between noise-induced permanent or temporary threshold shift (PTS or TTS) and increased neuronal activity in the IC. These noise-induced changes in neuronal activity may result from either pre- or post-synaptic plasticity. Recently, we have shown that L-type calcium channel (CaV) blockade prior to noise-induced TTS differentially affects peripheral and central synaptic function. Together these results suggest a role for dysregulation of CaVs in noise-induced hearing dysfunction. Comparing changes in CaVs may provide important evidence for identifying mechanisms contributing to the imbalance of neuronal activity that occurs after noise exposure. Therefore, in the current study we examined expression and distribution of three calcium channels, CaV1.2, CaV1.3, and RyR-3, in the IC following noise-induced hearing loss.
Methods: Sprague-Dawley rats were divided into three groups: control, TTS noise exposure, and PTS noise exposure. The noise groups were exposed to a 16 kHz, 106 dB SPL tone for one hour (TTS) or 10kHz, 118dB for four hours (PTS), while the control group was maintained in ambient noise conditions. Freshly dissected tissue from IC was used to isolate mRNA for quantitative real time PCR (qRT-PCR). Additionally, rats were transcardially perfused, and rat brains were collected, post-fixed, and cryoprotected. Serial cryostat sections (20 μm) were collected for immunolabeling. The density of immunolabeled CaV1.3 was compared across groups and subdivisions of the inferior colliculus.
Results: In the IC qRT-PCR demonstrated expression of CaV1.2, CaV1.3, and RYR-3 - which has not been previously reported. In the saline-only group, localization of CaV1.3 in the IC was most abundant in the external cortex and the dorsolateral portion of the central nucleus of the IC (CIC), compared to the dorsal cortex of the IC. In the CIC no differences were found in the low frequency dorsal regions across groups. However, in the high frequency ventral CIC, significantly more CaV1.3 labeling was observed in the TTS noise group compared to the control group (p LESS THAN 0.05).
Conclusions: Noise exposure revealed a frequency dependent increase in CaV1.3 distribution in the CIC. The current results combined with RYR-3 expression in the IC suggest a mechanism for the changes in neuronal synchrony and neuronal activity observed after noise exposure. Since our previous studies suggest that CaV blockade alone does not prevent noise-induced central changes in synaptic transmission, future studies should further delineate the contributions of L-type and RyR-3 calcium channels to hearing and their modulation across auditory nuclei, comparing differential noise trauma.

SA18. Inferior Colliculus Responses to Synthetic and Instrumental Timbre

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Category: Midbrain: Structure & Function

Background: Timbre is the quality of sound that allows subjects to distinguish between stimuli identical in pitch, duration, and loudness. Perceptual attributes of timbre, such as 'brightness', are correlated with characteristics such as spectral centroid and frequencies of spectral peaks.

However, it is unknown how timbre is represented in the inferior colliculus (IC). Spectral peaks can saturate inner-hair-cells, and when a peak harmonic near CF dominates (captures) the response, neural fluctuations of auditory-nerve (AN) fibers tuned near the peak are reduced. Conversely, responses of AN fibers tuned away from spectral peaks have larger fluctuations. Most IC neurons are sensitive to amplitude modulation, with responses that are band-enhanced (BE) or band-suppressed (BS) compared to unmodulated responses. We hypothesized that BE cells tuned near spectral peaks have reduced rates, due to capture, whereas BS cells have increased rates. This study investigated IC representation of spectral peaks in both synthetic and natural timbre stimuli, focusing on suprathreshold levels and exploring underlying mechanisms through physiological recordings and computational modeling.
Methods: Extracellular single-unit recordings were made in the central nucleus of the IC of awake Dutch-belted rabbits using tetrodes. Synthetic timbre stimuli consisted of a reference 300-ms harmonic tone complex (fundamental frequency (F0) = 200 Hz) with a triangular spectral envelope (24 dB/octave) (Allen and Oxenham, 2014 JASA 135:1371). The peak harmonic was a multiple of F0, initially positioned near a neuron's CF. Then, the spectrum was shifted in 50 Hz increments to infer a population response that included CFs at, below, or above the spectral peak frequency. Stimuli were presented at 43-83 dB SPL. Natural timbre stimuli consisted of 200-ms steady-state recordings of bassoon and oboe sounds with F0s spanning 58-1161 Hz, presented at 73 dB SPL. Stimuli were presented either diotically or contralaterally. Computational IC models featuring energy tuning, amplitude-modulation sensitivity, and broad inhibition mechanisms were tested against IC responses.
Results: For synthetic stimuli, BS neurons responded with robust peaks at the CF over a large CF range at suprathreshold levels. BE neurons had more varied responses. Most computational models accurately predicted BS neural responses, whereas energy-based and lateral-inhibition models better predicted BE responses. Diotic presentations resulted in a sharpened rate-profile compared to contralateral stimuli for many neurons. For natural stimuli, responses to bassoon and oboe sounds with large fundamental frequencies were best predicted by an energy-based model, as expected, because widely spaced components are less likely to cause beating for neural-fluctuation cues within the range of amplitude-modulation sensitivity.
Conclusions: Spectral peaks were robustly encoded in the rates of IC neurons across a wide range of CFs and sound levels for timbre stimuli. A mixture of mechanisms, including energy tuning, amplitude-modulation sensitivity, and broad inhibition, appear to interact to create these robust responses.

SA19. Cortical Layer-Specific Differences in Auditory Responses Between Young and Old Mice

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Category: Primary Auditory Cortex

Background: Age-related auditory processing changes affect the quality of life of older adults and may increase the risk factor for cognitive decline. These changes may include significant hearing loss but can also be more subtle and difficult to diagnose, such as an age-related decline of auditory temporal processing, independent of hearing loss. Processing throughout the auditory pathway is

affected, including in the auditory cortex as observed through EEG in mouse models and humans. In FVB mice, a model that experiences mild hearing loss with age, EEG analysis showed that age-related changes in temporal processing are more apparent when subjects are presented more complex stimuli, such as shorter and shallower gaps between noise. A potential treatment to mediate or reverse these auditory processing changes, nicotine, is an area of increasing investigation. It has been shown in young adult mice that systemic treatment of nicotine enhances and improves timing consistency of cortical layer IV responses to thalamic input while subjects were presented ≤ 10 Hz stimulus trains. What is not currently known is what changes occur in the auditory temporal responses across cortical layers over age, and how treatment of nicotine changes cortical processing in aged mice. Understanding subtle processing deficits over age in the cortical microcircuitry may better link suspected nicotinic receptor deficits to macroscopic scale EEG and behavioral outcomes.

Methods: We present auditory stimuli to awake, head-fixed FVB mice, over 8 groups to account for age (2-6 and 16-18 months), sex, and treatment (nicotine and saline). We record from the depth of the primary auditory cortex with a 32-channel, single-array electrode and calculate current source density (CSD) to investigate the differences in local auditory microcircuitry over these conditions.

Results: We have found weaker inter-trial phase coherence in aged mice to sound stimuli in layers I, Vb, and VI. These discrepancies are most apparent in response to 40 Hz click trains inducing auditory steady state response and in 100 ms noise bursts. The amplitude of population response, determined by CSD, is reduced in aged mice down the cortical column when stimuli are presented at the same SPL for both groups. When we increase SPL for the aged group, the column population response amplitude between young and aged mice is equivalent, but aged mice still have a weaker response to stimuli in thalamic input layer IV.

Conclusions: We predict that acute nicotine administration will have differing effects on aged and young mice, as well as male and female mice based on changes in age- and sex-related auditory cortical circuitry, with nicotine enhancing activity in layers where deficits are present. Funded by National Institute of Aging.

SA20. Layer 6 is a Hub for Cholinergic Modulation in the Mouse Primary Auditory Cortex

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Category: Primary Auditory Cortex

Background: The cholinergic basal forebrain (CBF) densely innervates auditory cortex (ACtx) and conveys signals related to internal state and behavior (Reimer et al., 2016, Robert et al., 2021; Zhu et al., 2023). Previous studies have focused on cholinergic inputs to cortical layer 1 (L1), where acetylcholine (ACh) engages L1 interneurons (L1-INs) that disinhibit deeper layer pyramidal neurons (Letzkus et al., 2011; Pi et al., 2013). Deeper cortical layers also receive direct cholinergic input (Clayton et al., 2021), although the functional relevance of these inputs has not been studied. Here, we characterize the ACh receptor subunit expression across primary ACtx (A1) layers, show that CBF axons can modulate the in vivo activity of pyramidal neurons in L6

(L6-PNs) and characterize the pharmacological properties of these synaptic inputs in an acute slice preparation.
Methods: Histology
Cholera Toxin subunit-B Alexa Fluor-647 was injected into the right medial geniculate body of Chat-Cre:Cdh23 mice to label corticothalamic neurons. Following perfusion and sectioning, multiplex fluorescent in situ hybridization was performed to label nicotinic and muscarinic ACh receptor (nAChR and mAChR) transcripts, along with markers for excitatory and inhibitory neurons.

Electrophysiology

Chat-Cre:Cdh23 mice were injected with channelrhodopsin in the right CBF to activate cholinergic axon terminals in ACtx in vivo and in vitro. For in vivo recordings, the L4/5 sink of the current source density was used to approximate depth for each unit. The trough to peak delay was used to sort units into fast spiking or regular spiking (RS). For in vitro recordings, we performed whole-cell current clamp experiments in visually identified L1-INs and L6-PNs. Light-evoked ACh-mediated postsynaptic potentials (PSPs) were recorded in the presence of glutamatergic receptor blockers, and abolished with antagonists for nAChRs and mAChRs.

Results: Our anatomical results show that L6 excitatory neurons are enriched in nAChR and mAChR transcripts compared to L1-INs, therefore pointing towards L6-PNs as major targets of CBF inputs. Our in vivo electrophysiological recordings during CBF axon activation show bidirectional modulation of spontaneous firing rate in L6-RS units with variable kinetics, in agreement with the diverse nAChR and mAChR subunit transcripts found in L6 excitatory neurons, known to trigger diverse cellular pathways in different time domains. Consistently, our in vitro recordings of L6-PNs in response to CBF axon stimulation reveal nAChR-mediated PSPs comparable to those found in L1-INs, as well as mAChR-mediated PSPs, confirming that L6-PNs receive bidirectional modulatory functional inputs from CBF.
Conclusions: We have identified L6-PNs as major targets of CBF cholinergic inputs suggesting that, together with L1, L6 is also a hub for cholinergic modulation. Corticothalamic PNs in L6 strongly recruit parvalbumin-expressing interneurons (PV-INs), whereas L1-INs predominantly inhibit PV-INs. Our study therefore suggests that CBF inputs could engage complementary microcircuits to mediate distinct forms of auditory processing.

SA21. Increases in Attentional Intensity Shift Auditory Cortical Responses Towards Object-Oriented Coding

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Category: Primary Auditory Cortex

Background: Temporal coherence (TC) between frequency channels is crucial for differentiating a target sound from its background. Attention plays an important role in the TC processing, as demonstrated by classic 'cocktail party' scenarios, where individuals can focus their attention on a

specific sound source, such as a target speaker, to selectively filter out background noise. We recently developed a sustained attention value (SAV) task in which mice were trained to detect the unpredictable emergence of TC in an otherwise random cloud of tones. Reward size alternated between high and low values in blocks of trials. We found behavioral evidence that increased task utility led to increased attentional intensity, indicated by improved signal evidence accumulation, including reduced signal neglect (see de Gee et al., 2024 for details). Here, we investigate the neural correlates in auditory cortex of improved TC processing with heightened attentional intensity.

According to the theory of Krishnan et al., TC encoding is achieved through the integration of multiple fundamental, but high-order features, such as temporal continuity, pitch, and spectral separation (see Krishnan et al.,2014). Basic spectral-temporal features play a critical role in this integration and can be captured by simple spectro-temporal receptive field (STRF) models. However, it is not clear how, and where in the brain, the TC is constructed from these low-level features.

Methods: We performed high-channel count laminar probe recordings in auditory cortex of head-fixed mice engaged in the SAV task and compared responses between the high and low task utility contexts. We estimated the STRF of individual neurons during the random tone cloud component of each trial and identified those whose spiking patterns can be well explained by STRF models (Huang et al; 2021).

Results: We found that the STRFs of these neurons was relatively stable across task utility contexts. However, the performance of the STRF model was significantly lower during TC than for hold-out tone cloud data, particularly in the high reward context. This suggests that even these 'good STRF' neurons engage an alternate encoding scheme particularly in high attention states.

Conclusions: To further understand the encoding of TC at the population level, we used Linear Discriminant Analysis (LDA) and Tensor Component Analysis (TCA) and compared results between utility contexts. Our results revealed multiple, distinct neural subpopulations with utility context-dependent responses. These included a population with overall increased activity in the high utility context and a population that exhibited slowly ramping activity after coherence start. In ongoing work, we understand how these auditory cortical neural subpopulations interact with each to engage object-oriented processing when it is useful.

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SA22. An Auditory Cortex Network Represents Both Vocal Categories and Family Dialects

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Category: Primary Auditory Cortex

Background: In some species, variability in the acoustic features of specific vocalizations can convey information about an individual's identity or family, sometimes referred to as vocal dialects. Thus, a fundamental question is how the central nervous system represents a general vocal category, while also discriminating between the subtle acoustic differences that may communicate social information.
Although there is a rich understanding of vocalization representations throughout the auditory neuraxis, the general approach has been to probe auditory neurons with relatively few vocalization exemplars, and often from vocalizers with an unknown relationship to the receiver. Here, we address the issue of call categorization by analyzing the response of adult gerbil auditory cortex (AC) neurons to a large array of variants recorded from the animal's own family and those of two other families.

Methods: We investigated the ability of AC neural populations to categorize 4 vocalization types in awake, freely-moving adult Mongolian gerbils (Meriones unguiculatus) (n=5; 3M, 2F). Gerbils are a highly social rodent species that live as multi-generational families and produce a rich vocal repertoire. We used chronically-implanted high-density silicon probes to record wirelessly from single AC neurons while presenting a large set of variants (n=300) for each of 4 vocalization categories (n=1200 stimuli, 5 trials each). The vocalizations were obtained from overnight audio recordings of individual gerbil families, one of which was always the implanted animal's own family. The response of each neuron to pure tones and amplitude modulated (AM) white noise was also used to characterize spectral and envelope responses. Initial analyses focused on AC neuron rate coding using a population decoder (support vector machine).
Results: Single unit responses generally displayed a highly variable response to the 300 variants within a call category. Despite this within-group variance at the single-neuron level, AC populations were able to decode categories significantly above chance (compared to shuffled trials; paired t-test, p LESS THAN 0.001). The sensitivity for decoding each call type was measured using the area under the curve (AUC) of the ROC curve, comparing trials of a given category versus all other categories. The average AUC across individual animals was 0.89 (range: 0.78-0.94). AC populations were also able to decode the family identity of each of the 4 call types, with an average AUC across individual animals of 0.63 (range: 0.57-0.69). Current research is investigating the extent to which AC neuron spectrotemporal tuning contributes to call and family categorization.
Conclusions: Our results suggest that vocal categories and family differences are represented by the same auditory cortex network, even for single syllables. Future work will investigate whether family information is more potent when vocalizations are presented in their natural bout structure.

SA23. Nuclear Translocation Coinciding With the Onset of Hearing in Rat and Mouse Inner Haircells

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Category: Hair Cells: Anatomy & Physiology

Background: Nuclear position is precisely orchestrated during cell division, migration, and maturation of cells and tissues. We previously reported a programmed movement of the nucleus in rat and mouse cochlear inner hair cells (IHCs) coinciding with the functional maturation of inner hair cells around the onset of hearing. : In early post-natal days, the IHC experiences a period of sustained growth, during which the nucleus sits at the very basal pole of the cell, far from the apically located mechano-transducing stereocilia, but close to where synapses with primary afferent and efferent neurons are forming. After IHCs reach their final length, the nucleus moves to occupy a new position half-way along the length of the cell. Our overall goal is to understand the cell-intrinsic and cell-extrinsic mechanism that drive this final phase of hair cell maturation.
Methods: Here, we asked if inner hair cells cultured in various conditions starting a few days before the onset of hearing continue to mature in culture, where maturation was indicated by simple anatomical measures such as nuclear position and the emergence of the big-conductance calcium-gated potassium channel (BK) in the plasma membrane. Culture conditions included high-K+ media, low-Ca+ media, media supplemented with thyroid hormone, and channel-rhodopsin-expressing inner hair cells subjected to pulsed depolarization periods driven by light-based excitation. After each culture experiment, we measured hair cell length, nuclear position, and BK channel expression from confocal scans of immunofluorescence-labeled hair cells in whole-mount cochlear preparations. Culturing started at P (post-natal day) 7, P8, and P9, and culture duration ranged from 3 – 7 days.
Results: IHCs cultured in low-K+ or thyroid hormone-supplemented solutions did not grow, express BK channels, or experience nuclear movement. IHCs cultured in high K+ solutions or depolarized by pulsatile light stimulation grew in culture, with the greatest sensitivity for cultures starting at P7, but none of the cultured hair cells experienced nuclear movement or acquired BK channels.
Conclusions: Nuclear migration and the acquisition of specific groups of potassium channels mark a dramatic biophysical transformation in the maturational sequence of IHCs. While we remain to understand how nuclear position impacts the function of hair cells, our results show that the in vitro conditions of cultured hair cells lack the necessary signals to promote inner hair cells' maturation. Future work focuses on elucidating the role of nuclear position in hair cell function and on identifying the cell-intrinsic and extrinsic signals that drive nuclear movement.

SA24. Latency Differences Between Lateral Line and Inner-Ear Evoked Startle Responses in Larval Zebrafish

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Category: Hair Cells: Anatomy & Physiology

Background: Zebrafish display an acoustic startle response known as the C-start that is initiated by hair cells in the inner ear. C-starts are primarily based on two hindbrain pathways: a Mauthner-cell (M-cell) circuit, resulting in faster, short-latency C-starts (SLCs), and a non-M-cell circuit that results in long-latency C-starts (LLCs). Recent work from our lab shows that the lateral line (LL) is capable of evoking SLCs and LLCs with latencies that are significantly slower than ear-evoked C-starts. Furthermore, when the optogenetic protein, Channelrhodopsin (ChR2) is expressed in both hair cells of the ear and lateral line, simultaneous activation with low intensity stimuli results

in SLCs and LLCs with mixed latencies, some in the range of ear-evoked and some in the range of LL-evoked C-starts. How these two hair-cell systems (ear and LL) come together to bring the SLC and LLC circuits to threshold is not well known. Here, we used optogenetic proteins with different rates of activation along with hair-cell and lateral-line specific promoters to selectively stimulate either or both of the two hair-cell systems. Further, using aminoglycosides to ablate hair cells (and remove spontaneous inputs), we compared the activation of one sensory system in the absence of the other.
Methods: Hair cells of the ear (lhfpl5a promoter) and lateral line (lhfpl5b promoter) were activated by expression of ChR2 and the faster activating, Chronos, to study C-start latencies and probabilities, which were quantified using both field potentials (to sort SLCs and LLCs) and simultaneous high-speed videos of C-starts in head-mounted or free-swimming larvae. Additionally, the aminoglycoside antibiotic, neomycin, was used to ablate hair cells to study LL or inner-ear evoked C-starts without spontaneous hair cell activity from the other system.
Results: To examine the role of ear and LL inputs on C-start excitability, SLC and LLC latencies were collected under the following conditions: (1) the ear is activated alongside the LL, (2) the LL is present and only contributing spontaneous activity, and (3) the LL is ablated. Additionally, experiments with ear activation using lhfpl5a:ChR2 combined with faster LL activation via lhfpl5b:Chronos (and vice versa) probed how timing differences between the arrival of the two inputs in the hindbrain impact C-start latency. Finally, comparison of latencies in free-swimming versus head-fixed larvae can further elucidate the role of ear and LL inputs on startle responses.
Conclusions: Our preliminary results suggest that the ear and LL systems interact to determine C-start latency depending on stimulus strength and modality. Given that in natural environments, corollary discharge from self-generated swimming movements can reduce LL activity, our study provides insight on how hair cell systems may interact in a context dependent manner to determine latency of startle responses, possibly providing for rapid escape from threat or necessary time for decision making.

SA25. Designing a Fluid-Jet Suitable for both Fast and Prolonged Deflections of Stereocilia in Mammalian Auditory Hair Cells

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Category: Hair Cells: Anatomy & Physiology

Background: Mechanosensory hair cells of the cochlea detect sound through the opening of mechano-electrical transduction (MET) channels located at the tips of shorter row stereocilia. To study MET currents ex vivo, stereocilia bundles must be experimentally deflected. The most used techniques for hair bundle deflection are: i) a piezo-driven rigid probe that pushes the stereocilia, and ii) a fluid-jet that generates laminar flow of fluid deflecting stereocilia. A rigid probe produces the fastest deflections, which is essential for resolving MET current adaptation. However, it fails to generate prolonged negative stereocilia deflections and suffers from the artificial widening of MET current-displacement (I/X) curve due to any mismatch between probe's shape and the shape of the hair bundle. In contrast, a fluid-jet provides a more synchronous stereocilia deflection, resulting in a steeper I/X curve, and is suited for both positive and negative deflections. However, it is significantly slower than the rigid probe and, depending on design, may not hold steady

pressure required for seconds-long bundle deflections. The goal of this study was to test different fluid-jet designs in order to develop a faster fluid-jet that would be also suitable for prolonged bundle deflections.
Methods: Four different fluid-jet systems were compared in their ability to deflect stereocilia bundles in the mouse auditory hair cells: 1-2) two "classical" fluid-jets driven either by a 27 mm piezoelectric disk or by the high-speed pressure clamp (HSPC); 3) the fluid-jet driven by a voice-coil that generates constant pressure within a liquid-filled compartment; and 4) the fluid-jet driven by a combination of HSPC and a high-speed solenoid valve placed near the pipette to ensure rapid pressure changes. Organ of Corti explants from young postnatal mice (P4-P7) were used for measuring hair bundle deflection. Sub-micrometer stereocilia movements were recorded with a medium-speed camera at ~1,500 fps and quantified off-line with a custom MATLAB script.
Results: As expected, the "classical" piezo-driven fluid-jet generated fast stimuli with the rise time of a bit less than a millisecond but was not able to generate sustainable seconds-long deflections, apparently due to the accumulated fluid outflow through the tip of the fluid-jet. The HSPC-driven fluid-jet delivered sustained second-long stimuli but was significantly slower. The voice coil system struggled with friction and had challenges achieving precise control over small negative and positive pressure changes. However, the HSPC/solenoid valve system produced fast stereocilia deflections with sub-millisecond rise time and stable force over several seconds. Interestingly, prolonged (3s) deflections of stereocilia bundles in mouse outer hair cells produced a hitherto undescribed phenomenon: a very large rightward shift of the MET-current-displacement (I/X) curves that, however, recovered within tens of seconds.
Conclusions: Development of new fluid-jet stimulators may reveal new phenomena in MET channel function.

SA26. Lipid Composition of Outer Hair Cells

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Category: Hair Cells: Anatomy & Physiology

Background: Outer Hair Cells (OHCs) through their electomotile properties are responsible for cochlea amplification. Our cryoEM structure of prestin, reveal cholesterol lying at the interface of the prestin dimers. Furthermore, the presence of stereociliary mechanosensitive channels makes it likely that lipids at the apical membrane play a critical role in its sensitivity to mechanical load. While 21 classes and over 400 different lipid isoforms have been identified in brain cells, the lipidome of OHCs is unknown.
Methods: OHCs were isolated from Prestin-YFP mice. Cochlea were dissected and the apical and middle turn micro-dissected. After brief treatment in 0.05% trypsin, tissue was gently triturated, filtered through 100 micron nylon mesh and FACS sorted cells were used for lipid analysis.
After quenching and extraction, lipids were analyzed by Creative Proteomics using ACQUITY UPLC separation was combined with AB SCIEX 5500 MS analysis. MultiQuant software was used for peak integration and internal standard calibration. To quantify analytes, a ratio of the peak

area and concentration of the target compound and the peak area and concentration of the internal standard was generated.

Results: As the number of OHCs was small, we limited our analysis to comparisons within lipid classes owing to the variability in ionization and detection by MS/MS. From this analysis, a total of 20 lipid classes and 1182 lipid types were indexed. Key findings were that the abundance of negatively charged lipids exceeded positively charged ones by almost 10-fold, despite the similar number of isoform types (556 versus 626, respectively). The most abundant positively charged lipids are CE and DAG. The negatively charged phospholipids PC, PE and PG showed the greatest variation in lipid isomers. With PC, that is found predominantly in the trans Golgi and plasma membrane, the most abundant species were PC(16:0_16:0) GREATER THAN PC(16:1_18:1) GREATER THAN GREATER THAN PC(16:0_17:0). For cholesterol, that too is found most abundantly with PC and sphingolipids in the plasma membrane, the most abundant species were CE(18:0) GREATER THAN CE(20:4) GREATER THAN CE(20:5) GREATER THAN CE(14:0). This contrasts to the adult brain which contains scant levels of cholesterol esters. The several sphingolipids including GlcCer(d18:1/24:1) GREATER THAN GlcCer(d18:1/16:0) GREATER THAN GlcCer(d18:1/24:0) GREATER THAN GlcCer(d18:1/22:0) GREATER THAN GlcCer(d18:1/22:1) were of relatively low abundance. Cardiolipins, the most abundant lipid detected and likely largely derived from the inner mitochondrial membrane are CL(72:4_18:1) GREATER THAN CL(72:1_18:1) GREATER THAN CL(72:8_20:4) GREATER THAN CL(72:5_18:2). DAG(18:0_18:0) GREATER THAN DAG(16:0_18:0) GREATER THAN DAG(16:0_16:0) were the most abundant diacylglycerols. Phosphatidylethanolamine (PE(18:1_18:1) GREATER THAN PE(16:0_18:1) GREATER THAN PE(18:0_18:1) vastly exceeded the amounts of phosphatidylserine and phosphatidylinositol (GREATER THAN 200 fold greater than each).

Conclusions: We present for the first time the lipidome of mouse OHCs. Of the abundant plasma membrane lipids, 2-5 specific isoforms of each lipid class dominate. These data have important implications for OHC membrane biophysics like membrane fluidity and putative lipid-protein interactions that might impact the phase of prestin's voltage-sensor charge movements.

SA27. Effects Tubulin Depolymerization on OHC and Deiter Cell Membrane Tension Measured by Flipper-TR

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Category: Hair Cells: Anatomy & Physiology

Background: Outer Hair Cells through their electomotile properties are responsible for cochlea amplification. A key determinant of OHC electromotility is the voltage of maximal voltage sensitivity of prestin its lateral wall motor. Membrane tension is one of the physiological determinants of Vh. In this study we sought to determine how tubulin depolymerization on membrane tension of OHCs and Deiter cells using the membrane tension sensing dye Flipper-TR.
Methods: The apical turn of mouse cochlea were isolated and imaged on a Leica STELLARIS 8 TauSTED Confocal Microscope with a HC FLUOTAR 25x/0.95 W VISIR lens after adding

Flipper-TR for 20 minutes. Colchicine was added to disrupt microtubule networks and Cytochalasin D to disrupt actin networks in Deiters cells and OHCs, followed by Fluorescence Lifetime Imaging Microscopy (FLIM) to quantify changes in membrane tension. We measured FLIM at 5 minute intervals with 8-10 z stacks after application of drug. Flipper fluorescence was excited using the white light laser (Leica Microsystems) with repetition rates of 80 MHz, and emission set to 488 nm in one photon mode. Fluorescence was collected between 600 nm and 700 nm on internal HyD-SMD detector (at 600 nm). Images were acquired at 700 Hz scanning frequency. Laser powers were constant. All lifetime data were obtained by applying a tailfit to ROI of the lateral wall of OHC or Deiter cells. We fitted FLIM data with two exponentials. Actin and tubulin depolymerization was assessed in tissue that were fixed immediately after by confocal immunofluorescence.

Results: We found that adding colchicine but not cytochalasin D resulted in increase in the first exponential of FLIM in OHCs. The increase in FLIM tau1 progressed with time upto 30 minutes and reached significance after 20 minutes. In contrast, Tau2 remained invariant with time. Within OHCs we found no significant difference in Tau1 when imaged at three z locations within each hair cell before the addition of cochicine. While Tau1 increased with time after adding colchicine, there was no variation of tau1 within hair cells in the z frame. In Deiters cells, we find a parallel increase in Tau1 that was significant at 30 minutes. We note significant reduction in tubulin but not actin staining of Deiters cells in preparations that were fixed at 30 minutes after addition of colchicine.

Conclusions: Tubulin depolymerization in Deiters cells significantly altered membrane tension and caused notable changes in OHC membrane mechanics compared to controls. These findings offer insights into the biomechanical interactions between Deiters cells and OHCs, contributing to a better understanding of cochlear mechanics. This has potential implications for hearing disorders associated with cytoskeletal disruptions.

SA28. Quantified Measures of Outer Hair Cell Structure from Fib_sem

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Category: Hair Cells: Anatomy & Physiology

Background: Outer Hair Cells, through their electomotile properties, are responsible for cochlea amplification. They can be divided on anatomical and physiological grounds into an apical transduction apparatus, a lateral wall housing prestin and a cytoskeletal network responsible for electromotility, and a basal end that receives synaptic input from efferent nerves. Using FIB-SEM we quantified the many subcellular structures within these cells.

Methods: The apical turns of mouse cochlea were fixed, embedded, and trimmed to a small block containing OHCs with the width perpendicular to the ion beam. The trimming was guided by x-ray tomography data obtained by a Zeiss Versa XRM-510 and optical inspection under a microtome. Thin layers of conductive material of 10-nm gold followed by 100-nm carbon were coated on the trimmed samples using a Gatan Ion Beam Coater, and the FIB-SEM prepared samples were imaged sequentially. The x-y pixel resolution was set at 4 nm. A subsequently applied focused Ga+ beam of 15 nA at 30 keV strafed across the top surface and ablated away 4

nm of the surface. The newly exposed surface was then imaged again. The acquired image stack formed a raw imaged volume, followed by postprocessing of image registration and alignment using a Scale Invariant Feature Transform–based algorithm. The voxel size of 4 × 4 × 4 nm3 was maintained throughout the entire volume and processed using Arivis 4.2.1 (Zeiss) software.
Results: We quantified volumes of the different compartments of the cell. At the apex of the cell we noted membranous organelles that were arranged circumferentially around the edge of the cuticular plate and linked the apical plasma membrane with several membranous organelles beneath the plasma membrane. In addition, we noted an unusual link between the subsurface cisternae and mitochondria on the lateral surface of the cell. The outer mitochondrial membrane and the inner subsurface cisternae were seen to merge and, at times, linked by electron-dense structures. At the base of the cell, we observed that efferent synapses contained vesicles of varying sizes and some that were larger and more electron-dense, likely containing co-transmitter peptides (CGRP). We noted no clear relationship between the size of the vesicles and distance from the synaptic cleft. We also quantified the volumes of the synaptic cistern.
Conclusions: We show for the first time FIB SEM data from mouse OHCs. These data allow us to make quantitative estimates of the various subcompartments of the cell that have physiological bearing. In addition, we note for the first time anchoring of the mitochondria to overlying subsurface cisternae.

SA29. Alternate Structures of Prestin, the Motor Responsible for the Electromotility of Outer Hair Cells

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Category: Hair Cells: Anatomy & Physiology

Background: Outer Hair Cells (OHCs), through their electromotile properties are responsible for cochlea amplification. Prestin is the motor protein located in the lateral wall of OHCs. We previously solved the Cryo-EM structure of prestin, resolving a single structure at 3.6 Å and one we believed to be in the contracted state. However, how the expansion of the protein occurs remains unknown. In the present work, we reanalyzed our data and identified a second structure that shows comparable resolution and small but significant variations from our original structure (PDB: 7SUN).
Methods: Purified prestin was applied to Quantifoil holey carbon grids and frozen in liquid N_2. Cryo-EM micrographs were obtained on a Titan Krios G2 transmission electron microscope at 300 kV with a K3 Summit direct electron detector. A total of 4,680 dose-fractionated super-resolution movies, with 36 images per stack, were recorded with defocus values varying from –1.15 to –2.15 µm. Data were processed in CryoSPARC, and the structure was solved using COOT program.
Results: We obtained an alternate structure of prestin in the contracted state at a resolution of 3.8 Å. Key features of the alternate structure of prestin were similar to those of our previously published structure. These include oligomerization as a dimer, with each protomer comprising 14 transmembrane helices (named TM1-TM14), showing an inverted 7-segment repeat organization with a core and gate domain, a C-terminal cytosolic STAS domain, and a short cytosolic N-terminal region. When compared to one another, the two structures show an overall RMSD of 0.74

Å. The structures differ in several places, including the C-terminus of TM6, the N-terminus of TM1, TM2, TM3, and TM11. The C-terminal cytosolic STAS domain and the cytosolic N-terminal region both show minimal differences in structure with 7SUN. Interestingly, TM10, which in our simulations was the most mobile region, shows minimal differences between the two structures. These data suggest that the TM domain and specific parts of it are more mobile and, therefore, likely the site of the voltage sensor location.
Conclusions: Our data show significant differences between two alternate forms of the contracted prestin. These differences are limited to specific areas in the transmembrane region and suggest that these sequences of the protein are more mobile than the remainder of the protein.

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SA30. Regulation of Myosin-Dependent Stereocilia Trafficking by Centrin-2

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Category: Hair Cells: Anatomy & Physiology

Background: Inner ear hair cells detect sound using mechanosensitive hair cell stereocilia. Each stereocilium is formed of a para-crystalline array of highly cross-linked actin filaments that create a rigid scaffold determining their size and shape. The molecular motor myosin 15 (MYO15A) is required for stereocilia elongation and contributes to this by trafficking WHRN, EPS8, GPSM2 and GNAI3 along actin filaments of the growing stereocilia core to the tips of stereocilia. Disruption of MYO15A-based protein trafficking perturbs stereocilia structure and causes human hereditary hearing loss, DFNB3. How MYO15A traffics and navigates along stereocilia actin filaments is unknown. In general, myosin motors must cluster or oligomerize to move processively along a filament, however to date there is no evidence that MYO15A is capable of this. It is thus a major unresolved question: how does MYO15A traffic along actin filaments of the stereocilia?
Methods: The baculovirus / Sf9 insect cell system was used to co-express truncated MYO15A-mEGFP-FLAG and CETN2. Protein constructs were purified via FLAG affinity capture followed by ion-exchange chromatography. We used dynamic light scattering and mass-photometry to assess oligomeric states, steady-state NADH assays to measure enzymatic activity and single-molecule fluorescence microscopy to interrogate actin filament binding.
Results: Here, we identify the centrosome – associated protein, centrin-2 (CETN2), as specifically binding to MYO15A and investigate how this impacts motor activity. MYO15A was expressed either as the full neck domain (MYO15A-3IQ) or with the third IQ domain truncated (MYO15A-2IQ). CETN2 co-purified with MYO15A-3IQ, but not with MYO15A-2IQ, confirming that CETN2 binds specifically to the third IQ domain. CETN2 is an EF-hand domain containing protein that may provide Ca2+ dependent regulation of MYO15A. To test this, steady state ATPase assays were performed on MYO15A-3IQ (with CETN2) and MYO15A-2IQ (without CETN2). We find that CETN2 binding does not affect MYO15A enzymatic activity, with or without Ca2+. CETN2 has previously been shown to oligomerize when bound to divalent cations, and we hypothesized that CETN2 might similarly drive oligomerization when bound to MYO15A. To test this, we

incubated MYO15A-3IQ (with CETN2), or MYO15A-2IQ (w/o CETN2), with varying concentrations of Ca2+ / Mg2+ and measured hydrodynamic radius using dynamic light scattering. We found that that MYO15A-3IQ (w CETN2) formed large particles GREATER THAN 1000 nm in the presence of 1 mM Ca2+, and separately with 5 mM Mg2+, whereas MYO15A-2IQ (w/o CETN2) did not.
Conclusions: We have determined the specific binding site of the stereocilia protein CETN2 within MYO15A. The ability of CETN2 to drive oligomerization of MYO15A in the presence of divalent cations provides a mechanism to cluster multiple motors, and we are currently exploring whether this is able to drive processive movement on actin filaments in vitro.

SA31. G-A Interacting Protein, C-Terminus 3 (GIPC3) Regulates Intracellular Vesicle Transport in Mammalian Auditory Hair Cells

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Category: Hair Cells: Anatomy & Physiology

Background: GIPC3 is a small adaptor protein essential for hearing in both mice and humans (Rehman et al., 2011; Charizopoulou et al., 2011). In many other systems, GIPC proteins interact with myosin VI (MYO6) and are involved in vesicular trafficking (Naccache et. al., 2006; Reed et. al., 2005). GIPC proteins have a unique ability to facilitate both clathrin-mediated (coated) and clathrin-independent endocytosis though coupling of MYO6 to its cargo (Naccache et. al., 2006). In cochlear hair cells (HCs), both GIPC3 and MYO6 are enriched along the cuticular plate and pericuticular necklace (Hasson, 1997, Chatterjee et. al., 2023), where vesicle trafficking and endocytosis are highly active (Kachar et. al., 1997, Griesinger et. al., 2004). Previous reports also suggest that apex-to-base vesicle transport in HCs is MYO6-dependent (Harasztosi et. al., 2020). While the role of GIPC3 and MYO6 in shaping the cuticular plate of HCs was recently demonstrated (Chatterjee et. al., 2023), here we asked the question whether GIPC3-MYO6 complex may have additional role in vesicular trafficking and endocytosis in the apical region of mammalian auditory HCs.
Methods: We generated a mouse model lacking GIPC3, Gipc3-/-. Organ of Corti explants were harvested from young (P7-P8) or adult (P21) mice and prepared for electron or confocal microscopy. To investigate intracellular trafficking, we used focused-ion beam (FIB) scanning electron microscopy (SEM) with 20 nm serial sectioning to visualize the apical region of Gipc3+/+ and Gipc3-/- HCs in fast-frozen freeze-substituted preparations. SEM was used to visualize the pericuticular surface of hair cells. Mis-localization of proteins associated with vesicular transport was investigated with immunolabeling and fluorescent confocal microscopy of motor and adaptor proteins (AP): MYO6, AP-2, AP-4, and APPL2. We are currently using FM1-43X to study endocytosis in vivo in Gipc3-/- HCs.
Results: We observed increased membrane blebbing (with SEM) and abnormal clustering of MYO6 in Gipc3-/- HCs along the pericuticular necklace, a well-known site of endocytosis. Immunolabeling of AP-2 and AP-4, which mark early endosomes and vesicles from the trans-Golgi network (TGN), label the canaliculi reticulum in Gipc3+/+ HCs but are not detectable in Gipc3-/- HCs. The canaliculi reticulum resides below the cuticular plate and is known to be involved in ion transport and intracellular trafficking pathways (Spicer et. al., 1999). FIB-SEM

imaging revealed the abnormal accumulation of both vesicles and disrupted Golgi apparatuses in Gipc3-/- HCs. It is possible that GIPC3 loss results in, i) failure to "label" vesicles with proper sorting machinery and/or ii) lack of proper transport machinery, either of which could result in vesicle accumulation in the apical region of the cell.
Conclusions: We conclude that GIPC3 is required for proper vesicular transport within HCs, most likely through its interaction with MYO6.

SA32. The Role of Membrane Cholesterol in Cochlear Hair Cell Mechano-Electrical Transduction

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Category: Hair Cells: Anatomy & Physiology

Background: Most mechanically gated ion channels are sensitive to forces propagated through the membrane. The lipid bilayer can modulate channel function directly through lipid/protein interactions or indirectly through membrane mechanics. Cholesterol is an important component of the membrane and a major modulator of membrane mechanics. While stretch activated ion channels have been found to close upon reduction in membrane cholesterol levels, the functional role of cholesterol for the cochlear mechano-electrical transduction (MET) channel has not been investigated.
Methods: Using whole-cell patch clamping and live-cell fluorescence lifetime imaging (FLIM) of a viscosity-sensitive molecular rotor BODIPY 1c in the inner ear, we investigated the role of membrane cholesterol in modulating the MET channel response properties of rat cochlear hair cells.
Results: Unlike in bullfrog vestibular hair bundles where cholesterol was localized near the tips of the stereocilia, cholesterol in rat P10 inner and outer hair cells hair bundles was uniformly distributed along the stereocilia, based on filipin labeling. We confirmed extraction of cholesterol, using methyl β cyclodextrin (MβCD), which reduced filipin staining in both inner and outer hair cell hair bundles within 30 seconds of application. MβCD treatment led to a reversible reduction in fluorescence lifetime in hair bundles. Membrane lifetime reduced with a similar time course as the filipin labeling. Inner hair cell bundles showed a 15% change while outer hair cell bundles showed ~8% change in 30 sec. Both recovered incompletely over the next 5 minutes. MβCD treatment reversibly increased the MET channel resting open probability while reducing the maximally elicitable MET current. Current amplitude could be recovered for short duration applications. Adaptation was also impacted both in the rate and extent.
Conclusions: Together these data suggest that the cell membrane mechanical properties impact force relay to the MET channel and potentially directly impact the energy required for the channel to transition between open and closed states.

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SA33. Macro-Patch Voltage Clamp Evaluation of Sub-Membranous Chloride Levels at the Outer Hair Cell Lateral Membrane

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Category: Hair Cells: Anatomy & Physiology

Background: Prestin is responsible for cochlear amplification which supports high frequency hearing. A fundamental property of prestin is its sensitivity to intracellular chloride. Boltzmann fits (2-state-Csa; Santos-Sacchi and Navarrette, Pflugers Arch, 2002) determine Qmax, Vh, z and delta-Csa, the latter thought to correspond to changes in membrane surface area/thickness that occur in sync with prestin conformational change across voltage. Here we explore the influence of chloride binding on prestin characteristics with the macro-patch technique and compare to whole cell measures.

Methods: In guinea pig membrane patches, first on-cell then excised, we measured prestin's Boltzmann parameters during changes under voltage clamp using AC measures of NLC (nonlinear capacitance) at 1 kHz. By comparing on-cell patch characteristics to subsequent excised inside-out patch characteristics perfused with 1, 10 and 150 mM chloride, we are able to estimate chloride levels in the intact OHC.

Results: Our data indicates that on-cell Vh is closest to those of excised patches when 1 mM chloride is perfused. Interestingly, delta-Csa appears unaffected by changes in chloride concentration. Since changes in chloride levels also shift Vh levels (indicative of prestin state distribution change), we tested whether increases in patch membrane tension, which shifts Vh to positive levels, is associated with changes in delta-Csa. It is not. These observations indicate that although chloride and membrane tension control Vh (thus, prestin state distribution) an expected change in delta-Csa is absent, and the parameter is not intrinsically dependent on the number of active prestin dimers, where N= Qmax/(z*e-). Boltzmann characteristics are also evaluated at frequencies of 2, 4 and 8 kHz. Delta-Csa, while variable, remains between 20 and 35 fF across frequency in our patches, with Qmax ranging between 15 and 22 fC at 1 kHz. Qmax and z roll off in frequency for all recording conditions, with Qmax decreasing by 8.3 dB from 1 to 8 kHz for on-cell conditions, like our previous macro-patch measures (Santos-Sacchi et al., JNeuro, 2023).

Conclusions: Most evaluations of prestin properties have been garnered with whole cell patch clamp, for example, we estimated sub-membranous chloride to be less than 10 mM by leveraging the relationship between prestin's salicylate sensitivity and whole cell chloride levels (Santos-Sacchi et al., JNeuro, 2006). Our new data indicate levels near 1 mM chloride and suggest that the sub-plasmalemmal space between the lateral membrane and subsurface cisternae is tightly buffered by the high density of prestin, an anion transporter, within the lateral plasma membrane.

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SA34. The Role of Alternative Splicing on the Otoferlin C-Terminal Transmembrane Domain in Auditory Hair Cell Synaptic Transmission

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Category: Hair Cells: Anatomy & Physiology

Background: Mutations in the OTOF gene are linked to DFNB9, a nonsyndromic recessive form of deafness classified as a cochlear neuropathy/ synaptopathy. Otoferlin-induced synaptopathy is due to a defective Ca2+-evoked exocytosis of synaptic vesicles by inner hair cells (IHCs). Otoferlin includes a single C-terminal transmembrane domain (TMD) anchoring the protein to the vesicular membrane and six C2 domains. Remarkably, brain and inner ear tissues express two different alternatively spliced forms of the otoferlin C-terminal TMD, encoded by either exon-47 or exon-48, respectively. While the function of otoferlin TMD-isoform exon-48 in IHCs is clearly established as a calcium-sensor, the role of the otoferlin TMD isoform encoded by exon-47 remains unknown.
Methods: To address this question, we generated a knock-in mouse model Otof exon47-mScarlet-flx/flx in which exon-47 is tagged with mScarlet and flanked by two loxP sites. This mouse model allowed a direct fluorescence imaging of the expression of the TMD-exon-47 otoferlin isoform in cochlear tissues. Mice with constitutive deletion of exon-47 were obtained by Cre/lox recombination when crossing Otof exon47-mScarlet-flx/flx mice with Tg(Pgk1-cre)1Lni mice while mice with hair cell specific deletion were obtained when crossing with with Myo15-Cre mice. To assess auditory function, we recorded ABRs and DPOAEs in P25-P40 mice using a TDT RZ6/BioSigRZ system (Tucker-Davis Technologies).
Results: Direct observation of live organs of Corti freshly dissected from Otof exon47-mScarlet-flx/flx mice showed specific fluorescent expression of mScarlet-tagged otoferlin in IHCs, in particular at the ribbon synaptic regions. Furthermore, Otof exon47flx/flx Cre+/- mice, in which exon-47 is excised in hair cells or constitutively, displayed apparent normal ABRs and DPOAEs thresholds. However, when analyzing ABR wave-1 amplitude as a function of sound intensity, these mice showed a significant reduction of wave-1 at high sound levels above 60 dB SPL, suggesting an "hidden-like" IHC synaptopathy. Remarkably, these mice lacking exon-47, unlike WT controls, showed a great decrease in ABR responses during prolonged sound stimulations (strong fatigability). At the cellular level, under patch-clamp recording, IHCs lacking exon-47 displayed reduced Ca2+ currents associated with a defect in sustained exocytosis. Confocal immuno-microscopy analysis showed a 30% reduced number of ribbon synapses as compared to wild-type controls. Also, these hair cells lacking exon-47 displayed smaller presynaptic ribbons associated with larger postsynaptic AMPAR clusters as compared to wild-type mice.
Conclusions: Overall, our results underline the importance of the exon-47 TMD splicing isoform of otoferlin in IHCs and suggest that this isoform is essential for the function and maintenance of a particular subtype of ribbon synapses, presumably the ones associated with the high threshold fibers. These findings should have important implications for future clinical diagnosis of otoferlin neuropathies and the design of efficient AAV rescue strategies.

Funding source: Fondation pour l'Audition, FPA IDA09

SA35. A Mitochondrially Associated Myosin Motor is Necessary for Hearing

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Category: Hair Cells: Anatomy & Physiology

Background: Inner ear hair cells use actin-based stereocilia to assemble a mechanosensitive bundle that transduces sounds and accelerations. Stereocilia are tuned into rows of ascending heights, and myosin-15 (MYO15A) establishes this gradient using multiple protein isoforms. The longest isoform (MYO15A-1) maintains the size of mechanotransducing stereocilia in postnatal hair cells, whilst the shortest isoform (MYO15A-2) traffics the elongation complex (EC) necessary for initial stereocilia growth in neonatal hair cells. We recently characterized a third isoform (MYO15A-3) that traffics the EC in postnatal and adult stereocilia. Though MYO15A-2 and -3 both traffic the EC, they are non-redundant; an isoform-specific deletion of Myo15a-3 causes stereocilia degeneration and progressive hearing loss in mouse. MYO15A-3 has a unique 6 kDa N-terminal domain, but is otherwise identical to MYO15A-2. We therefore hypothesized that the 6 kDa N-terminal domain of MYO15A-3 provides a unique function that is essential for maintaining postnatal stereocilia architecture.
Methods: To investigate the physical-chemical properties of the 6 kDa N-terminal domain unique to MYO15A-3, bioinformatic analyses were performed using AlphaFold 3, IPSORT and Mito Fates. Human embryonic kidney 293 (HEK293) cells were transiently transfected with enhanced green fluorescent protein (EGFP) fused to either full-length MYO15A-2, MYO15A-3, or truncated sub-domains. To specifically reveal mitochondria, transfected cells were stained with MitoTracker Deep Red FM dye. Cells were imaged live on a confocal spinning-disk microscope.
Results: Using AlphaFold 3 we identified an amphipathic helix within the 6 kDa N-terminal domain, that was further predicted to contain a mitochondrial targeting sequence (MTS) and mitochondrial processing peptidase (MPP) cleavage site. To test if the 6 kDa domain could allow for mitochondrial import, we expressed full-length MYO15A-3 (+ N-terminus) with a C-terminal EGFP tag that should not interfere with potential MTS processing. In HEK293 cells, MYO15A-3-EGFP fluorescence was positively correlated with MitoTracker signal, indicating association with, or uptake into mitochondria. By comparison, MYO15A-2-EGFP or EGFP alone were anti-correlated with MitoTracker, indicating their exclusion. Expression of the truncated 6 kDa N-terminal domain alone was also sufficient to drive strong accumulation of EGFP within mitochondria.
Conclusions: Our data show that the 6 kDa N-terminal domain contains a targeting sequence that is necessary and sufficient to drive import and accumulation of full-length MYO15A-3 within mitochondria. In contrast to other known myosin motors attached to the mitochondria outer membrane, our findings indicate that MYO15A-3 can be imported into mitochondria via an experimentally verified MTS. We speculate this mechanism may couple and regulate the function of MYO15A-3 in stereocilia with mitochondrial activity within the hair cell body.

SA36. High-Resolution Flat-Panel Ct Analysis of Intrascalar Cochlear Implant Electrode Position

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Category: Auditory Prostheses

Background: The cochlea has significant anatomic variability, though cochlear implantation has long adopted a one-size-fits-all schema. Recent work has increasingly focused on patient-specific anatomy; however, in vivo evaluation of cochlear implant (CI) electrodes has been underutilized. With the advancement of high-definition flat-panel computed tomographic imaging (FPCT) allowing for in vivo electrode visualization, our group has shown that FPCT data can be used in the postoperative setting and that image-guided postoperative activation may improve pitch perception (Limb et al, 2014; 2018). This study aims to describe a novel assessment of intrascalar CI electrode array location using in vivo FPCT data.

Methods: We conducted a retrospective, observational study among 16 participants with MED-EL FLEX 28 CI. All patients underwent FPCT postoperatively with secondary reconstruction and transformation to a standard cochlear coordinate system. Reformatted mid-modiolar image slices were used to measure electrode array angular insertion depths and to place landmarks on the lateral wall, scala tympani modiolar wall, and electrode array centers at 30º intervals. Landmarks were interpolated to provide coordinates of each structure at 1º intervals. The lateral wall and modiolar wall landmarks were used to estimate the angulation of the scalae relative to the basal turn plane at each angle. The radii of the lateral wall, modiolar wall, and electrode center were determined at each angular depth (measured from the mid-modiolar axis). The radii measurements were used to assess the relative location of the electrode array within the scala tympani.

Results: Average insertion degrees was 511º (433-630), in line with company-reported average of 500-550 degrees. The angulation of the scala relative to the basal turn plane was noted to have a negative slope immediately past the round window niche—consistent with a known "sharp turn" of the scala tympani at the round window, with an expected positive deflection at around 200 degrees. The average lateral wall to electrode radial distance was 1.1 mm at 90 degrees, decreasing to 0.7 mm at 180 degrees and 0.6 mm at 360 degrees. All 16 of these lateral wall electrodes were consistently found to have smaller radial distances from the lateral wall compared to the modiolar wall at depths greater than 100 degrees.

Conclusions: To our knowledge, this is the first study to use post-operative FPCT in vivo to map patient-specific electrode coordinates and intrascalar distances along the full depth of electrode insertion. Electrode-to-modiolus distance is known to be correlated with programmed stimulation levels (Noble et al, 2016); therefore, we anticipate that intrascalar distances may have implications for electrode-level CI function. Additional measurements such as basal turn angle may have implications for post-insertion trauma. Future studies will focus on correlating these newly available precision measurements to CI function in hopes of improving user-specific CI optimization.

SA37. Voltage-Driven Bundle Movements in Mammalian Cochlear Hair Cells Support Common Underlying Mechanisms Across Species and End Organs

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Category: Hair Cells: Anatomy & Physiology

Background: Mechanically sensitive bundles of interconnected protrusions called stereocilia transduce mechanical energy into electrical signals, allowing us to hear and sense acceleration (e.g., head movement, gravity). The sensory cells harboring these bundles are called hair cells. Their direction of sensitivity is determined by the orientations of the bundles' staircase-like patterns and the extracellular tip-links connecting stereocilia. Pushing bundles toward their taller edge tensions links and opens ion channels. In contrast, pulling the bundle slacks links and closes channels. This conversion of mechanical energy into electrical signals is mechano-electrical transduction (MET) and underlies the common function of all hair cells. Resting tension in the links keeps some MET channels open even at rest. In reptiles, amphibians, and birds, rapidly altering resting conditions by depolarizing the cell shifts the bundle position by causing mechanical changes in MET-related machinery. Upon depolarization, there is a "fast" bundle offset in the negative direction, superimposed on a "slow", opposing offset. A slower third component is dependent on bundle resting position.
Methods: Voltage-driven bundle-derived movements have not been thoroughly examined in mammals. Given that mammalian cochlear inner hair cells (IHCs) have less tightly coupled stereocilia and since slow adaptation appears to contribute less to MET in IHCs, voltage responses might manifest differently. Similar movements in mammalian cochlear cells would mean the processes fundamental to hair cells' ability to respond to tip-link tension and current are conserved across species. To address this, we provided a voltage step to P7-P9 rat and mice IHCs while imaging stereocilia to track movements. To investigate channel contributions, we blocked them using curare. To assess the role of the cadherin-based links, we disrupted connections using a calcium-chelator.
Results: We found IHCs have voltage-driven movements, and they move similarly to previously investigated hair cell types. The slow component was abolished by pharmacologically blocking channels, and the fast component required intact links. We have not characterized a third component, but similar movements were observed. Contrary to expectations, 2/5 cells still moved upon depolarization after breaking links. These results would suggest there is an unexplored, "link-free" component of the voltage-driven movements. We are actively investigating this further to validate these findings and, if confirmed, pursue their origins.
Conclusions: Our results indicate the fundamental mechanisms underlying the voltage-driven responses are shared across hair cells responding to different sensory modalities and in different species. Unlike other model organisms used to investigate hair bundle mechanics, mice provide a robust toolbox to investigate the molecular underpinnings of the voltage-driven response and allow us to better understand how forces are conveyed to the MET complex. In the future, we plan to examine the role of calcium in the voltage-driven response, particularly the slow component which requires MET current.

SA38. The Calcium and Integrin-Binding Protein 2 (CIB2) Provides Fast Kinetics to the Met Complex through Localization of BAIAP2L2 to Stereocilia Tips

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Category: Hair Cells: Anatomy & Physiology

Background: Calcium and Integrin-Binding protein 2 (CIB2) is a cytosolic protein that interacts with TMC1/2 and is required for hearing and mechano-electrical transduction (MET), presumably by retaining and/or transporting TMC1/2 subunits to stereocilia tips in auditory hair cells (HCs) (Riazzudin et al., 2012, Giese et al., 2017, Liang et al., 2021). Deafness-associated mutations in Cib2 disrupting the interaction with TMCs (e.g., p.F91S) result in complete loss of MET currents in mouse auditory HCs. Therefore, these mutants cannot be used to determine whether CIB2 has additional functions within the MET complex. Fortunately, other mutations that preserve the CIB2-TMC1/2 interaction have small but detectable MET currents. Here, we use an independently generated knock-in mouse carrying one of these variants (p.R186W) to clarify the function of CIB2 in mechanotransduction.

Methods: Freshly isolated organ of Corti explants from early postnatal mice (P4-P7) were used for MET current recordings. Stereocilia bundles were deflected with a customized stiff-probe capable of producing 1 μm displacement in ~5 μs to resolve MET channel activation and adaptation. Video recordings at 90,000 fps confirmed that our stiff probe deflects stereocilia bundles significantly faster than the rise time of MET currents in wild-type HCs. Fluid-jet evoked MET currents were recorded to measure resting open MET channel probability (Popen) and current-displacement relationships. Serial sectioning with Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) was used to examine stereocilia ultrastructure. Since BAIAP2L2 is absent from stereocilia of Cib2-/- mice (Yang et al., 2022), we used immunolabeling and confocal imaging to investigate BAIAP2L2 localization in Cib2R186W/R186W bundles. Finally, to explore how BAIAP2L2 removal could change MET current kinetics in wild-type HCs without Cib2 mutations, we used a MET channel blocker to mis-localize BAIAP2L2 from stereocilia.

Results: Consistent with previous results (Liang et al., 2021), MET current amplitude is reduced in Cib2R186W/R186W hair cells. Despite the smaller size of the MET currents, the Cib2R186W/R186W HCs have larger Popen and steeper MET current-displacement curves, indicating an increase in MET complex sensitivity. In addition, the p.R186W variant results in at least two-fold slower MET current activation compared to wild-type littermates and complete loss of fast adaptation. FIB-SEM imaging revealed disruption of the lower tip-link density in every 2nd-row stereocilium in cochlear inner HCs of Cib2R186W/R186W mice. Consistently, we found that BAIAP2L2, a self-aggregating protein interacting with the plasma membrane, is mis-localized from stereocilia tips in Cib2R186W/R186W HCs. Pharmacological mis-localization of BAIAP2L2 from stereocilia tips in wild-type HCs resulted in slower MET current activation like the one observed in Cib2R186W/R186W HCs.

Conclusions: We concluded that the p.R186W variant of CIB2 slows down force transmission to the MET complex due to mis-localization of the membrane-associated protein BAIAP2L2.

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SA39. Comparative Analysis of Temporal Bone Anatomy and Whole-Mount Dissection in the Inner Ear in Mice Vs. Common Marmosets

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Category: Inner Ear: Anatomy & Physiology

Background: To investigate hair cell loss and transduction efficiency when delivering vectors for therapeutic studies, whole mount or surface preparation of the cochlea and vestibule is useful. Although the mouse has been a promising animal model for studying the inner ear, the common marmoset (Callithrix jacchus), a New World monkey, is increasingly considered a preclinical animal model to bridge the species differences between humans and rodents. Here, we summarize anatomical knowledge and methods for whole-mount dissection of the inner ear of mice and common marmosets.
Methods: Adult C57/BL6 mice (8 weeks old) and common marmosets were used. Cochleae were harvested, fixed in 4% paraformaldehyde, and stored at 4 °C in preparation for immunohistochemistry. Specimens were visualized with a dissection microscope and dissected without decalcification for whole-mount analysis.
Results: Unlike the mice, the configuration and shape of the components of the ossicles, cochlea, semicircular canals, and facial nerve appeared homologous to their human counterparts. The murine cochlea has 1.75 turns, while the common marmoset's cochlea has 2.75 turns which is similar to that of human beings. A drill was used to dissect both cochleae without damage.
Conclusions: The anatomical images presented provide a practical guide for advancing studies of the middle and inner ear using mice and common marmosets.

SA40. A Novel Clearing and Analysis Pipeline for Quantitative Cellular Analysis within the Cochlea

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Category: Inner Ear: Anatomy & Physiology

Background: Two-dimensional (2D) staining and imaging have long been crucial histological techniques for studying the intricate morphology of the cochlear membranous labyrinth. However, the fluid-filled spaces within the otic capsule and the delicate nature of the sensory epithelium make the tissue highly prone to damage during freezing and sectioning. In recent years, three-dimensional (3D) tissue clearing and staining methods have emerged as valuable alternatives for preserving the fine 3D structure of the cochlea. Among these, iDISCO, an organic solvent-based clearing method, has been successfully applied to cochleae from various species. However, its reliance on methanol dehydration limits compatibility with many antibody staining protocols and significantly reduces endogenous fluorescent signals. Here, we present an optimized aqueous clearing protocol that combines SHIELD and CUBIC-HistoVIsion, designed to preserve endogenous fluorescence and minimize tissue expansion while maintaining compatibility with existing antibody staining protocols.

Methods: Using the Lfng-eGFP mouse line, where Deiters cells and Inner Phalangeal/Border cells express eGFP and Myo7a immunostaining was used to label the hair cells, we directly compared tissue shrinkage and expansion effects induced by iDISCO and SHIELD+CUBIC clearing protocols. Furthermore, unlike iDISCO, the CUBIC clearing method is compatible with DAPI staining. We then used the supervised machine learning algorithm, Cellpose, to segment individual nuclei and developed a custom Vision Transformer-based classifier to separate different cell-types based on their nucleus staining.
Results: The iDISCO protocol resulted in tissue shrinkage, whereas the CUBIC clearing protocol caused tissue expansion, with the expansion effect being particularly pronounced in younger tissues. However, incorporating SHIELD reagents for additional tissue preservation effectively mitigated this expansion. Implementation of automated segmentation allowed accurate detection of hair cells and defined their precise position along the sensory epithelium.
Conclusions: The optimized aqueous clearing protocol, combined with this custom automated analysis pipeline, minimizes the dependence on antibodies for identifying morphological landmarks, enabling quantitative cellular analysis within the cochlea.

SA41. Localization of Perineuronal Nets in the Contralesional Cochlea Following Unilateral Cochlear Ablation

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Category: Inner Ear: Anatomy & Physiology

Background: Nearly 1.5 billion people worldwide suffer from hearing loss in at least one ear and 200 people per million worldwide are newly diagnosed with single-sided deafness (SSD) every year. SSD is severe to profound deafness in one ear that is clinically unmanageable. While the non-deaf ear has often been considered to be 'normal,' there is evidence, including from our own studies, that suggest that the ear contralateral to the dysfunctional ear itself is no longer the same as under normal bilateral hearing conditions. We have previously reported that in the cochlea contralateral to a surgically induced unilateral cochlear ablation, there is upregulation of corticotropin releasing factor (CRF), a molecule that is involved in a local cochlear stress response (Graham et al., 2010, 2011). When visualized with a red fluorescent protein reporter mouse line, CRF is observed in hair cells, support cells, the spiral limbus and spiral ligament. Prior collaborative work has demonstrated changes in extracellular matrix molecule localization following traumas of noise exposure and mild traumatic brain injury. Given our previous findings revealing CRF upregulation in the contralesional cochlea, we were interested in assessing whether aggregates of extracellular matrix molecules in perineuronal nets change in their distribution following unilateral cochlear ablation. Here we report on our results assessing perineuronal net localization contralesional to a unilateral cochlear ablation.
Methods: Adult CRFcre:tdTomato (Ai14) male and female mice were anesthetized with ketamine (70-100mg/kg) and xylazine (10-20 mg/kg) and subjected to unilateral cochlear ablation. A trans-tympanic approach was used to access the cochlea which was pierced with a Beaverblade ophthalmic knife. The bony capsule inferior to the stapedial artery was broken and removed. The middle ear was packed and the ear canal sealed using VetBond cyanoacrylic glue. Age-matched male and female CRFcre:tdTomato mice without cochlear ablation served as controls. One to 21

days following cochlear ablation, animals were transcardially perfused and fixed (4% paraformaldehyde). Temporal bones were isolated, decalcified, cryoprotected, cryostat cut and histologically processed (anti- RFP, Rockland Immunochemicals; biotin-Wisteria Floribunda, Millipore Sigma) and appropriate secondary antibodies conjugated to Alexa 488 and Alexa 594 or Cy3 were used. Sections were counterstained with DAPI and imaged (Zeiss LSM880 Confocal).
Results: Compared to mice not subjected to cochlear ablation, we find elevated localization of wisteria floribunda, which binds perineuronal nets, within the cochlear epithelium as early as 1 day following cochlear ablation through 21 days survival. Localization is focused primarily around support cells.
Conclusions: Our results indicate that extracellular matrix molecules undergo remodeling, even within the contralesional cochlea, an organ often presumed to be 'normal' under conditions of single sided deafness.

SA42. Developmental Expression of Membrane Bound and Secreted Corticotropin Releasing Factor Receptors in the Cochlea

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Category: Inner Ear: Anatomy & Physiology

Background: We have previously shown that corticotropin releasing factor (CRF), a 41 amino acid neuropeptide involved in the systemic stress response that also modulates effects on glutamatergic neurotransmission in the nervous system, is expressed in the auditory periphery by support cells in the cochlea. Additionally, our work has shown that CRFR1 and CRFR2 function in concert to modulate / set levels of hearing sensitivity (Graham et al. 2010, 2011). Studies of CRFR1 loss of function at maturity have revealed morphological phenotypes that are suggestive of deficits that occurred during the development (Graham et al. 2010). However, a developmental role for CRF signaling in the cochlea is currently based only on inference. To investigate the potential for CRF signaling involvement in normal development of the cochlea, we investigated the developmental expression of two CRF receptors, CRFR1 and CRF binding protein (CRFBP), during early developmental stages of the cochlea.
Methods: CRFR1-GFP transgenic mice (BAC transgenic mice that express GFP under the control of CRFR1 promoter and enhancer elements) were used at postnatal day (P) 0, P3, P17 and at adulthood. Animals were perfused and fixed (4% paraformaldehyde), temporal bones isolated, decalcified, cryoprotected and cryostat cut. Temporal bone sections were processed for immunohistochemistry (GFP, Rockland Immunochemicals; CRFBP, Santa Cruz Biotechnology), DAPI stained and imaged by confocal microscopy (Zeiss LSM 880).
Results: We utilized BAC transgenic mice that express GFP under the control of CRFR1 promoter and enhancer elements. These mice exhibit faithful recapitulation of endogenous expression patterns, but with greater sensitivity than standard immunohistochemical approaches (Justice et al, 2008). No CRFR1-GFP is detected in the cochlea at postnatal day (P) 0 or P3. At P7, robust CRFR1-GFP is detected in Deiter's cells located basal to outer hair cells and is the only detectable signal in some regions of the cochlea. In other nearby areas, in addition to strong staining of Deiter's cells, fainter GFP localization is seen in border cells and inner and outer sulcus cells. By P17, robust GFP localization is detectable in all of these cell types. In adult animals, the P17

expression pattern is maintained along with additional labeling of root cells of the lateral wall. Interestingly, immunohistochemical labeling with CRFBP at P7, at a developmental timepoint showing strongest CRFR1 protein localization in Dieter's cells, and weaker staining in border, inner, and outer sulcus cells, shows co-localization only within Deiter's cells.
Conclusions: The mature phenotype observed in CRFR1 loss of function mutants that is suggestive of impacts on developmental events may be due not only to the progressive temporal onset of CRFR1 expression in varied support cell types in the cochlea, but also independently regulated expression of corticotropin releasing factor binding protein.

SA43. Novel Deep Learning-Based Tools for Inner Ear Research

Abhijeeth Erra[1], Cayla Miller[1], Kenta Ninomiya[2], Jeffrey Chen[3], Elena Chrysostomou[1], Lauren Sullivan[1], Yuzuru Ninoyu[1], Yasmin Kassim[1], Shannon Barrett[1], Federico Ceriani[4], Rick Friedman[1], Walter Marcotti[4], Artur Indzhykulian[5], Cody Carroll[3], Alexey Terskikh[6], Uri Manor*[1]

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Category: Inner Ear: Anatomy & Physiology

Background: The analysis of auditory brainstem response (ABR) recordings and high-resolution microscopy images of cochlear hair cells is crucial for understanding hearing function and its decline. However, traditional analysis methods are often labor-intensive, time-consuming, and prone to human bias. Moreover, the complexity of 3D imaging data, particularly in characterizing subcellular structures like synapses and epigenetic landscapes, often precludes the use of simple heuristic approaches. This necessitates the development of automated, machine learning-based tools to accelerate research and enable more comprehensive and unbiased analyses. Manual analysis of synapse counts and locations in many labs involves time-consuming manual counting, which can take hours to complete. Similarly, traditional ABR analysis requires manual annotation and visual estimation of hearing thresholds, also taking about an hour for a small number of recordings. We posit that deep learning-based automation tools can significantly accelerate these analyses, reducing processing time to mere seconds or minutes, thereby enabling high-throughput studies and freeing researchers to focus on experimental design and interpretation.
Methods: We developed a suite of open-source deep learning tools for the automated analysis of auditory brainstem response (ABR) waveforms and cochlear hair cell images. Additionally, we developed a deep learning-based super-resolution and segmentation workflow for confocal images of cochlear hair cell synapses. Finally, we introduce the use of CODE (colocalization of chromatin determinants) tensors for inner ear hair cell nuclei, a novel computational approach to quantify chromatin organization and epigenetic landscapes in single cells in situ.
Results: Our CNN-based ABR analysis tool, ABRA, achieved high accuracy in threshold detection, comparable to human experts, and significantly reduced analysis time. The U-net model successfully segmented cochlear ribbon synapses in 3D, facilitating automated quantification of synapse counts, sizes, and locations. Using CODE tensors, we achieved highly accurate classification of inner hair cells based on age and tonotopic position.

Conclusions: The development of these open-source tools for automated analysis of ABR waveforms, cochlear hair cell images, and epigenetic landscapes offers exciting possibilities for future research. By combining these tools, we can gain a deeper understanding of the complex changes that occur in the cochlea during aging and in the context of congenital deafness mutations. The ability to integrate functional measurements of hearing with structural and epigenetic information at the single-cell level has the potential to uncover novel insights into the mechanisms of hearing loss and identify new therapeutic targets. Furthermore, the development of multi-modal deep learning models that integrate these diverse datasets could lead to more accurate and comprehensive predictions of hearing function and hair cell health, paving the way for personalized medicine approaches to hearing loss prevention and treatment.

SA44. Passive Mechanics in the Human Cochlea Appears to be Sharply Tuned

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Category: Inner Ear: Cochlear Mechanics

Background: Psychophysical results from masking experiments and otoacoustic emission studies indicate that the tuning of the human cochlea is sharper than in common animal models (Shera, C. A., and Charaziak, K. K. (2019). Cochlear Frequency Tuning and Otoacoustic Emissions. Cold Spring Harbor perspectives in medicine, 9(2)). It is known that the passive cochlear tuning sharpness, or Q, as measured in a dead or compromised cochlea or at high sound pressure is less than when the active processes are present and working. This suggests that cochlear tuning originates in passive mechanics and active phenomenon sharpens it. We aim to investigate the origin of the tuning in the human cochlea.

Methods: Optical coherence tomography (OCT) imaging and vibrometry were used to measure radial cross sections of the cochlear partition in fresh (LESS THAN 24 hours) postmortem human cochleae (Thorlabs Ganymede, OCTG9). Tissue was sourced from donors with permission at Massachusetts General Hospital. The cadavers and temporal bones were maintained in a chilled environment during all stages of processing. Phosphate buffered saline and culture media were used to maintain the specimens. A surgical drill was used to make a facial recess to expose the round window for basal turn measurements. In second turn measurements, the cochlea was approached with the surgical drill from the scala vestibuli side. Acoustic stimulation was presented to the tympanic membrane via an ear canal mounted probe tube sound source and reference microphone. Cochlear partition transverse displacement was measured in response to sinusoidal acoustic stimulation at frequencies from 100 Hz to 25 kHz at multiple radial locations for each longitudinal place measured on the cochlea. At each radial location, the sound driven displacement of the cochlear structures such as the basilar membrane, pillar cells, supporting cells were measured.

Results: This is an ongoing study with limited numbers of measurements (N=3) made at this point. The results show longitudinal varying tuning that is generally in agreement with the passive Greenwood map for human. The results indicate that the passive tuning in the human cochlea is sharper than the passive tuning in animal models. At the most apical measured location, the tuned

frequency was 834 Hz with a Q of 2.2. ½ turn more basal was tuned to 2.3 kHz with a Q of 2.9, In the base within the round window, the tuned frequency was 16 kHz with a Q of 5.3.
Conclusions: These preliminary results suggest the sharper psychophysical tuning of the human may be partially attributed to sharper passive mechanical tuning in the cochlea. [Work supported by R01 DC013303 from the NIDCD of NIH.]

SA45. Salicylate-Induced Changes in Low-Frequency Organ of Corti Vibrations

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Category: Inner Ear: Cochlear Mechanics

Background: Intracochlear vibrations depend on active processes and are sensitive to acoustic trauma and pharmacological manipulations. Here, we introduce salicylate —a drug that is ototoxic primarily by blocking outer hair cell (OHC) motility— into the cochlea and monitor its effects on the sound-induced, organ-of-Corti (ooC) vibrations in the second turn of the gerbil cochlea.
Methods: Thus far, we have made recordings from n=12 tonotopic locations in the left ears of 9 gerbils. Vibratory recordings in the middle turn of the cochlea (best frequency (BF) ≈ 2 kHz) were obtained over a range of frequencies (0.4–4 kHz) and intensities (30–70 dB SPL) using optical coherence tomography. We combined recordings from multiple, closely spaced (10 μm) A-lines at one tonotopic location to map the vibrations across the ooC. Salicylate was introduced into the cochlea via the round window. Its dispersion along the cochlear duct was monitored using distortion product otoacoustic emissions (DPOAEs; f2/f1=1.25, L1=L2=55 dB SPL). After 60–120 minutes, 2f1–f2 DPOAEs with f2=BF had substantially reduced, and the vibratory recordings were repeated.
Results: At baseline (i.e., before salicylate was introduced), low-intensity basilar membrane (BM) and OHC vibrations were tuned, and responses throughout the ooC grew compressively over most of the tested frequency range. Salicylate had a level- and frequency dependent effect on the vibrations. No, or little, effect at 70 dB SPL, modest reductions in vibration amplitude at 50 dB SPL, and reduced the response amplitudes at 30 dB SPL. These reductions were frequency dependent, with the effect size increasing with frequency. As a consequence, the 30–70 dB cochlear gains were reduced.
Conclusions: Thus far, our data analysis has been focused on the BM and OHC responses, evaluation of the salicylate-effects on other regions within the ooC are in progress. Although these two regions exhibit different tuning characteristics, we have found that they are identically affected by salicylate. That is, changes in the level-dependent frequency response curves (and therefore the gain curves) for these two regions are the same. It seems that "the spread" of salicylate-induced changes in the active outer hair cell processes to other regions of the ooC (e.g., the BM) is unaltered by salicylate.
We thank our lab technicians, Ana K. Gallegos-Anchondo and Laura M. Anchondo, for their help with collecting the data. This work was supported by NIDCD R21DC019998 and VA RRD Merit 1 I01 RX003491. These contents do not represent the views of the U.S. Department of Veterans Affairs or the United States Government.

SA46. Organ of Corti Responses to Tones With Low Side-Suppression, and in the Post Mortem Condition

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Category: Inner Ear: Cochlear Mechanics

Background: Optical coherence tomography (OCT) has revealed that the outer hair cell (OHC) body region vibrates with a pattern unlike the basilar membrane (BM). In response to pure tones of moderate level, the OHC-region's vibrations show linear growth below the best frequency (BF) but with a higher amplitude than the BM. In contrast, when driven by multi-tone stimuli, the sub-BF frequency band shows a pronounced broad-band nonlinearity. The difference between the single and multi-tone responses is likely due to saturation of OHC-MET current, which begins at moderate SPL (noted per tone) for multi-tones, and at high SPL for single tones. We explored this expectation by combining pure tone stimuli with an intense low-side suppressor.

Methods: Organ of Corti (OoC) vibrations were measured with OCT in the base of the gerbil cochlea. Responses were measured either near the 25 kHz BF place (with a primarily longitudinal/transverse optical axis) or in the hook region where the BF was 45 kHz (with a primarily transverse optical axis). Vibrations were measured in response to: (1) a pure tone frequency sweep, (2) the sweep in the presence of an intense (3 kHz 100 dB SPL) low-side suppressor, and (3) a multi-tone Zwuis complex. Distortion product otoacoustic emissions (DPOAEs) were measured in response to swept two-tone stimuli throughout each experiment to monitor the cochlear condition and postmortem (PM) responses were measured shortly after death. Robust DPOAEs and healthy responses to low-level stimuli throughout each experiment indicated the suppressor's effects were transient.

Results: The amplitude of the suppressor tone was ~ 2 – 3 times larger at the 25 kHz place than at the 45 kHz place.

In the sub-BF band, OHC-region responses fell in the presence of the suppressor tone despite this region responding linearly to pure tones. The results can be qualitatively understood with a Boltzmann-type input/output function, arising from the mechanoelectrical transduction channels.

At both locations the PM responses were reduced and showed further loss of amplitude than the suppressed vibrations. At the 45 kHz place, the BM and structures within the organ of Corti moved similarly PM. In contrast, at the 25 kHz place, the BM and OHC-region vibrations were different, evincing persistence of internal motion within the organ of Corti PM.

At the 25 kHz place, suppression and death eliminated the BF peak, whereas at the 45 kHz place the BF-peak often persisted.

Conclusions: Both the presence of an intense low side suppressor and the PM condition demonstrate the presence of OHC-region active gain at frequencies well below the BF. The postmortem condition did not, however, eliminate internal OoC motion at the 25 kHz location.

SA47. Complex Difference Analysis Provides Insight into Internal Organ of Corti Motion

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Category: Inner Ear: Cochlear Mechanics

Background: Optical coherence tomography (OCT) has allowed for simultaneous in vivo recordings of structures within the organ of Corti (OoC). In gerbils, outer hair cell (OHC) motion contains larger amplitudes across frequencies than the basilar membrane (BM). The angle between the OCT optical axis and the anatomical coordinate system of the cochlea has been shown to greatly impact the measured motion. Here, we perform a complex difference analysis between OCT-measured internal OoC motion and basilar membrane (BM) motion to extract the "internal" motion.

Methods: In the case of a transverse optical view, the BM (whose motion is transverse) is the substrate for OoC motion. To extract the internal OoC motion, we take the complex difference between the measured OoC motion and BM motion, such that internal OoC motion = measured OHC motion - BM motion. Due to the motion being characterized by both magnitude and phase, the complex difference was taken. This calculation can be performed on any OoC structure or region, including the outer hair cell/Deiters cell (OHC/DC) junction or the reticular lamina (RL) region. We explore these relationships at several best frequency (BF) locations and in gerbils and guinea pigs.

Results: Compared to the gerbil, internal motion (found via the complex difference) in the guinea pig was usually lower in magnitude and similar in phase to the BM. In some instances, there was a small region near the RL that contained sub-BF nonlinearity. When the complex difference was taken at this location, internal motion was similar in magnitude and phase to BM motion near the BF at moderate sound pressure levels (SPLs). At higher SPLs, internal motion decreased and became out of phase with BM motion. These traits differ from the hotspot traits, which are robustly found in the gerbil. Through a cochleostomy, the OHC/DC junction in the guinea pig occasionally more closely resembled the gerbil hotspot. OHC/DC motion was similar in magnitude but out of phase with BM in these instances, resulting in the measured motion of the region being small in amplitude and flat with frequency.

Conclusions: Taking the complex difference between structures within the OCC is vital for accurately representing the motion of internal OoC structures. Without such calculations, finer details of internal OoC motion across frequencies can be lost. In the guinea pig, the measured motion within the OoC was generally equal to the measured motion of the BM, thus the calculated internal OoC motion was small. While the guinea pig sometimes presents with substantial internal OoC motions, the guinea pig hotspot appears more localized/elusive than the prominent, robust hotspot found in gerbils.

SA48. The Wonder of Evolution: Optimization of Bone Layer Thickness Between Turns to Minimize Cochlear Volume

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Category: Inner Ear: Cochlear Mechanics

Background: The cochlea consists of three tubular structures (scala vestibuli, scala media, and scala tympani), which are bundled together to form a spiral shape with 2.75 turns. The mechanism for sound perception is concentrated in the organ of Corti within the scala media. Sound waves, excited at the oval window, create a pressure difference above and below the basilar membrane, producing a traveling wave that propagates to different positions along the cochlea depending on the sound frequency. Upon examining a cochlea specimen cut along its axis, we see that the thickness of the bone separating each turn becomes extremely thin at the cochlear apex. Why did the cochlea evolve into such a shape? This study aims to explore the acoustic reasons underlying this evolution.

Methods: We propose a cochlear fluid dynamics model that rigorously addresses the acoustic phenomena within the cochlea by accounting for the compressibility of the lymphatic fluid. This model has been used to elucidate auditory mechanisms and to hypothesize the causes of various auditory disorders. To model the bone layer separating each cochlear turn, thin bone layers made of elastic material were placed on the upper surface of the scala vestibuli in the lower turn and on the lower surface of the scala tympani in the upper turn. Boundary conditions were set to ensure acoustic continuity between these layers. To investigate the effects of bone density loss due to aging, bone characteristics were expressed using Young's modulus, and hearing levels were estimated based on the maximum displacement of the basilar membrane.

Results: The cochlear fluid dynamics model mentioned above was modified by adding bone layers (thickness: 0.2 mm at base, 0.1 mm at apex) separating each turn. The hearing levels were evaluated by varying the Young's modulus in the order of 1.0E+10 Pa (normal hearing), 1.0E+9 Pa (mild osteoporosis), and 1.0E+8 Pa (severe osteoporosis). Compared to an ideal model without sound leakage through the bone layers, hearing loss of 0.22 dB (normal hearing), 0.30 dB (mild), and 3.88 dB (severe) at 4000 Hz, and 0.18 dB (normal hearing), 0.89 dB (mild), and 6.78 dB (severe) at 16000 Hz were observed.

Conclusions: Generally, lower-frequency sounds more easily pass through softer and thinner elastic materials. The cochlea detects high-frequency sounds near the base and low-frequency sounds near the apex. In normal, high-frequency sounds do not leak through the thicker-bone-layers at the base, and low-frequency sounds cannot escape through the harder-bone-layers at the apex. However, as the Young's modulus of the bone layers decreases with age, the sound-trapping effect is gradually weakened, leading to hearing loss. Based on this analysis, we conclude that in a healthy state, the bony labyrinth is designed to minimize volume while preserving sound-trapping effects across all frequency ranges.

SA49. Optical Coherence Tomography Vibrometry in Low Signal-to-Noise Ratio Regime

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Category: Inner Ear: Cochlear Mechanics

Background: Optical coherence tomography (OCT) is used to image and measure vibrations of the organ of Corti and is a common imaging modality in studying hearing mechanics. The visibility of cochlear tissue depends on the imaging system as well as the experimental setup. In phase-sensitive OCT vibrometry (OCTV), vibrations are recovered from the phase of a complex-valued variable, and thus have a finite codomain. Prior studies have focused on how noise affects OCTV in extreme low and high signal-to-noise ratios (SNR), but most data fall between these limits, where noise effects are not well described. Subsequently, filtering data prior to mechanical analysis lacks standardization, with different research groups using different criteria. We describe and show how noise can bias Fourier analysis and provide techniques to correct for noise-induced biases. This technique, along with a new way of acquiring data, has allowed us to better image deeper or less visible structures. The results provide grounds for objective filtering criteria.
Methods: Noise effects on vibrometry were investigated through simulations, experiments on a single scattering surface, and on tissue in situ. An image pixel was modeled as a bivariate Gaussian distribution in complex plane, with magnitude (reflectivity) following a Rician distribution and phase a Von Mises distribution. SNR was defined as the logarithm of the mean distance from the origin, normalized by the distribution's spread.
Results: At low SNR, reflectivity was Rayleigh-like, and phase was dispersed, including a uniform noise component. This uniform component of the noise lead to underestimation of vibration magnitude in Fourier analysis, indicating that the retrieved value of vibration magnitude could vary with pixel's brightness. The bias occurred even when displacement-correlated metrics were arbitrarily large. We used simulations to determine a bias correction technique in post-processing and defined objective filters to either fix or remove such data. We showed how omitting bias correction can lead to artifacts in mechanical analysis that may misinform underlying physics, especially in structures that have anisotropic optical properties.
To further reduce bias, OCTV was performed at sub-Nyquist rates to increase reflectivity due to longer exposure time, thereby increasing SNR. An analytical form of the OCT system's filtering effect was obtained to account for the integration of spectral interferograms, allowing accurate measurements at acquisition rates 10 times lower than the vibration frequency. We termed this protocol wideband OCTV.

Conclusions: Noise, particularly in pixels with low reflectivity or high temporal variance, biases OCTV results. These biases occur at reflectivity levels typical of cochlear tissue. A bias correction protocol was developed to minimize this effect. Wideband OCTV reduces biasing effects, enabling more reliable measurements from fainter structures. Our findings provide objective methods for filtering, correcting, and improving OCTV data without hardware modifications to the OCT system.

SA50. Application of a Novel Capsid Engineered Vector to Rescue Hearing Loss in TMPRSS3 Mutant Mice

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Category: Gene Therapy

Background: Despite the development of novel AAV vector systems such as Anc80 and AAV9.PHP.B there are still limitations in delivering genes to certain cell types in adult animal models. In particular outer hair cells have been difficult to transduce. Patients with TMPRSS3 gene mutations suffer from recessive progressive deafness (DFNB8), with cochlear implantation being their only treatment option, though outcomes can be poor. This is due to the distribution of the TMPRSS3 gene in inner and outer hair cells as well as in the spiral ganglion. A vector that transduces all three of these target cells is therefore needed for optimal delivery.
Methods: A novel capsid-engineered AAV vector, V6, which efficiently transduces inner and outer hair cells as well as spiral ganglion neurons across the entire cochlea already at low dose (1e6 per cochlea), was evaluated in a knock-in mouse model carrying a common human DFNB8 TMPRSS3 mutation. These TMPRSS3A306T/A306T homozygous mice exhibit delayed-onset progressive hearing loss similar to human DFNB8 patients.
Results: A single V6-hTMPRSS3 injection into the inner ear of 6-month-old TMPRSS3A306T/A306T mice demonstrated long term improvement in hearing thresholds compared with untreated control ears. A comparison with the benchmark AAV2-hTMPRSS3 revealed that achieving the same level of hearing rescue required a 3-log fold higher concentration of the vector compared to V6-hTMPRSS3. To further characterize V6, our novel capsid was compared to AAV-Anc80 and AAV2. V6 demonstrated higher transgene expression in HEI-OC1 cells, compared to AAV-Anc80 and AAV2 (GOI 2000, 93 %, 29 % and 46 %, respectively), despite significantly lower entry efficiency, indicating enhanced intracellular processing. Subsequent IF-FISH analysis at single-cell level visualized V6 uncoating to approximately 4-fold higher levels than AAV2 after 24h. In contrast to AAV2, V6 uncoating appeared independent of nucleolar reorganization, which has been shown to correlate with cell cycle progression for AAV2. This characteristic potentially offers an advantage for V6 in post-mitotic auditory cells. Furthermore, V6 outperformed AAV2 with 2-fold higher uncoating in vitro (HEI-OC1) in an indirect assay, correlating with corresponding transduction efficiencies.
Conclusions: Our novel AAV vector V6 demonstrated effective and consistent inner ear cell transduction in the mature inner ear, likely driven by enhanced uncoating, and achieved successful hearing rescue in an aged mouse model of human genetic deafness at a low dose. This lays the groundwork for developing V6-hTMPRSS3 gene therapy to treat DFNB8 patients.

SA51. Tailoring AAV Vectors for Gene Therapy of Inner Ear Disorders by Directed Evolution

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Category: Gene Therapy

Background: Hearing loss (HL) affects approximately 20% of the global population and the treatments are currently limited to hearing aids and cochlea implants. Gene therapy offers a possibility to prevent or even cure HL. With the aim to optimize the adeno-associated virus (AAV) vector system for inner ear directed gene therapy, we generated AAV peptide display libraries based on the AAV1, AAV2 and AAV6 capsid backbones. Thus far, AAV variants being tested in the inner ear today originate from libraries screened in other target tissues (e.g. CNS, eye) or have been in silico generated (e.g. Anc80L65), therefore a specific directed evolution of AAV libraries in the inner ear may yield AAV variants with higher infectivity of inner ear cell types.
Methods: Libraries were constructed to present random unique 7-mer peptide inserts at variable region VIII of the capsid protein with diversities ranging from 80,000-622,000 (maximum likelihood estimate, MLE). We conducted high-throughput in vivo selection screens in the inner ear of adult mice (n = 4, per library), testing alternative administration routes that demand overcoming robust biological barriers.
Results: Distinct variants were found to be accumulated to up to 5% for AAV2-based variants and up to 2.5% for AAV1-derived capsids after two rounds of in vivo selection. A total of 20 top candidates from the AAV1 and AAV2 libraries were produced as vectors packaging the dTomato transgene. They outperform the parental serotypes and show distinct expression patterns in the adult mouse cochlea. Three promising variants had the ability to transduce outer HCs, a challenging cell type to infect, and many also targeted inner HCs. Almost half of the variants also strongly transduced all layers of the stria vascularis – a viable target tissue for the treatment of age-related HL – and the SGNs were targeted, with 4 variants being highly specific for SGNs.Top AAV candidates have also been confirmed to reach the cells in 10-11 week old pig cochleas (n = 4) using our less invasive alternative administration route, with two clear top performers, Var1 and Var3.The top OHC-targeting variant, Var1, is also being tested as a dual-AAV to deliver the large stereocilin transgene to the cochlea. Success has already been seen in vitro, with full-length stereocilin protein already detectable at 48 hours. Clinically, this can be utilised as a treatment for the second most common cause of SNHL, DFNB16.
Conclusions: We report on a set of promising new AAV variants with distinct features developed by in vivo high throughput selection screens for improving inner ear directed gene therapy.

SA52. Optimizing Antisense Oligonucleotide Chemistry for the Treatment of Hearing Loss and Imbalance in Usher Syndrome

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Category: Gene Therapy

Background: Usher syndrome (Usher) is a rare genetic disorder characterized by the loss of hearing, vestibular, and visual function. There are 3 main clinical types (Usher 1, 2, 3) based on the severity and age of onset of the hearing and vision loss. Usher type 1C (USH1C) is a severe form caused by mutations in the USH1C gene which encodes harmonin, a structural protein important for the function of hair cells and photoreceptors. Mutations in USH1C account for ~3.5% of all Usher cases; however, the frequency of this subtype is significantly increased in the Acadian population of Louisiana and Canada due to the USH1C c.216G GREATER THAN A (216A)

founder mutation. The 216A mutation causes aberrant splicing that results in a severely truncated harmonin protein. We have previously shown that a novel antisense oligonucleotide (ASO-29) therapy targeting the 216A mutation transiently restores hearing, balance behavior, and vision in a mouse model of USH1C. To improve upon these results, various ASO chemistries were designed to target the 216A mutation and tested for their effectiveness to improve hearing and balance in USH1C mice.
Methods: USH1C mice were treated via intraperitoneal injection at postnatal day 2 with 216A-targeting ASOs on a 2' methoxyethyl (ASO-29) or morpholino (MO-29) chemistry, or a combination of the two chemistries (COMBO). Hearing function and balance behavior were assessed in 1- and 3-month-old USH1C-treated, -untreated, and wildtype (WT) littermates using auditory-evoked brainstem response (ABR), and a rotarod and balance beam analyses respectively.
Results: USH1C mice treated with ASO-29, MO-29, or COMBO therapy showed moderate rescue of ABR thresholds at 1- and 3-months of age compared with untreated USH1C mice. Additionally, USH1C mice treated with ASO-29, MO-29, or COMBO therapy showed improved latency-to-fall on the rotarod and time-to-traverse on the balance beam compared with untreated USH1C littermates. Latency-to-fall on the rotarod was not statistically different from WT mice at either age following treatment with ASO-29 or MO-29 whereas mice treated with the COMBO were statistically different from the WT mice.
Conclusions: These preliminary data show that ASO-29, MO-29, and COMBO therapy provide therapeutic benefits to hearing and balance behavior in USH1C mice and supports the potential of antisense treatment to restore hearing and vestibular function in Usher syndrome.

SA53. Template-Independent Genome Editing for Restoring Inherited Deafness Caused by Frameshift Mutations

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Category: Gene Therapy

Background: Insertion or deletion (InDel) mutations account for 22% of Mendelian inherited diseases, posing significant challenges to human health due to lack of effective therapeutics.
Methods: Here, we utilized non-homologous end joining (NHEJ) mediated biased DNA repair for in vivo gene therapy in mouse models with InDel mutations.
Results: To achieve this, we first profiled editing outcomes of homologous gRNA pairs across cell lines and cochlea tissues, revealing properties of in-frame products with specific nucleotide-level preferences. These repair principles then guided our cochlea-based analyses to assess therapeutic potential of gRNAs targeting InDel mutations associated with human deafness. Remarkably, certain loci exhibited a high restoration score when utilizing the optimal sgRNA, achieving near wild-type restoration of hearing thresholds in diseased mouse models. This was accomplished through dual adeno-associated virus (AAV) delivery of gRNA and spCas9 in animals.
Conclusions: Our findings indicate that this template-independent gene editing for restoration (TIGER), when combined with spCas9 and thorough evaluation, holds promise as an in vivo gene therapy strategy for disorders induced by frameshift mutations.

SA54. Hair Cell Patterning During Zebrafish Utricular Development

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Category: Development: Cellular/Systems

Background: In zebrafish, like in amniotic species, the vestibular sensory epithelia in the otolith organs are organized into central and peripheral zones, called the striola and extrastriola. Striolar and extrastriolar hair cells drive distinct but parallel vestibular sensory circuits and behaviors, which are functionally matured in the fish's first 5 days. In mouse it has been shown correct zonal patterning depends on differential retinoic acid (RA) signaling, but hair cell identity during epithelial patterning in development has not been described in detail. We assessed the spatiotemporal development of these two hair cells types during epithelial patterning, and in correlation with the expression patterns of RA synthesizing and degrading enzymes, in the developing zebrafish utricle.
Methods: To determine perinatal hair cell identity, we use hybridization chain reaction fluorescent in situ hybridization (HCR FISH) probes informed by single-cell RNA sequencing in larval zebrafish, where calcium binding protein genes cabp1b and cabp2b are exclusively expressed in extrastriolar and striolar hair cells, respectively. We examined hair cell development in various transgenic zebrafish lines, including wildtype myo6b:GFP and myo6b:nlsEOS, and fish with the cdh23tj264 mutation. We also use HCR FISH probes to assess expression patterns of RA enzyme encoding genes cytochrome P450 26 (cyp26b1) and aldehyde dehydrogenases family 1, subfamily A (aldh1a3).
Results: In wildtype fish utricles, hair cells that develop in the first 48 hours express markers for both striolar and extrastriolar hair cells, indicating a transient incipient hair cell type. Hair cells that are added after 2 days are already specified as striolar or extrastriolar. This change in the mechanism of addition is accompanied by complementary expression of cyp26b1 in the striolar zone and aldh1a3 in the extrastriolar zone. By 5 days, the initial incipient hair cell population has transitioned to become extrastriolar hair cells. In cdh23 mutant fish, where hair cells do not mechanotransduce, incipient hair cells never transition. However, complementary patterning of cyp26b1/aldh1a3 in striolar/extrastriolar zones is normal in cdh23 mutant utricles at 5 days.
Conclusions: We provide evidence for two pathways of early hair cell development: hair cells born in the first 2 days begin in a transient state that is neither striolar nor extrastriolar and take up to five days to acquire extrastriolar characteristics, a process that is dependent on functional activity. Hair cells born after 2 days develop as either striolar or extrastriolar, corresponding to their contact with cyp26b1- or aldh1a3-expressing supporting cells, suggesting evolutionary conservation of zonal molecular logic.

SA55. Epigenetic Modulation of Cochlear Organoids: Investigating the Role of CHD4 in Sensorineural Hearing Impairment Associated With Sifrim-Hitz-Weiss Syndrome

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Category: Development: Cellular/Systems

Background: Sensorineural hearing loss (SNHL) is a significant cause of functional disability worldwide, affecting millions globally. The development of targeted therapies requires an in depth understanding the underlying molecular mechanisms of cochlear development and its disruption by mutated genes affected in SNHL patients. Recently, pluripotent stem cell-derived inner ear organoids were established as a scalable and high-fidelity alternative for studying human auditory biology.
Methods: Chromodomain helicase DNA-binding protein 4 (CHD4) is an ATP-dependent chromatin remodeler that plays a central role in epigenetic gene regulation, DNA repair, and cell cycle progression. Several mutations were identified in CHD4, resulting in patients manifesting hearing loss under the syndrome of Sifrim-Hitz-Weiss (SIHIWES). In this study, we investigated the impact of CHD4 mutation on cochlear organoid development derived from human pluripotent stem cells (hESCs). To achieve this, we initially established a PAX2-mCherry reporter hESC line, allowing us to isolate otic progenitors efficiently. Subsequently, CRISPR / Cas9 technology was employed to generate hESC lines from mutant CHD4 p.G1003D and CHD4 knockout (KO) hESC lines.
Results: Mutant cochlear organoids show reduced number of hair cells, increased proliferation in both pluripotent state and during differentiation, and increased apoptosis in KO lines at pluripotent state. RNA-seq data on day 20 otic progenitors show disruption of developmental, survival, cell death and proliferation pathways. Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) data on otic progenitors show disruption of similar pathways.
Conclusions: This study provides valuable insights into the specific role of CHD4 during the development of cochlear organoids.

SA56. Characterization of Potential Roles for Glial Precursors During Wiring of the Developing Cochlea

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Category: Development: Cellular/Systems

Background: Neuronal connectivity in the cochlea is optimized for transducing auditory stimuli in a tone-specific manner while preserving fine scale temporal information. Whereas spiral ganglion neurons arise from the otocyst, the glia that associate with them develop from neural crest-derived glial precursors (GPs) that must travel long distances and then take on Schwann cell and satellite glial cell identities appropriate for the cochlea. To traverse these distances efficiently, GPs migrate in group formation, including as they invade the cochlea. In many systems, GPs are thought to travel travel behind developing axons to reach their destination without contributing to pathfinding by those axons. This is also true in the early embryo, where GPs interdigitate with the earliest growing spiral ganglion neuron (SGN) neurites (Sandell et al., 2014). However, our lab demonstrated that a subset of GPs in the developing cochlea migrate into the surrounding mesenchymal tissue ahead of most SGN neurites (Druckenbrod et al., 2020). Additionally, SGN neurites grow faster when they contact migrating GPs, raising the possibility that GPs contribute to cochlear wiring, either by forming a conducive scaffold or by actively shaping neurite behavior.

Methods: To unravel how GPs may shape cochlear wiring, we characterized GP morphologies and investigated how they interact with each other and with SGN neurites. To reveal potential roles in the developing cochlea, we induced sparse labeling of cochlear GPs via tamoxifen administration to PLPCre-ERT mice also carrying the Ai14 tdTomato reporter at embryonic day (E) 11 and collected at E13, when the GPs emerge from the spiral ganglion into the surrounding mesenchymal tissue. To begin to distinguish between passive and active roles for GPs and assess whether glial morphology is dynamic over time, we performed time-lapse imaging of embryonic cochlear explants at E13 and E14, when the GPs assemble into chain-like formation. GPs were identified by expression of GFP in the PLP locus (PLPGFP/+). SGNs were labeled with tdTomato using Bhlhe22Cre and Ai14.
Results: Our preliminary time lapse imaging studies captured the initial formation of glial protrusions, with examples of glia and neurites co-invading the mesenchymal space from the spiral ganglion. Dynamic changes in morphology suggest that GPs grow as chains and hint at possible roles in maintaining the spiral ganglion border and shaping SGN neurite outgrowth.
Conclusions: Cochlear GPs assume a variety of morphologies that predict behaviors that may contribute to the proper organization of SGN peripheral processes in the cochlea.

SA57. Impact of FGF8 Secreted by Inner Hair Cells on Supporting Cell Identity and Distribution in the Organ of Corti

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Category: Development: Cellular/Systems

Background: The organ of Corti has two types of mechanosensory hair cells (HCs) forming one row of inner hair cells (IHCs) in the inner compartment and three rows of outer hair cells (OHCs) in the outer compartment. These cells are surrounded by uniquely specialized supporting cells (SCs). Inner border cells (IBCs) and inner phalangeal cells (IPhCs) surround IHCs; outer pillar cells (OPCs) and three rows of Deiters' cells (DCs) surround OHCs. Inner pillar cells (IPCs) separate both compartments. It is described that FGF8 released from IHCs regulate pillar cell development. However, it is yet to be resolved whether FGF8 secreted by IHCs is the only morphogen that induces PCs differentiation and whether this FGF8 affects other SCs. We wondered if by removing FGF8 from different locations in the inner ear during embryonic development we can define the effects that this factor secreted by IHCs has on the SCs.
Methods: We deleted Fgf8(F/F) embryonically with TgPax2-Cre for ablation in all otocyst-derived cells (spiral ganglion neurons and cochlear epithelium); with Emx2-Cre for ablation in the entire cochlear epithelium but not the neurons; with Insm1-GFP-Cre for ablation in neurons and with; Atoh1-Cre for ablation in HCs. Due to early postnatal lethality TgPax2-Cre mice were examined at P0 and Emx2-Cre mice up to P4. Then, to determine the effect of the FGF8 elimination from these different locations we examined the effect on SCs analyzing the expression of FABP7 for IBCs and IPhCs; p75, ACE and NPY for IPCs; CD44 for OPCs; and PROX1 for the nuclei of PCs and DCs.
Results: Preliminary results indicate that: (1) In all these Fgf8-cKOs, IBCs and IPhCs surrounding the IHCs are normal in number and structure. (2) With elimination of FGF8 in the entire otocyst or in the cochlear epithelium, IPCs are formed and express all three markers (p75, ACE and NPY).

However, eliminating FGF8 in the entire otocyst affected the number of IPCs, which align in a row without the characteristic high density and packing of oblong nuclei. (3) The TgPax-Cre (otocyst) and Emx2-Cre (cochlear epithelium) Fgf8-cKO mice also displayed a great reduction of OPCs, possibly due to a conversion to DCs.
Conclusions: Our results suggest that FGF8 secreted from IHCs is not required for the identity and formation of IBCs, IPhCs and IPCs but might induce the high density and packing of IPCs. Finally, we conclude that FGF8 secreted from IHCs promotes OPC differentiation.

SA58. Repopulating Microglia in the Auditory Brainstem Recapitulate Developmental Properties following Cessation of CSF1R Inhibitor Treatment

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Category: Development: Cellular/Systems

Background: Neuroglial signaling is imperative for proper neural circuit formation. The brain's primary immune cells, microglia, are involved in the development and maturation of connections in the auditory brainstem. In the first postnatal week, microglia populate the brainstem, appearing in the ventral cochlear nucleus by postnatal day (P) 0 and in the medial nucleus of the trapezoid body (MNTB) by P6. Microglia first enter the brainstem lateromedially and display an ameboid morphology, characterized by large cell bodies and short branches. Just after hearing onset, microglial quantity increases, and they display more complex branching and smaller somata. Microglial elimination with a colony stimulating factor-1 receptor inhibitor, BLZ945, in the first ten postnatal days halts calyceal pruning and diminishes levels of glial fibrillary acidic protein (GFAP), a marker for mature or reactive astrocytes, in the MNTB. BLZ945 treatment also results in elevated auditory brainstem response (ABR) thresholds, decreased amplitudes, and increased ABR inter-peak latencies. After cessation of BLZ945 treatment, microglia return to the brainstem in a lateromedial progression, similar to normal development. Remarkably, microglial return rescues impairments from microglial depletion several weeks after normal development. Once microglia fully repopulate the brainstem, at four weeks of age, monoinnervation is restored in MNTB, but GFAP levels remain diminished. By seven weeks of age, following an extended period of microglial return, GFAP levels are comparable to controls and ABR amplitudes and inter-peak latencies largely recover. These functions do not recover with long-term microglial elimination. Our findings point to a role for microglia in circuit sculpting in the auditory brainstem and suggest that repopulating microglia take on characteristics of normally developing microglia.
Methods: To test this hypothesis, we compared morphology and phagocytosis in developing and repopulating microglia. We used CX3CR1+/EGFP mice in which microglia express enhanced green fluorescent protein. We characterized microglia at different ages in control and BLZ945-treated mice. Microglial qualities such as volume, branching, and expression of the lysosomal marker CD68 were measured through 3D reconstruction.
Results: We found that during a period of circuit optimization, developing microglia have larger somata, stunted processes, and higher lysosome marker expression.

Conclusions: After temporary microglial ablation with the CSF1R inhibitor, BLZ945, repopulating microglia reinstate some active characteristics as they rapidly recover damage from their depletion.

SA59. Expansion and Differentiation of Inner Ear Organoid Progenitors

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Category: Development: Cellular/Systems

Background: Cultures of inner ear organoids from pluripotent stem cells may one day provide suitable donor material for regenerative approaches to hearing and balance disorders. However, the cultures remain inefficient and heterogeneous, necessitating strategies to identify and isolate donor progenitors. As a next step toward developing useful donor material, we have also sought methods to isolate cells from stem cell-derived otic vesicles, expand these progenitors in vitro, and demonstrate their capacity to reassemble into organoids with sensory epithelia.
Methods: Mouse embryonic stem cells from a Pax2-EGFP reporter line were used to generate inner ear organoids and their intermediate otic vesicles. Aggregates with otocysts on in vitro day 14 (D14) were dissociated and subjected to fluorescence-activated cell sorting using the GFP reporter. Sorted cells were then introduced into domes of 100% Matrigel and cultured in Maturation Media (DMEM/F12, HEPES, N2, Glutamax) with or without various growth factor and small molecule supplements (EGF, IGF1, FGF2, valproic acid, ascorbic acid, CHIR99021, and Repsox). After another 14 days in culture within the domes, the number of vesicles or cysts and degree of expansion were quantified along with the percentage of cells continuing to express Pax2-EGFP. Some cultures were also examined by immunofluorescence for organoid production and differentiation of sensory hair cells using the hair cell marker MyoVIIa.
Results: Between 5 and 10% of the cells from D14 spheroids were GFP-positive, with most of this signal arising from otocyst-like structures within the larger spheroid aggregate. Once introduced into Matrigel domes, the progenitor cells remained viable and GFP-positive but did not proliferate or assemble into vesicles or cysts. However, when progenitors were exposed to a cocktail of growth factors and small molecules critical for expansion and differentiation of LGR5-positive cochlear stem cells, we observed widespread proliferation (50 to 500% increase in cell number) and the production of numerous vesicles and cysts within the domes. However, the percentage of GFP-positive cells decreased over time, suggesting a combination of proliferation and differentiation. Many of the large cysts produced within the domes also generated MyoVIIA-positive hair cells.
Conclusions: In this study, we found that isolated Pax2-positive progenitors from organoid cultures do not have the capacity to proliferate, reassemble into organoids, and differentiate into sensory tissue, unless treated with a cocktail of growth factors and small molecules used elsewhere to expand cochlear stem cells. By refining this approach, we hope to identify the factors that enable expansion while maintaining progenitor status in order to produce plentiful donor material for regenerative medicine approaches. We also hope to identify the factors that then guide those expanded cells to self-assemble and differentiate into sensory epithelia to inform the regeneration strategies.

SA60. PEA3 Transcription Factors Role in Cochlear Epithelial Development

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Category: Development: Cellular/Systems

Background: The PEA3 subfamily of ETS transcription factors (ETV1, ETV4 and ETV5) contribute to the development of various tissues including nervous tissue, kidneys and lungs. They function through their evolutionary-conserved ETS domain that mediates DNA binding and thereby regulates gene expression. In the cochlea, mesenchymal ETV4 and ETV5 operate downstream FGF signaling to regulate cochlear lengthening during early development. While epithelial FGF signaling has been shown to regulate multiple processes during cochlear development including placode induction, progenitor proliferation and differentiation, the role of potentially downstream PEA3 transcription factors has not been studied. In this work, we study the impact of epithelial-specific ETV1/4/5 single and combined deletions on cochlear development.

Methods: We generated single, double and triple mutant mouse models for the PEA3 family members through a conventional deletion of ETV4 and conditional deletions of ETV1 and ETV5 utilizing SOX2-drevin inducible CreERT2. Mutant mouse embryos from pregnant females induced by tamoxifen at embryonic day (E) 12.5 were collected at E19.5 and analyzed using immunohistochemistry of whole mount tissues and cryosections. We analyzed single, double and triple mutant cochlea at E19.5 (n=3-7/genotype) utilizing the following markers: SOX2 (supporting cells), NGFR (inner pillar cells), CD44 (outer pillar cells), MYO6 (hair cells), phalloidin (F-actin enriched stereocilia bundles), and TUJ1 (neuronal marker). One-Way ANOVA test followed by Tukey's multiple comparisons test were used to detect statistical significance and adjusted P value LESS THAN 0.05 was considered significant.

Results: ETV4/5 double and ETV1/4/5 triple mutants showed 22-35% increase in inner hair cell density throughout all cochlear turns with no change in outer hair cell density. TUJ1 staining showed a normal innervation pattern. Hair cell densities in single mutant cochleae were comparable to controls. As for supporting cells, ETV4/5 double mutants showed 30-40% reduction in NGFR+ cell density and 5-33% increase in CD44+ cell density without any change in SOX2+ cell density. ETV1/4/5 triple mutants showed a more severe phenotype including 85-93% reduction in NGFR+ cell density and 64-78% increase in CD44+ cell density indicating a possible dose-dependent role of the PEA3 subfamily members in pillar cell development. Sox2-CRE positive control cochleae were similar to Sox2-CRE negative cochleae and displayed no change in hair cell or supporting cell densities. Subsequent analysis of progenitor proliferation and gene expression changes in triple mutants during development is underway.

Conclusions: PEA3 family of transcription factors regulate epithelial development in mammalian cochlea impacting both hair cells and supporting cells.

SA61. EBF1 is Necessary for Sensory Domain Establishment Within the Organ of Corti

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Category: Development: Cellular/Systems

Background: Hearing depends on the precise patterning of hair cells (HCs) and support cells (SCs) along the length of the organ of Corti. Large gaps remain in our understanding of the signals that drive this developmental process. A recent bulk ATACseq study from our lab identified that the frequency of the EBF consensus motif is greater in the open chromatin of prosensory cells than nonsensory cells collected from embryonic cochleae. Complimentary single cell RNAseq analyses performed by our lab revealed that Ebf1 is expressed in the developing cochlear epithelium while Ebf2-4 show little to no expression. Here, we further characterize how EBF1 regulates cochlear development.

Methods: To identify EBF1's role in cochlear development and auditory function, our lab designed a conditional knockout (cKO) mouse model in which the Slc26a9 promoter directs Cre-mediated excision of Ebf1 in the otocyst at embryonic day (E) 9.5. Ebf1-cKO mice are fertile and survive into adulthood. We treated E15.5-E17.5 Cre-negative control and Ebf1-cKO littermates with EdU to identify EBF1-dependent changes in the timing of sensory cell cycle exit and domain establishment. We also performed immunolabeling on embryonic and neonatal cochlear wholemounts and sections to characterize changes in sensory cell patterning. To assess hearing in adult mice, we tested auditory brainstem response (ABR). Lastly, we are using a multiomic approach that combines single nucleus RNAseq and ATACseq into a single workflow to characterize EBF1's role as a transcriptional activator or repressor for target genes in distinct cell populations present at E14.5.

Results: Our single cell RNAseq, in situ hybridization, and immunolabeling experiments involving E12-E18 cochleae revealed Ebf1 is expressed in the Kölliker's organ, prosensory cells, HCs, and SCs. Loss of EBF1 leads to dramatic sensory expansion consisting of supernumerary HCs and SCs in the sensory domain in addition to ectopic sensory patches randomly distributed throughout the Kölliker's organ. Embryonic Ebf1-cKO cochleae exhibit prosensory cell proliferation beyond the stages that these cells typically drop out of the cell cycle in addition to a delay in differentiation relative to the cochleae of Cre-negative control littermates. By postnatal day 1, Ebf1-cKO cochleae possess approximately triple the number of inner HCs and double the number of outer HCs observed in Cre-negative control littermates. The supernumerary HCs in Ebf1-cKO cochleae are accompanied by supernumerary FABP7-positive inner border/phalangeal cells, CD44-positive outer pillar cells, and PROX1-positive Deiters' cells but not p75-positive inner pillar cells. Ebf1-cKO cochleae have abnormal innervation patterns that lack clear spiral bundles and include neuronal projections that extend to the ectopic sensory patches. In adult Ebf1-cKOs, supernumerary HCs and SCs persist. Adult Ebf1-cKO mice show significantly elevated ABR thresholds for all tested frequencies except 60 kHz, indicating that these mice are deaf.

Conclusions: EBF1 restricts cochlear sensory epithelium establishment and is necessary for hearing.

SA62. Determining the Impact of Igf2bp1 Re-Expression on Hair Cell Regeneration in the Mouse Cochlea

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Category: Regeneration

Background: Cochlear hair cells (HCs) are not replaced after damage in adult mammals. However, HC regeneration has been documented in mice during the first postnatal week in vitro and in vivo. Igf2bp1 is an RNA-binding protein that coordinates the transition from a fetal to a mature state. During embryonic development, Igf2bp1 is expressed in pro-sensory progenitor cells of the developing cochlea but is downregulated as the progenitors differentiate into HCs and supporting cells (SCs). SCs neighbor HCs and play multiple roles including acting as the source of regenerated HCs in the neonatal mouse cochlea. We propose that re-expression of Ig2bp1 in SCs would induce progenitor cell-like plasticity and render SCs responsive to HC damage in the mature cochlea.

Methods: Rosa26-loxP-stop-loxP-Igf2bp1 (Rosa26Igf2bp1) mice were used to re-express Igf2bp1 and Rosa26-loxP-stop-loxP-tdTomato (Rosa26tdTomato) mice were used to express tdTomato in Cre+ cells for fate-mapping. Pou4f3DTR/+ mice were used to express the human diphtheria toxin receptor (DTR) in HCs, allowing HC death to be induced by injection of diphtheria toxin (DT). We generated Pou4f3DTR/+::Plp-CreER::Rosa26Igf2bp1::Rosa26tdTomato mice and Pou4f3DTR/+::Prox1-CreER::Rosa26Igf2bp1::Rosa26tdTomato mice to examine effects of Igf2bp1 re-expression after HC loss. The two CreER lines were used to assess differences between two different SC subpopulations that neighbor the inner HCs (Plp-CreER) and those that surround the outer HCs (Prox1-CreER). Tamoxifen was injected at postnatal day (P) 0 to induce expression of Igf2bp1 and tdTomato in SCs, and DT was injected at either P1 or P7 to kill HCs. Samples were collected 1 week post-DT. Littermates lacking the Rosa26Igf2bp1 allele and littermates without HC damage (Pou4f3DTR/+-negative) were used as controls. Cochlea was immunostained using anti-myosin VIIA antibodies, while tdTomato was detected by endogenous fluorescence. Confocal microscopy was used to quantify tdTomato+ HCs (the Cre+ fate-mapped SCs) in each of our samples to measure HC regeneration.

Results: Mice with re-expression of Igf2bp1 in Prox1+ SCs had a significant number of tdTomato-positive HCs (regenerated HCs) in the apical turn of the cochlea after HC damage was induced at P7. Yet there was no evidence of HC regeneration present in control samples. Notably, in mice with re-expression of Igf2bp1 in Prox1+ SCs and without HC damage, we observed a small number of tdTomato-positive HCs. However, there were no differences compared to controls when HC death was induced at P0 or when Igf2bp1 was re-expressed in Plp+ SCs.

Conclusions: Our findings show that HC regeneration can be induced in a more mature cochlea when the SCs located near the outer HCs maintain expression of Igf2bp1. These observations suggest that the re-expression of Ig2bp1 may be preventing these SCs from maturing and/or promoting the activation of pro-regenerative signals that are activated by HC damage.

SA63. Tracing Inner Ear Progenitor Cells in Cochlear Organoids

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Category: Regeneration

Background: Hearing loss is one of the most common sensory impairments in humans, primarily due to the lack of regenerative ability in mammalian cochlear hair cells (HCs). Despite the presence of approximately 15,000 cochlear HCs in the inner ear, genetic mutations or environmental damage can lead to irreversible cell loss and consequent deafness. Atoh1, a key gene for HC differentiation, becomes progressively downregulated during the postnatal period due to the accumulation of repressive epigenetic marks. Our studies indicate that the epigenetic silencing of Atoh1 can be partially reversed by specific epigenetic modifiers.
Methods: Here, we use organoids derived from the postnatal mouse cochlea to examine trajectories of inner ear progenitor cells to HCs. We tag lateral and medial supporting cells, using Fgfr3-CreER and Glast-CreER, respectively, as well as an Lgr5-CreER strain to trace Lgr5+ cells. We followed the tagged cells through proliferation to form the organoids and differentiation to the hair cell lineages, including cells with transcriptomic profiles of outer and inner hair cells, as well as vestibular hair cells. We analyzed single-cell RNA/ATAC-seq multiomes at days 0, 2, 4, and 10 of organoid differentiation.
Results: We crossed a newly developed Atoh1-mCherry mouse strain to obtain mice with red cochlear HCs. We showed that medial and lateral compartment origin of cochlear supporting cells largely determines IHC and OHC fate. Myo7a+ cells derived from the lateral compartment of the cochlea co-labeled for the OHC marker prestin (87% of reporter-positive organoids), and these cells expressed significantly higher levels of the OHC genes Ocm and prestin than reporter-negative cells, whereas Myo7a+ cells derived from the medial compartment of the cochlea labeled for the IHC marker vGlut3 (85% of reporter-positive organoids), and these cells expressed higher levels of the IHC gene vGlut3 than reporter-negative cells.
Conclusions: This work helps to unravel the transcriptional programs underlying the differentiation of HCs and address future hearing loss treatment.

SA64. Unlocking Regeneration of Auditory Neuroprogenitors Using the Phoenix Platform

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Category: Regeneration

Background: Sensorineural hearing loss, a permanent condition, results from the irreversible damage of cochlear hair cells and auditory neurons. The limited regenerative capacity of cochlear progenitors is a significant barrier to the development of effective in vitro models, slowing progress in therapeutic innovations. While animal models are commonly used for preclinical testing, they are limited by low throughput, high variability, and poor predictive value for human outcomes. Overcoming these limitations could dramatically accelerate the development of novel hearing loss therapeutics.
Methods: We introduce Phoenix auditory neuroprogenitors (ANPGs), highly regenerative stem cells derived from the A/J mouse cochlea. The phoenix cells offer an abundant source of auditory neurons in a primary culture-like environment, enabling high-throughput screening, reducing assay variability, and significantly lowering the need for animal use. Based on these cells, our

research aims to identify and understand the pathways governing mammalian ANPGs proliferation and differentiation to advance therapeutic strategies for sensorineural hearing loss.
Results: By comparing transcriptome (RNAseq) and epigenome (ATACseq) of Phoenix progenitors and traditionally low stemness ANPGs, we identified key stemness pathways. To provide functional validation of identified regenerative pathways, we performed a targeted screen on differentially expressed pathways. Epigenomic studies further revealed additional target loci crucial for ANPG regeneration. Through targeted modulation of the WNT and TGFβ/Smad pathways using small molecules or genetic methods, we achieved sustained expansion of ANPGs in vitro, while maintaining their ability to differentiate into functional auditory neurons. This stemness reprogramming method, initially optimized in mouse auditory cells, is now being translated to human fetal otic neural stem cells.
Conclusions: The Phoenix platform represents a breakthrough in auditory research, offering a scalable, high-throughput, cost-effective, and ethically responsible (3R-compatible) method for screening otoprotective and otoregenerative drugs. Additionally, our work sheds new light on the regenerative mechanisms of auditory neuroprogenitors, opening new avenues for inner ear regeneration therapies. Future work will focus on leveraging this platform to identify and develop novel therapeutic candidates for hearing loss treatment.

SA65. Novel Small Molecule-Mediated Restoration of the Surface Expression and Anion Exchange Activity of Mutated Pendrin Causing Pendred Syndrome and DFNB4

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Category: Genetics A: Genomic and Gene Regulation

Background: The background of the paper focuses on genetic hearing loss, particularly Pendred syndrome and DFNB4. It highlights that the SLC26A4 gene, which encodes the pendrin protein, is one of the most common causes of genetic hearing loss among East Asians. Pendred syndrome is characterized by congenital hearing loss, often accompanied by subclinical hypothyroidism, while DFNB4 is a nonsyndromic form of hearing loss. Both conditions are associated with inner ear malformations. Although patients exhibit residual hearing at birth, their hearing progressively worsens over time.
Methods: Identification of Correctors for the H723R Variant via High-Throughput Screening, Cell Culture and Transfection, Surface Protein Expression Analysis, Experiments on Human Nasal Epithelial Cells, Chloride/Bicarbonate Exchange Activity Measurement, Protein Stability Test, Evaluation of Drug Permeability and Metabolic Stability, Safety Assessments
Results: Through high-throughput screening, PC2-1 was shown to enhance the correct folding and trafficking of the mutant protein to the plasma membrane in a dose-dependent manner. In both CHO and human nasal epithelial cells, PC2-1 restored the pendrin's function by increasing chloride/bicarbonate exchange activity. Additionally, PC2-1 improved the structural stability of H723R-PDS under proteolytic conditions. The compound exhibited high permeability and metabolic stability, with no observed cytotoxicity or cardiac side effects (as shown in hERG assays). Furthermore, when applied locally in the cochlea, PC2-1 reached micromolar concentrations in the perilymph, demonstrating its potential for inner ear drug delivery.

Conclusions: This study identified PC2-1 as a potent small-molecule corrector that restores the folding defect of the H723R pendrin mutant (H723R-PDS) and recovers its surface expression and anion exchange activity. PC2-1 demonstrated high permeability and metabolic stability, with no observed cytotoxicity or cardiac side effects. These findings suggest that PC2-1 has great potential as a therapeutic agent for treating genetic hearing loss disorders such as Pendred syndrome and DFNB4. Further research and therapeutic development based on PC2-1 could play a significant role in alleviating symptoms for patients with hearing loss.

SA66. Novel Variant in CEP250 Causes Protein Mislocalization and Leads to Nonsyndromic Autosomal Recessive Type of Progressive Hearing Loss

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Category: Genetics B: General

Background: Hearing loss is the most common sensory disorder in humans. Hearing loss caused by autosomal dominant diseases accounts for 22-25% of hereditary hearing loss, and about 30 causative genes have been identified. However, the exact association and mechanism for this are not yet known. In addition, there are many related genes that have not been discovered yet, and research is underway to discover them. In this study, we uncovered CEP250 as a newly discovered deafness gene through whole exome sequencing in a family where affected individuals showed hearing loss. (YUHL cohort)

Methods: Patients and Diagnosis of Genetic Hearing Loss, WES and Analyses, pRK5-Myc-CEP250 Recombinant Vector Construction and Mutagenesis, Cell Culture and Transfection, Immunoblotting, Immunocytochemistry, CEP250 Knock mouse Inner Ear Immunoblotting and Immunohistochemistry, Auditory Brainstem Response Test

Results: We studied how it affects centrosome and primary cilia when CEP250 clone transfection into NIH3T3 cells through immunocytochemistry.

In this study, we found CEP250, a newly discovered deaf gene, through whole exome sequencing in families with progressive hearing loss with onset of infected individuals.

In order to investigate the effect of CEP250 as a deaf gene on cilia and centrosomes, ICC experiments were conducted using NIH3T3 cells derived from mouse fibroblasts. It was confirmed that CEP250 Wild type was localized to the centrosome in the transfected cells. On the other hand, it was confirmed that CEP250 Q1171X was expressed without being localized to the centrosome in the transfected cells. Also, the expression of CEP250 mutation does not completely block the generation of primary cilia, but it is presumed that it affects the length and the distance between centrosomes.

When the location of CEP250 was confirmed through IHC in cochlea of WT mouse, it was confirmed that it was expressed in hair cells and spiral ganglion.

In the case of the CEP250 Q1171X mutant discovered by the research team, it is thought that when a mutation occurs in the CEP250 gene, it interferes with the expression of the CEP250 protein at the appropriate location.

Conclusions: A nonsense variant in CEP250 results in a deficit of centrosome localization and hair cell degeneration in the cochlea, which is associated with the progression of hearing loss in humans and mice.

SA67. POLD3 Haploinsufficiency is Linked to Non-Syndromic Sensorineural Adult-Onset Progressive Hearing and Balance Impairments

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Category: Hair Cells: Anatomy & Physiology

Background: Hearing impairment (HI) is a significant health concern globally, influenced by genetic and environmental factors. We had identified a homozygous pathogenic variant in POLD3 in a Lebanese patient with an autosomal congenital recessive syndromic hearing loss. This variant was found at heterozygous state in the parents, who developed progressive hearing impairment around age 40.

Methods: We conducted a thorough clinical and genetic assessment of sixteen family members, including physical exams, audiometry and vestibular function evaluations. Additionally, gene expression analysis of the Pold3 gene was performed in mice using RNAscope.

Results: Twelve individuals were heterozygous for the variant in POLD3, of whom eight showed bilateral adult-onset HI, typically starting around ages 40-50, and two older patients displaying unilateral vestibular weakness. Additionally, two carriers of the variant developed cancer at an early age. RNAscope confirmed Pold3 expression in auditory and vestibular neurons. Exome sequencing analysis excluded the presence of pathogenic variants in any known hearing impairment or cancer predisposition genes.

Conclusions: We present herein evidence of a heterozygous pathogenic POLD3 variant that may be associated with a novel form of autosomal dominant progressive adult-onset hearing and vestibular impairments. We also highlight the necessity for further exploration of the role of POLD3 in cancer predisposition.

SA68. Long-Read Sequencing of a STRC Deletion

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Category: Genetics B: General

Background: Copy number variants (CNVs) are a well-established cause of hearing loss. Most frequently, these CNVs are associated with deletions involving STRC, the detecting and mapping of which is complicated by the local genomic architecture and presence of pseudogenes. Specifically, STRC is one of four genes (PPIP5K1, CKMT1B, STRC, CATSPER2) in a tandem duplication and is frequently impacted by deletions also involving the adjacent CATSPER2 gene. Because mapping of short sequence reads is hindered by alignment challenges, we hypothesized that long-read sequencing (LRS) technologies may be useful to characterize this genomic region.
Methods: We completed LRS using Oxford Nanopore Technologies (ONT) with adaptive sampling on a child of consanguineous parentage to evaluate the size and breakpoints of a homozygous contiguous gene deletion of STRC-CATSPER2 identified through gene panel testing. Read alignment and variant calling was done with a bioinformatics pipeline from ONT with manual alignment where necessary.
Results: LRS data showed that the CNV deletion in this patient extended beyond STRC-CATSPER2 and into the surrounding genes and tandem duplication areas. Even though ONT enabled sequencing of larger continuous sections, alignment remained problematic, most frequently due to mis-mapping between the gene and pseudogene. Although structural variant callers could not definitively identify deletion breakpoints, visual inspection placed them within completely identical regions between the CKMT1B gene and its tandem duplication, CKMT1A.
Conclusions: This study demonstrates that while LRS improves resolution of the STRC region, genomic complexities remain that preclude precise mapping of the CNV deletion breakpoints.

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SA69. Exploring the Contribution of MYO15A Towards Alzheimer's Disease: Is DFNB3 Really Non-Syndromic?

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Category: Genetics B: General

Background: Mutations in the gene MYO15A, encoding the molecular motor myosin 15, cause human non-syndromic autosomal recessive deafness, DFNB3. MYO15A is expressed by inner ear hair cells and is well characterized to orchestrate the assembly and maintenance of mechano-sensory stereocilia. MYO15A is also expressed in other tissues throughout the body, but a syndromic phenotype has yet to be reported in patients with DFNB3. A recent GWAS study identified a high-confidence, single-nucleotide polymorphism (SNP) in MYO15A that was associated with a significantly reduced risk of developing Alzheimer's Disease (AD). The goal of this study was to explore the function of MYO15A in the brain and how it contributes to the onset of Alzheimer's Disease pathology.
Methods: We used RNAScope in situ hybridization and immunofluorescence to detect Myo15a transcripts and protein in coronal sections of fixed, C57/BL6 mouse brains. Testing of

hippocampal-dependent short-term learning and memory was conducted using the Y-maze test in Myo15a(shaker 2) mutant mice. To model tauopathy, a hallmark pathology of AD, we used the transgenic rTg4510 mouse model that overexpresses human tau (MAPT) with the familial frontotemporal dementia (FTD) variant, p.P301L.
Results: Myo15a transcripts were detected in pyramidal cells of CA1, CA2 and CA3 regions of the hippocampus, in addition to granule cells that project mossy fibers from the dentate gyrus to CA3. Immunofluorescence confirmed these observations and localized endogenous MYO15A protein to synaptic terminals. To test our hypothesis that MYO15A is necessary for hippocampal processing and function, we used the Y-maze behavioral test to assess short-term working memory in the Myo15a(shaker 2) mutant mouse and control littermates. Mutant shaker 2 mice displayed a statistically impaired recognition of the maze novel arm compared to wild-type littermates, suggesting that MYO15A has a physiological function in learning and memory. Finally, we studied the localization of MYO15A in a mouse model of tauopathy that exhibits cognitive decline and forms pathological neurofibrillary tangles (NFTs). In rTg4510 mice, MYO15A protein was significantly reduced in mossy fibers synapses projecting to CA3, and instead was strikingly accumulated into mature NFTs that co-labeled with hyper-phosphorylated tau protein.
Conclusions: Our study reveals a function for MYO15A in hippocampal-dependent learning and memory, and raises the possibility that DFNB3 - associated variants may have syndromic phenotypes. We further uncover a direct association of MYO15A with NFTs, a central neuropathology in AD. Future work is aimed at understanding the function of MYO15A within NFTs and how this can modify disease severity.

SA70. Genetic Evaluation of a Large Cohort of Pediatric Cochlear Implant Patients: Correlation With Outcomes

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Category: Genetics B: General

Background: Cochlear implantation (CI) has revolutionized the care of children with hearing loss: CIs allow children with hearing loss to attain speech outcomes on par with their normal-hearing peers and improve quality of life in affected children and their families. While data show that most CI users obtain excellent hearing and speech perception outcomes, some children do not achieve expected hearing levels. In addition, up to 7% of pediatric CI patients ultimately become non-users, likely because they receive little or no benefit from the device. For these children, access to sensory stimuli is lost during a key period in neurocognitive development. Therefore, there is a critical need to identify causes of poor CI outcomes so that alternative, better treatment modalities may be developed for these children. We and others have previously shown correlations between damaging genetic variants that affect the relevant nerval structures and CI outcomes, termed the spiral ganglion hypothesis, but only in small cohort studies. To date, this hypothesis has not been tested in a large (GREATER THAN 100 patient) cohort of pediatric patients with definitive genetic testing results.

Methods: We performed genetic testing of pediatric CI patients using gene panels, exome sequencing, and genome sequencing. We tracked postoperative audiometric outcome, including word scores, using standard measures and considered the most recent test result GREATER THAN 3 months after CI surgery in our analyses. Disparate age-appropriate word scores were combined after normalization around the mean (Z score).
Results: We performed genetic testing for 138 pediatric CI patients. The diagnostic yield was 59% (82/138). The most common genetic diagnosis was pathogenic variants in GJB2 comprising 34% of all patients (47/138). Patients with GJB2 hearing loss had, on average, excellent CI outcomes though there were three outliers. Further analysis will identify genes associated with poorer CI outcomes. Ongoing analysis includes evaluating damaging genetic mutations that may negatively affect spiral ganglion neuron function.
Conclusions: This is the largest cohort reported to date correlating objective CI outcomes in pediatric CI patients. Correlations with outcomes will improve pre-operative counseling for CI patients.

SA71. Proportional Differences in Alternative Splicing Isoforms of SLC12A2 Across Tissues and Their Role in Disease Phenotypes

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Category: Genetics B: General

Background: The SLC12A2 gene has been implicated in three distinct disorders: Delpire-McNeill Syndrome, Kilquist Syndrome, and nonsyndromic hearing loss (DFNA78). The mechanisms underlying these diverse phenotypes remain unclear. In this, we propose to investigate the pathomechanisms by which mutations in SLC12A2 cause different phenotypic outcomes.
Methods: Next-generation sequencing was used to identify variants in SLC12A2 in a large Chinese cohort of individuals with hearing loss, named from the Chinese Deafness Genetics Consortium (CDGC). Sanger sequencing and segregation analysis were performed to validate the identified variants. Long-read sequencing (PacBio Hi-Fi) was employed to investigate the full-length transcripts of SLC12A2 in cochlear and vestibular tissues from humans and mice. Immunofluorescence was used to assess SLC12A2 protein expression and localization in various mouse tissues, while qRT-PCR quantified the relative expression of SLC12A2 isoforms across tissues.
Results: We identified three novel pathogenic variants of SLC12A2 affecting exon 21 splicing in patients with nonsyndromic hearing loss. Two additional novel pathogenic variants in exons 1 and 11 were found in patients with syndromic features including hearing loss and neurological abnormalities. Full-length transcript sequencing of human and mouse inner ear tissues revealed two alternative splicing isoforms, differentiated by the inclusion or exclusion of exon 21. qRT-PCR analysis demonstrated significant variation in the proportion of these isoforms across tissues. In mouse inner ear, the isoform including exon 21 was predominant accounting for over 97%, 70%, and 95% of transcripts in the stria vascularis and spiral ligament, spiral ganglion neurons,

and vestibule, respectively. However, in the central nervous system, this isoform represented only ~50%.
Conclusions: The proportion of alternative splicing isoforms of SLC12A2, in addition to differences in the transcripts themselves, plays a key role in determining disease phenotypes. This finding is particularly important for genotypic and phenotypic correlation analyses of SLC12A2, improving prognosis, and offering insights into the underlying molecular mechanisms.

SA72. Investigating the Relationship Between Apolipoprotein E (APOE) and Auditory Abilities

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Category: Genetics B: General

Background: Apolipoprotein E (Apo-E) is a protein that facilitates transport and metabolism of lipids as well as neuronal maintenance and repair. The APOE gene has three alleles, ε2, ε3, and ε4 with six possible permutations. The ε4 allele has been previously shown to be associated with a higher risk for Alzheimer's Disease (AD). In animal models, APOE knock-out mice have significant sensorineural hearing loss (Ma et al, 2022), suggesting a common mechanism linking hearing loss and AD. While human studies have reported no differences in behavioral hearing thresholds between APOE ε4 carriers and non-carriers (Yuka et al, 2019), others have reported longer latencies of P300 cortical responses in APOE ε4 carriers compared to non-carriers (Pedroso et al, 2020). Here we investigated the relationship between APOE ε4 genotype and behavioral auditory abilities.
Methods: We conducted a retrospective analysis of auditory and genomic data on 114 adults between the age of 63 and 98 years. The participants were enrolled in community-based studies through the Rush Alzheimer's Disease Center and consisted of similar numbers of Black/African American (n = 51) and White/Caucasians (n = 63) but primarily women (n = 100). Hearing was assessed using pure tone audiometry, and a four-frequency pure-tone average (PTA) was calculated for better-hearing ear. Speech in noise ability was assessed using the Quick Speech in Noise (QuickSIN) test. Two additional tests of spectral pattern discrimination were performed: ripple-phase discrimination and frequency-modulated signal discrimination in noise (Sheft et al, 2015).
Results: Overall, we found that having at least one copy of ε4 (n = 19) was associated with slightly better hearing abilities across all measures, whereas at least one copy of ε2 (n = 16) was associated with overall poorer hearing abilities ($F(1,33) = 8.685$, $p = 0.006$). In a small subgroup with both ε2 and ε4 alleles (n = 5), protective effect of ε4 on hearing persisted across all but one measure (frequency-modulated tone discrimination in noise).
Conclusions: APOE ε4 allele was associated with better performance on all hearing tasks in our sample. Future research is needed to understand the mechanism of action for Apo-E on hearing and auditory processing.

SA73. Success of Targeted Sequencing in the Search for Genetic Causes of Usher Syndrome Type 2

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Category: Genetics B: General

Background: Usher syndrome is one of the most common rare diseases in which both hearing impairment and retinitis pigmentosa coexist. Currently, four types of Usher syndrome are known. They are genetically heterogenous and clinically characterized based on the age of hearing loss and retinitis pigmentosa diagnosis, the degree of hearing loss and the presence of vestibular dysfunction. The aim of the study was to characterize the genetic background of Usher syndrome type 2 (USH2) in a group of Polish patients.
Methods: A total of 55 patients with a clinical diagnosis of USH2 were recruited to the study. The DNA was isolated from peripheral blood and genetic testing was performed using three different methods: real-time genotyping with TaqMan probes, high-throughput sequencing of the USH2A gene, and a panel of 237 hearing-related genes. Bioinformatic and expert analysis focused on the search for single nucleotide variants (SNVs) and copy number variants (CNVs). Segregation analysis was performed using Sanger sequencing and quantitative real-time PCR. Selected novel variants probably affecting splicing were tested using minigene assay.
Results: The cause of USH2 was identified in all patients. In 98% (54/55) of the individuals, causative variants were located in the USH2A gene. In one patient (2%; 1/55), a new homozygous terminating variant in the ADGRV1 gene was identified. In the USH2A gene, 42 different genetic variants were identified (28 known and 14 novel). A total of 74% (31/42) of the variants were deleterious. The most frequently identified genetic cause of USH2 was c.11864G GREATER THAN A (p.Trp3955Ter), present in 29 of the studied alleles. Deletions of exons 22-24 (17 alleles) and 10-11 (8 alleles) of the USH2A gene also played a significant role in USH2 development.
Conclusions: The obtained results characterize the mutation profile responsible for USH2 development in Polish patients. Genetic testing of USH2 patients should be based on high-throughput tests that enable simultaneous identification of SNVs and CNVs. The gathered data can serve as a starting point for further genotype-phenotype association analyses and may, in the future, identify patient groups that could benefit from developing molecular and cellular therapies.
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SA74. Investigation of the Regulation of Innate Immunity via Modulating Cochlin and Lccl Domain Peptide

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Category: Immunology

Background: Hearing loss is a common sensory disorder, and more than half of congenital or childhood-onset cases are linked to genetic factors, including mutations in the COCH gene.

COCH, highly expressed in the cochlea, encodes the cochlin protein, which is critical for auditory function. Mutations in cochlin, associated with DFNA9, can impair auditory signal transmission. Cochlin contains the LCCL domain, involved in immune responses such as bacterial entrapment and macrophage recruitment. Research suggests that treating macrophages with LCCL can promote M1 macrophage polarization, which triggers inflammatory responses through cytokine production. Genes like Ticam2 and CD40 play key roles in macrophage differentiation and inflammation, making cochlin's interaction with M1 macrophages relevant for understanding and potentially treating inflammatory and immune-related diseases.
Methods: ELISA was conducted to investigate the differences in cytokine secretion between Raw 264.7 cells treated only with PAO1 and those treated together with LccL. RNA sequencing revealed that the NF-kB signaling pathway was increased after 24 hours of LccL treatment. Ticam2 and CD40 genes, which are involved in M1 polarization, were targeted to conduct qPCR, which showed an increase in Ticam2 and CD40 genes in cells treated with LccL compared to the control group. Western blot verified that Ticam2 increased significantly, and Flow cytometry analysis demonstrated that CD40, CD64, CD68, and iNOS were increased.
Results: When RAW 264.7 cells are stimulated with LccL and cLccL, Relative Exp of the target genes increase significantly compared to the control group. Additionally, Western blot verified the increase in the ticam2 gene, while Flow cytometry revealed that target probes increased as well
Conclusions: Based on the observation, Raw 264.7 cells stimulated by LccL and cLccL induces M1 Polarization

SA75. Hair Cell Dysfunction Promotes Cochlear Inflammation Independent of Trauma

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Category: Immunology

Background: Cochlear inflammation has been associated with various hearing loss disorders such as age-related hearing loss and noise-induced hearing loss. In these disorders, the cochlear response is marked by increased immune cell numbers either by proliferation of resident macrophages or infiltration of monocytes to the site of damage. However, the functional cues that lead to the activation of the inner ear immune system are not clearly elucidated. In this study, we investigated the possible role sensory hair cells play in priming the inner ear immune system to respond to damaging stimuli.
Methods: Using two mouse models of sensory hair cell dysfunction, we evaluated the distribution and morphology of tissue-resident macrophages in the young adult cochlea. In our first model, outer hair cells lack oncomodulin (OCM), an important calcium buffering protein associated with hearing loss. In our second model, MYO7A, a key component of the mechanotransduction complex is absent which compromises auditory function. Distortion product otoacoustic emissions and auditory brainstem responses were used as measures of hearing thresholds. Macrophage populations were identified by positive immunofluorescence against the pan-leukocyte marker, CD45, and the macrophage specific marker, Iba1.

Results: In Ocm knock-out (Ocm-/-) mice, the number of macrophages across all frequency regions was greater than in age-matched Ocm wild-type (Ocm+/+) mice. Although the number of macrophages was elevated in Ocm-/- mice, there was no observable damage to the cochlea in the form of OHC loss or loss of synapses. In mice without MYO7A (Myo7afl/fl Myo15 cre+/-) there was no clear difference in the distribution of macrophages, prior to elevated thresholds compared to age-matched control (Myo7afl/fl) mice. However, at the onset of hearing loss, there was an increase in the population of macrophages. To determine if there were any morphological changes in macrophages, single-cell analysis for shape descriptors such as cell size and ramification was performed using a Fiji-based algorithm. In both mouse models, macrophages presented with a ramified morphology and varying sizes.
Conclusions: Our findings suggest that hair cell dysfunction, and not hair cell loss, leads to changes in the immune system of the cochlea. These changes may influence the cochlea's ability to respond to damage in the form of aging or trauma. However, the consequences of this altered immune state are unknown. Further research will explore the activation states of macrophages, as well as other inflammatory mediators in our models of hair cell dysfunction, and their potential influence on the extent of damage to sensory structures in the inner ear after trauma.

SA76. Longitudinal Study of Otoacoustic Emissions in a Rat Model of Age-Related Hearing Loss

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Category: Aging

Background: As an early objective biomarker of subclinical hearing loss and cognitive dysfunction, outer hair cell (OHC) dysfunction receives increasing attention in age-related hearing loss (ARHL), speech discrimination and early onset dementia. ARHL progression has previously been reported in standard Wistar rats, including characterization of OHC function but limited to only non-linear distortion-product otoacoustic emissions (DPOAEs). Linear reflection-type stimulus-frequency otoacoustic emissions (SFOAEs) have not yet been established for this species and may provide additional information about cochlear amplification, tuning and onset of hearing loss. Based on human studies, inclusion of both OAE types in cochlear assessment may provide complementary information. Here we report a longitudinal study of male Wistar rats from 6 to 12 months of age, with an objective assessment of cochlear function using both swept-tone stimulus DPOAE and SFOAE measurements.
Methods: Thirteen male Wistar rats underwent monthly unilateral audiometric characterization in a soundproof chamber from 6 to 12 months: ABR (8/16/24/32 kHz, 90-10 dB SPL in 5 dB steps, closed field configuration), swept-tone stimulus OAEs from 4 to 42 kHz Including high and low level DPOAEs as well as SFOAEs. For DPOAEs parameters were: f2/f1 ratio = 1.2, L1/L2 = 80/70 and 50/40 dB SPL, for SFOAEs probe/suppressor level were 50/65 dB SPL, fs/fp ratio = 1.1.
Results: At 6 months, animals demonstrated normal hearing with ABR thresholds from 19.2□0.8 to 22.5.8□1.7 dB SPL between 8-32 kHz. High level DPOAEs showed mean amplitudes from 13.7□1.1 to 33.0□1.6 dB SPL between 4-42 kHz, low level DPOAEs amplitudes ranged between

-8.4□3.6 and 18.0□0.9 dB SPL. Half-octave averaged SFOAE amplitudes ranged from 2.7□2.0 to 10.3□1.0 dB SPL. Only measurements with SNR GREATER THAN 6 dB were included.
Whereas ABR threshold shifts increased statistically significantly for 8, 16 and 32 kHz frequencies from 12 months, DPOAE amplitudes significantly decreased across the 4-32 kHz frequency range already from 11 months and even 10 months for low level DPOAEs at 8 kHz. SFOAE amplitudes also showed statistically significant losses from 11 months for 5, 7 and 20 kHz center frequencies.

Conclusions: These data confirm that SFOAEs and DPOAEs can be measured consistently in Wistar rats, offering a higher sensitivity for detection of the hearing loss onset with aging, compared to ABR thresholds.
Furthermore, in this model, the joint-OAE profile of DPOAEs and SFOAEs seems in accordance with published human data where SFOAE amplitudes appear to be more preserved that DPOAEs with aging.

SA77. Multiomic Analysis Highlights an Aberrant Gut-Metabolome-Proteome Network in Mice with Age-Related Hearing Loss

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Category: Aging

Background: Age-related hearing loss (ARHL) is associated with high incidence rates, ambiguous pathogenesis and poor clinical efficacy. Recently, an increasing number of studies have suggested that hearing loss is associated with dysbiosis of the gut microflora, cochlear metabolites and protein regulation in the inner ear. However, the potential interactions among these three factors are still unknown.
Methods: We used the ARHL model here. Gut microbiota compositions in decal samples, metabolism and protein molecules (in cochlear samples) were detected comprehensively.
Results: (1) We provide the first comprehensive analysis of the structural and hearing threshold features of C57BL/6J mice with different ages and hearing levels.
(2) We revealed for the first time the differences in the composition of the gut microbiota among the 3 groups; the richness or diversity of the gut microbiota decreased, and the F/B ratio increased, with age and hearing level.

(3) We performed, for the first time, an integrated correlation analysis of the metagenomic, metabolomic and proteomic data, and numerous “bacterium–metabolite–protein” correlation pairs were identified that may act as important mediators in ARHL.

Conclusions: In summary, this comprehensive study employing metagenomics, metabolomics, and proteomics elucidated the intricate interplay among gut microflora dysbiosis, cochlear metabolites, and protein regulation in the inner ear, including multiple correlated "bacterium–metabolite–protein" interactions underlying the pathogenesis of ARHL. Our findings provide a robust foundation for further exploration of novel diagnostic tools, population-wide screening strategies, and precision-based therapeutic interventions for ARHL.

SA78. Morphological Changes Associated With Aging in the Mammalian Vestibular System

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Category: Aging

Background: The mammalian vestibular system quickly and adaptably processes gravitational forces, head movements, and accelerations. It is also crucial for maintaining balance and enabling safe movement in one's surroundings. Vestibular function has been found to deteriorate within aging humans and rodents, yet the underlying causes remain unclear (Bergstrom, 1972; Tung, et al, 2014; Huang, et al, 2022). This increased likelihood of vestibular dysfunction as humans age plays a substantial role in falls in the elderly, often leading to diminished quality of life or death (Gananca, et al, 2006; Agrawal, et al, 2009).
Methods: To identify the underlying causes of vestibular dysfunction associated with aging, we quantified morphological changes between mature (1-3 month) and aged (36-40 month) gerbils. We used immunofluorescence, tissue clearing, and 2-photon microscopy to visualize morphological differences. The entire temporal bone was dissected and decalcified, instead of dissecting individual vestibular organs, effectively minimizing tissue damage during dissection. We used a solvent-based ethyl cinnamate clearing method, found previously to successfully clear cochleae encased in the temporal bone (Brody, et al, 2020). Our goal was to quantify the number of hair cells, both Type I and Type II, and synaptic density changes within the epithelia of the otolithic and macular vestibular organs of mature and aged gerbils. We used Imaris v10.2 to create an automated analysis, involving machine-learning algorithms to quantify hair cell counts and synaptic density.
Results: Preliminary data indicate a decrease in hair cell density in the central zone and parts of the medial peripheral zone of the saccular organ in aged gerbils compared to mature ones (n=3, with 2 aged and 1 mature). Additionally, it appears that there is reduction in supporting cell density in those same regions. The volume of the saccular organ was also found to decrease with age.
Conclusions: More organs will need to be examined before determining if these differences hold significance. Our study will aid in a deeper understanding of the morphological changes associated with aging in the mammalian vestibular system.

SA79. Vestibular-Associated Shape Changes in the Prefrontal Cortex Depend on Cognitive Ability

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Category: Aging

Background: The role of brain structure in linking peripheral vestibular dysfunction to cognitive deficits, which contribute to daily living challenges in older adults, remains unclear [1,2]. Structural abnormalities have been observed in the frontal and sensorimotor cortices—two crucial nodes in the vestibular cognitive network [3]— in adults with vestibular dysfunction [4,5,6,7].

However, the evidence is inconsistent, likely due to limitations in brain mapping techniques, small sample sizes, or individual heterogeneity (e.g. multisensory function, health status, brain plasticity). To address these gaps, we examine the relationship between age-related vestibular dysfunction and the morphology of the frontal and sensorimotor cortices using diffeomorphometry, accounting for age, intracranial volume, sex, and cognitive ability (executive and spatial).

Methods: Data from 118 participants aged 60+ from the Baltimore Longitudinal Study of Aging, who underwent end organ-specific vestibular testing (cVEMP for the saccule and oVEMP for the utricle), cognitive testing, and T1-weighted MRI scanning on the same visit, were analyzed. MRI scans were segmented, surface meshes were generated, and shape descriptors (surface expansion/contraction) were estimated using the MRICloud pipeline [8,9]. Cognitive tests of executive function included the Forward and Backward Digit Span, Trail-Making Test Part B, Category and Letter Fluency, and Clock Drawing tests. Spatial cognitive tests included the Card Rotations, Benton Visual Retention, Trail-Making Parts A and B, and Purdue Pegboard tests. Executive and spatial cognitive functions were age-corrected and classified based on DSM-V criteria as Impaired ($\geq 1SD$ below average in ≥2 domains), Average (1SD GREATER THAN x GREATER THAN $-1SD$), or Above-Average ($\geq 1SD$ above average in ≥2 domains). The effects of vestibular function on cognitive groups were assessed using ordinal logistic regression. Shape descriptors were linearly regressed on categorical vestibular variables and covariates. Hypotheses were tested using permutation testing.

Results: Strong trends were observed between lower saccular function and a higher odds of having higher executive ability and between lower utricular function and a lower odds likelihood of having higher executive ability (p_perm LESS THAN 0.1). However, these trends did not persist when controlling for multisensory function, diabetes, hypertension, or smoking status (p_perm GREATER THAN 0.1). Otolith function and cognitive ability (spatial and executive) jointly influence the shape of the left posterior middle frontal gyrus (MFG) (p_perm LESS THAN 0.05). Utricular function and executive ability together affected the shape of the left dorsal prefrontal cortex MFG (MFG_DPFC) (p_perm LESS THAN 0.05). Intact otolith responses were linked to less compression or expansion, particularly at lower cognitive levels. As cognitive performance improved, compression increased in the left posterior MFG, especially in individuals with absent otolith responses. In contrast, the left MFG_DPFC

expanded with absent utricular function and above-average executive ability.

Conclusions: Brain plasticity or compensation may help maintain cognitive abilities as vestibular function declines with aging. Future longitudinal studies will need to determine whether peripheral vestibular dysfunction directly causes brain structure abnormalities and cognitive deficits.

SA80. Age-Related Alteration of Neural Correlates of Tone-In-Noise Detection in the Auditory Midbrain

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Category: Aging

Background: Age-related hearing loss, clinically termed presbycusis, is a progressive sensorineural hearing deterioration that is one of the three most prevalent chronic medical conditions of our elderly, impacting one-third of the global population over 65 years old. Furthermore, our auditory system is perpetually confronted with the challenge of understanding speech in the presence of background noise, or the "cocktail party problem". The loss of peripheral inputs and senescence-related alterations of central neurotransmission lead to decreased activity driving neurons in the inferior colliculus (IC), a major midbrain convergence site critical for processing complex sounds such as speech. However, how aging affects hearing in noisy environments at the level of neural encoding within the IC remains largely unresolved. To investigate this, we measured signal-in-noise detection in the IC of young and old CBA/CaJ mice.
Methods: We used in vivo extra-cellular electrophysiology to measure the responses of neuronal populations in the in the central nucleus of the IC of 10 young (5-10 months, 633 units) and 14 old (22-24 months, 1,014 units) CBA/CaJ mice while listening to tones in noisy backgrounds. The tone-in-noise stimulus consisted of 50 ms tone bursts at 8, 12, 16, 20, or 24 kHz and were presented at pseudo-random signal-to-noise ratios above 40 or 60 dB SPL wideband background noises. Tone detection was quantified via a d′ sensitivity index and characterized as either excitatory or inhibitory and temporally categorized into onset or sustained responses. Additionally, the IC was mapped and receptive fields were obtained to determine the characteristic frequency and minimum threshold of each unit.
Results: Response patterns determined via peri-stimulus time histograms were categorized into onset and sustained types based on a unit's response to a tone-in-quiet. Overall, there were 4.7% more onset responses in the old group compared to the young group (53.1% vs. 48.4%). Additionally, there were 6.2% more onset responses relative to sustained within the old group (53.1% vs. 46.9%) compared to 3.2% less onset responses relative to sustained in the young group (48.4% vs. 51.6%). Young mice had an increased proportion of tone detection and detection strength between both response patterns across the majority of frequency and SNR conditions. Two types of sustained patterns, excitatory and inhibitory, were observed in both the young and old age groups. However, we found that units in old mice which were robust tone detectors showed larger inhibitory-sustained responses to tones-in-noise.

Conclusions: Our findings suggest that the IC contains distinct subpopulations of neurons that preferentially encode different stimulus features and that aging selectively alters certain subpopulations. Future work determining the effectiveness of exposure to an enhanced acoustic environment containing signals-in-noise could offer a therapeutic strategy to ameliorate age-related deficits in signal in noise detection.

SA81. Memory Encoding for Middle-Age and Older Adults During Word Identification in Noise

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Category: Aging

Background: Remembering speech in noisy backgrounds can be challenging, particularly for middle-age and older adults. Previous studies identified medial temporal and frontal brain regions associated with successful memory encoding, but it is unclear if these putative neural systems for memory encoding are diminished with age and could explain poorer memory for words in noise among older adults. The current study measured brain activity during a word-identification-in-noise task that was associated with delayed memory performance for middle-age to older adults. Voicing and pitch manipulations for each word were used to modulate listening difficulty and memory.

Methods: Participants were a sample of 42 middle-age to older adults (30 females, 50-80 years of age) with normal hearing up to moderate hearing loss. First, participants performed a word-identification-in-noise task (encoding) during fMRI scanning, which presented speech at 88 dB SPL in speech-shaped noise (+15 dB signal-to-noise ratio). The task difficulty was manipulated by presenting words with 1) initial consonants /b/ (voiced) or /p/ (voiceless) and 2) increased or decreased fundamental frequency (i.e., pitch). After the fMRI task, participants were provided a list of 24 words and were instructed to select the words they remembered hearing from the identification task (50% were foils). Significant fMRI results were defined with combined p LESS THAN 0.01 statistic and Family-Wise Error corrected p LESS THAN 0.05 cluster extent thresholds. A-prime (bias-adjusted memory accuracy) and response bias were both estimated based on hits and false alarms.

Results: No significant associations between A-primes and age or hearing loss were observed [ps GREATER THAN 0.10]. Identification of /p/ words was significantly better than /b/ words [$t(41) = 10.30$, p LESS THAN 0.001], but memory A-primes were not significantly different between voicing conditions [$p = 0.89$]. The response bias parameters indicated that /b/ words were more likely to be reported as remembered than /p/ words [$t(41) = 3.48$, $p = 0.001$]. There were no significant pitch-related differences in A-prime or response bias [ps GREATER THAN 0.11]. Significantly elevated brain activity in bilateral hippocampus, basal forebrain, and precuneus was observed during the encoding task for later successfully remembered words relative to the other words. Significantly lower activity in the bilateral inferior frontal sulcus was also observed for correctly identified words that were later not remembered, compared to the other words.

Conclusions: Successful memory encoding for words in noise appeared to benefit from activity within a network of brain regions previously linked to recall. Additionally, lower activity in frontal brain regions was linked to unsuccessful memory encoding, suggesting that attention-related brain

regions were also important to memory for words in noise. Together, these findings suggest that memory for speech in challenging listening conditions among middle-age to older adults is supported by medial temporal and frontal regions.

SA82. Differential Age-Related Decline in Central Auditory Function in Adults Living With HIV

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Category: Aging

Background: The trajectory of central auditory function with age remains poorly understood. Identifying individuals who show accelerated central auditory aging, particularly on tests involving speech perception in noise, may offer valuable markers for potential cognitive decline. These markers could be crucial in guiding early interventions, especially for populations at higher risk of auditory or neurocognitive deficits, such as those living with HIV. This study examines the longitudinal trajectory of central auditory performance in adults living with and without HIV in Dar es Salaam, Tanzania, to assess whether faster are-related declines in central auditory function are associated with HIV status.

Methods: A cohort of 442 participants (106 HIV-negative and 336 HIV-positive), each with at least four visits and no peripheral hearing loss (GREATER THAN 25dB from 500-4000 Hz), was analyzed. All adults living with HIV were actively taking antiretroviral medications. The Triple Digit Test (TDT) was used as the primary measure of central auditory function across age. The longitudinal performance on the TDT was analyzed, by calculating the slope of the TDT/time relationship for each participant omitting the first visit. A linear mixed-effects model was employed to assess the impact of HIV status, age at the start of the study, and the interaction between these factors on TDT trajectory over time. Random effects were included to account for individual variation.

Results: In the people without HIV (PLWOH), the TDT slope decreased significantly with age at study entry indicating that older individuals in the PLWOH tended to improve their TDT performance while in the study (p LESS THAN .001). The interaction term describing the relationship between age and TDT slope between people living with and without HIV (PLWH/PLWOH) was very significant (p LESS THAN 0.001). The PLWH group showed a worsening in TDT slope with increasing age at study entry. This suggests HIV alters the progression of central auditory test performance with age.

Conclusions: Speech-in-noise performance seemed to improve over time in the older PLWOH, which may represent increased attention on the test with older individuals. Worsening in central auditory test performance during the study was seen in the older PLWH. This study supports the existence of a differential aging effect on central auditory function in people living with HIV. Further research is needed to determine whether central auditory tests can serve as predictors of HIV related deficits such a neurocognitive dysfunction.

SA83. Reduced Neural Adaptation for Encoding Complex Sounds in the Auditory Cortex With Aging

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Category: Aging

Background: Efficient auditory processing is crucial for effective communication. How the brain rapidly handles complex sensory mixtures and parses information has been one of the most sought questions in auditory neuroscience. The auditory cortex (AC) is known as a critical hub that enables stream segregation, ultimately helping us focus on a specific sound stream that we aim to attend. A sparse representation of AC neurons indicates that only a small group of neurons are engaged to process sensory input with reduced energy. Rather than all AC neurons being activated for every incoming sound input, only a group of relevant neurons is involved, and they would rapidly adapt to recurring sounds. However, with aging, such central auditory pathway processing appears to degrade, resulting in a diminished ability to segregate foreground and background sensory inputs. This leads to difficulty efficiently communicating in noisy environments. Importantly, this decline is not simply because peripheral hearing degradation. Even with intact peripheral hearing, central auditory processing degradation occurs.
Methods: Here, we test how AC neurons behave differently between young adult and aging populations during sound encoding, a critical phase that underlies efficient auditory processing. We focus specifically on changes in neural adaptation to re-occurring sounds due to aging. To mimic naturalistic acoustic environment for encoding newly presented sounds, we play a series of randomly generated complex spectrotemporal patterns (dynamic random chords; DRC) with a target, fixed DRC intermittently re-occurring to awake aging mice (~24 months). We use 2-photon Ca2+ imaging to trace response changes in a population of AC neurons. We target three different subfields of AC: Layer 4 of primary field (A1 L4), Layer 2/3 of A1, and Layer 2/3 of A2.
Results: In aging mice, neurons in subfields of the AC showed limited response amplitude decrease, an index of neural adaptation, to re-occurring sounds compared to other random sounds. Specifically, this amplitude decrease is significantly less pronounced than young adult mice. Higher correlation across neurons from aging population was also observed compared to young adults.
Conclusions: Out data suggest that the ability of neurons to rapidly adapt to re-occurring sounds deteriorates with aging, leading to diminished neural adaptation. The data also suggests that aging disrupts distinctive network dynamics in AC, further complicating efficient auditory processing.

SA84. Myelin Degeneration in the Aging Human Auditory System

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Category: Aging

Background: A defining feature of the auditory system is the extraordinary temporal precision that is crucial for speech understanding in challenging listening environments. However, deficits

in this temporal precision are one of the hallmarks of auditory aging. Myelin plays a fundamental role in ensuring temporal precision, particularly under conditions where temporal precision is especially important (e.g., speech recognition, binaural sound localization). To examine the effects of age and hearing loss, we first obtained traditional diffusion kurtosis imaging (DKI) metrics to characterize myelin in a large cohort of older adults. However, DKI metrics assess white matter (WM) microstructure and are sensitive to but not specific to myelin, and values may be influenced by non-myelin origins (e.g., fiber dispersion, inflammation, edema). Therefore, in a preliminary data set, we also used a novel imaging technique, myelin water fraction (MWF) imaging, to quantify myelin in the auditory brainstem (inferior colliculus, IC; medial geniculate body, MGB), and auditory cortex. MWF can detect and quantify changes in myelin not observable with conventional diffusion imaging. MWF values decreases with expected myelin deficits in aging and neurodegeneration. We predicted that age-related myelin degeneration would contribute to longer response latencies and decreased synchrony of neural responses measured from brainstem and cortex in response to auditory stimuli, and that these effects will be greater with temporally demanding stimuli (speech in noise (SIN), rapid clicks) than with simple stimuli (speech in quiet, slow rate clicks).

Methods: We used DKI to characterize WM microstructure in a large cohort of younger (N=53, 18-30 years) and older adults (N=103, 54-89+ years). In a subset of participants, we acquired WMF using the ViSTa sequence. Neural response latencies and phase-locking from brainstem (Wave V) and cortex were measured in response to clicks presented at various rates (slow and fast rate), and at the cortex in response to speech presented in quiet and in noise. Analyses examined the effects of age and hearing loss on DKI metrics, and associations between subcortical and cortical neural responses and MWF.

Results: DKI metrics changed with age and hearing loss in a pattern consistent with increased atrophy and myelin degeneration in auditory cortex. In older adults, decreased WMF in the brainstem was associated with poorer Wave V neural synchrony, and these associations were stronger at the faster click rate. Similarly, decreased auditory cortex MWF was associated with longer speech-evoked response latencies.

Conclusions: These preliminary data support our hypothesis that myelin degeneration occurs throughout the auditory system of older adults and contributes to neural dysynchrony and poorer temporal encoding.

SA85. Neural and Behavioral Changes from Auditory-Cognitive Speech-In-Noise Training in Older Adults

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Category: Aging

Background: Listening in noisy environments is a common challenge for older adults, even those with clinically normal hearing. Moreover, compared to younger adults, older adults report higher listening effort when listening to competing speakers. This suggests that auditory scene segregation is affected by more than just hearing loss and may also be influenced by cognitive processing, both of which decline with age. Recent research has shown that auditory-cognitive

training may be more beneficial for speech-in-noise perception than auditory-only or cognitive-only training programs alone. In this study, we aim to determine if auditory-cognitive training can improve speech-in-noise listening in normal-hearing, older adults by measures of neural and behavioral responses.
Methods: This study collected behavioral and neural data from older adults pre- and post-training, along with younger adults who did not undergo training. Magnetoencephalography (MEG) was used to record neural responses while subjects listened to narrated audiobooks under four different noise conditions. For each audio presentation, we logged listener-reported intelligibility and listening effort. All neural data was analyzed using encoding and decoding models using the temporal response function (TRF) framework. Additional behavioral data obtained include various tasks of working memory and audio segregation, such as stochastic figure-ground ("tone cloud") detection, the quick speech in noise test (QuickSIN), a speech perception in noise task (SPIN), and tests of working memory (RSPAN and N-back).
Results: Preliminary behavioral results for older adults show a post-training reduction in listening effort with competing speakers. Preliminary TRF results show a post-training increase in the late (M400) response to word onsets, which has been linked to enhanced intelligibility. Additionally, preliminary neural results for stimulus reconstruction features show a greater contrast between foreground and background speech features post-training—an indication that maladaptive overcompensation typically observed in older adults may be decreased. Critically, one pre-training behavioral measure may predict the level of potential neuroplasticity benefit, performance in a non-speech auditory scene segregation task: lower pre-training performance was associated with larger post-training foreground-background differences in neural (stimulus reconstruction) measures.
Conclusions: These results are promising for the incorporation of auditory-cognitive training in older adults who experience difficulty understanding speech in noise.

SA86. Economic and Social Burden of Tinnitus in France (2021-2022)

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Category: Tinnitus

Background: This study aims to evaluate the economic burden associated with the management of tinnitus and the potential therapeutic wandering, as well as to estimate the professional and social impact of tinnitus on individuals and their families.
Methods: A comprehensive survey consisting of approximately 137 questions was conducted with 1,563 respondents. The demographic profile included an average age of 55 years (±14.2), with 55.7% being female. The survey covered various aspects such as professional status, socio-economic background, and the impact of tinnitus on daily life.
Results: The study revealed that 55.3% of respondents were professionally active, while 31.3% were retired. Among those on sick leave, nearly half (44.9%) attributed their leave to tinnitus. Tinnitus was medically validated in 68.7% of cases, with identified causes including noise trauma

(12%) and other unspecified causes (88%). The impact on professional life was significant, with 16% of respondents reporting at least one day of work absence due to tinnitus, with a median of 15 days. Additionally, 11.4% reported job changes or adjustments, and 19.2% received disability recognition. The economic burden was substantial, with the average annual cost per patient for consultations and examinations being €840.75, of which €296.75 was covered by social security. Equipment costs averaged €1,512.75 annually, with €382.15 reimbursed. The total out-of-pocket expense per patient was estimated at €1,079.85 annually. Nationally, with an estimated 16 million tinnitus sufferers in France, the annual economic burden could reach up to €12 billion for the Healthcare part alone and above €24 billion adding the loss of productivity and sick leaves at work.
Conclusions: Tinnitus imposes a significant economic and social burden on individuals and the healthcare system in France. Effective management strategies and increased awareness are essential to mitigate these impacts.

SA87. Use of Opm-Meg for Auditory Research

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Category: Tinnitus

Background: Acquired auditory processing disorders including age dependent hearing loss, speech discrimination deficits, tinnitus or hyperacusis, require a personalized diagnosis to assign the individual cause within the auditory hierarchy to either the periphery, subcortical or distinct cortical or cortico-fugal neuronal dysfunctions. The well-functioning feedforward and feedback PV-IN network is an essential precondition for temporal intracortical network function in audition that above all senses relies on high speed of information flow (Zajac IT and Nettelbeck T, 2018). We hypothesize disease-specific deficits in temporal intracortical network function in auditory circuits. Therefore, the diagnostic of those should have a special significance.
Methods: We used time-sensitive MEG-OPM measurements and aimed to study different auditory stimulus paradigms to detect fast auditory processing in different groups of tinnitus with and without hyperacusis or presbycusis.
Results: We expect this method to become an efficient diagnostic strategy to fathom peripheral or central contribution of the distinct auditory impairments in the future to improve individualized targeted interventional therapies. Here we will present preliminary results demonstrating the usability and function of the OPM-MEG for hearing research.
Conclusions: The OPM offers new possibilities in the diagnosis of auditory disorders such as tinnitus.

SA88. Modified Tinnitus Relieving Sound Therapy for Chronic Subjective Tinnitus: Results of a Prospective Controlled Study

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Category: Tinnitus

Background: Tinnitus is a major health issue, yet there are currently no tinnitus elimination treatments for chronic subjective tinnitus. Acoustic therapy plays an increasingly important role in tinnitus treatment, especially personalized acoustic therapy. With the application of smartphones, personalized acoustic stimulation combined with smartphone applications will be more conducive to the individualized treatment and management of tinnitus patients. To evaluate the efficacy of a new personalized approach known as the modified tinnitus relieving sound (MTRS) for tinnitus treatment and explore the factors that may influence its therapeutic effect.

Methods: Patients with subjective tinnitus were enrolled in this study from 14 July 2020 to 24 May 2021, in the Tinnitus Specialist Clinic of Eye and ENT Hospital, Fudan University, Shanghai, China. The MTRS was generated according to the matched tinnitus pitch developed through our application, named the Fudan Tinnitus Relieving System (FTRS). A total of 107 participants were instructed to listen to the personalized MTRS for at least 2 h a day and other 77 participants to listen to the unmodified one. The patients who completed three consecutive months of assessment were included in the final analysis. Multi-dimensional assessment scales including tinnitus handicapped inventory (THI), hospital anxiety and distress scale (HADS), Athens insomnia scale (AIS), fear of tinnitus questionnaire (FTQ), and tinnitus catastrophizing scale (TCS) were used to evaluate the severity of tinnitus and the quality of life of patients with tinnitus from multiple dimensions. Statistical analysis of the results of the three follow-up visits with the baseline data allowed for an assessment of the efficacy of MTRS compared to the unmodified music (UM).

Results: Based on the results of the multi-dimensional assessment scale, after 3 months- of treatment, the MTRS-treated patients showed significant tinnitus relief compared to those from the UM group. In addition, the customized acoustic treatment effectiveness was positively correlated with total treatment time. The longer time the patients received customized acoustic therapy, the more noticeable the improvement in patients' subjective reports on the impact of tinnitus. As the experiment proceeded, post-intervention scores on each subscale, including THI, HADS, AIS, FTQ, and TCS, significantly and consistently declined compared to the baseline data. Patients with severe tinnitus before the clinical trial tended to have better acoustic treatment results.

Conclusions: Our study suggests that the modified tinnitus relieving sound is a new promising and non-invasive therapy for chronic tinnitus, and it can be delivered through mobile application to bring more convenience to tinnitus patients.

SA89. Place and Temporal Envelope Cues for Pitch, Music, and Emotion Perception with Cochlear Implants

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Category: Auditory Prostheses

Background: Emotion, music, and pitch cues are often poorly perceived in cochlear implant (CI) users. Two cues for pitch perception available to CI users are cochlear place of stimulation and temporal cues; temporal cues can be encoded either by pulse rate or by amplitude modulation (AM) of the pulse train. Previous studies have shown place of stimulation to be weighted more strongly than pulse rate for pitch perception. However, the weighting of place and AM for pitch perception in CI users has not yet been investigated. The goal of this study was to measure the relative weighting of these cues in bilateral CI users for pitch, musical contour, and spoken emotion perception.
Methods: Five bilateral CI users (4 females, 1 male; age range 14-33 years) were recruited. Electrode discrimination (place cue) and amplitude modulation (AM) rate discrimination (temporal envelope cue) were measured using direct stimulation in a 3-alternative forced choice, 2-up, 1-down adaptive procedure. Relative weighting of the two pitch cues was measured using a single-interval pitch scaling magnitude estimation procedure on a scale of 1 to 10. Stimuli were biphasic pulse trains presented to an apical, middle, or basal electrode at a 0, 75, 100, 150, 200, or 300 Hz AM rate. Spoken emotion identification and musical contour identification were assessed under three conditions: unprocessed, place-only, and temporal-only. Place- and temporal-only stimuli were created by use of a sine wave vocoder, specifically low-pass filtering temporal envelope cues to below 20 Hz for the place-only condition, and sending output only to a single middle electrode for the temporal-only condition.
Results: All participants showed better pitch discrimination with AM rate than electrode place, especially for AM rates below 150 Hz. In the pitch scaling task, weighting for AM rate was stronger than place. Preliminary findings from two participants suggest that spoken emotion and music identification are best in the unprocessed condition, and the temporal-only condition may be slightly better than the place-only condition, especially for lower F0s.
Conclusions: Weighting of temporal envelope cues could be stronger than place cues, especially for low AM rates and F0s. Future work will examine the role of temporal and place cues in real-world music and spoken emotion perception. This study was funded by a grant from House Institute Foundation and NIH STEMM-HEAR grant R25DC020698.

SA90. Frequency-Following Responses and Auditory Brainstem Responses in Individuals With a Vestibular Schwannoma

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Category: Hearing Loss: Consequences and Adaptation

Background: Vestibular schwannomas (VS) are benign tumors that develop from Schwann cells of the vestibulocochlear nerve and are commonly associated with progressive sensorineural hearing loss (SNHL). Prior research has indicated that auditory brainstem responses (ABRs) are altered in individuals with a VS, suggesting that ABRs may have high sensitivity for detecting these tumors and could serve as a potential screening tool. However, the impact of a VS on the frequency-following response (FFR), a valuable measure for studying (speech) sound processing, has not yet been explored.

Methods: Pure-tone audiometry, speech-in-noise tests, ABRs and FFRs are being conducted in 20 patients with VS and normal to moderately-severe SNHL. FFRs are recorded using a /dao/ stimulus and are presented at 60 dB SPL above the pure-tone average (PTA) or at the maximum comfortable loudness level if the initial intensity is uncomfortable. Stimuli are delivered monaurally in three blocks of 1,000 presentations per ear, allowing for a comparison between the ear affected by the VS and the contralateral control ear. The influence of the size of the schwannoma on the FFR parameters will also be investigated.
Results: To date, measurements have been completed on 18 subjects (mean age 50.85 years). Preliminary analysis indicates that the F0 response amplitude for both the /o/ segment and the entire /dao/ stimulus is reduced on the VS side compared to the contralateral side, though this difference is not statistically significant. When subjects are grouped by tumor size, no significant differences in any of the FFR parameters are observed in those with intracanalicular VS. However, in patients with larger VS extending beyond the internal auditory canal, the F0 response amplitude for both the /o/ segment (p LESS THAN 0.05) and the entire /dao/ stimulus (p LESS THAN 0.05) is significantly lower on the VS side compared to the contralateral side. This reduction is also present in the signal-to-noise ratio power (p LESS THAN 0.05 for both the /o/ segment as for the entire /dao/ stimulus). Additionally, pitch error is significantly higher on the VS side in this group (p LESS THAN 0.01), while pitch strength is significantly lower (p LESS THAN 0.01).
Conclusions: Preliminary results suggest that in subjects with larger VS, there is reduced ability to track the F0 of the stimulus on the side affected by the tumor compared to the contralateral side. By the time of the conference, we plan to present complete results from at least 20 subjects and conduct more in-depth analyses, including ABR analysis. In addition, a more detailed analysis of the influence of schwannoma size - according to the Koos classification - will be performed.

SA91. Hearing Aid Use and Speech Recognition in Older Adults: Preliminary Findings from a Six-Month Follow-Up

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Category: Hearing Loss: Consequences and Adaptation

Background: The benefits of using hearing aids vary substantially across users with age-related hearing loss. Whether some of this variability stems from individual differences in amplification-induced plasticity is unclear. In this ongoing longitudinal study, we examine the recognition of fast speech, speech in babble noise, and dichotic speech, with and without hearing-aid amplification, at three time points over a six-month period. We aim to assess changes in the effects of hearing aids over time in first-time hearing aid users compared to individuals with no hearing aids.
Methods: At present, 29 first-time hearing aid users (ages: 67 - 86, M = 77) have completed the study. The first phase was conducted shortly prior to hearing aid fitting, the second phase was conducted two to three months post-fitting, and the final phase six months post-fitting. Aided and unaided performance was assessed. We examined the relative contribution of phase, condition (aided and unaided), level of hearing loss, and their interactions to the perception of fast speech and speech in babble noise.

Results: In our preliminary analyses the recognition of fast speech was significantly and negatively influenced by hearing level (OR = 0.38) and by a complex interaction of amplification, test phase and hearing loss (OR = 0.73). This suggests that the contributions of both hearing and amplification to fast speech may change over time. It appears that the negative effect of hearing loss is partially offset by the use of hearing aids, but after six months of hearing aid use, amplification is more beneficial to listeners with milder levels of hearing loss. For speech in babble noise the effects of both hearing level (OR = 0.47) and amplification (OR = 1.79) were strong and depended on test phase. Whereas during the first phase the effects of amplification were particularly strong in listeners with poorer hearing, six months later they were similar across the range of hearing levels.
Conclusions: The contributions of hearing aids to speech recognition are complex and likely depend on the duration of hearing aid use and the severity of hearing loss.

SA92. Novel Stimulation Methods for Direct Intraneural Stimulation of the Auditory Nerve in Guinea Pig

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Category: Auditory Prostheses

Background: Auditory nerve implants (ANIs) offer the potential for lower stimulation thresholds and more focused activation of frequency regions compared to conventional cochlear implants (CIs). By improving the electrode-nerve interface and minimizing current spread, intraneural stimulation enables a greater number of independent frequency channels. This could lead to more precise sound encoding and better hearing experience. However, several challenges must be addressed before ANIs can be clinically implemented. The present work investigates the frequency response map activated by the ANI array, which can differ significantly in each individual case and demonstrates complex tonotopic organizations which must be accounted for. This research focuses on developing efficient stimulation strategies based on the frequency map, as well as coordinating pulse trains across multiple electrode sites.
Methods: A 2-shank 32-site electrode array (NeuroNexus) was placed along the tonotopic axis of the central nucleus of inferior colliculus (ICC) in ketamine-anesthetized guinea pigs to record multiunit activity. Responses to pure tones (1kHz to 40kHz, 0-70dB SPL) were recorded to generate tuning curves and validate accurate electrode placement. Next, a transcochlear surgical approach was performed to expose the modiolar portion of the auditory nerve. A single-shank 16-site edge electrode array (NeuroNexus) was inserted into the nerve bundle for stimulation. A charge-balanced, cathodic leading biphasic pulse (40μs/phase) was delivered from each site in random order. Current levels ranged from 0 μA to 120 μA, presented in randomized order at steps of 5uA. For bipolar stimulation, the pulse polarity (cathodic vs anodic) at each electrode was also varied. Additionally, current steering between two channels and dynamic activation of three stimulation sites were also tested.
Results: Frequency organization maps differed significantly across animals even when employing an identical surgical approach. Thresholds for monopolar intraneural stimulation were generally low, typically around 5-15μA. Bipolar stimulation required higher thresholds but resulted in much narrower activation patterns. Furthermore, the extent of threshold increase varied between

stimulation sites and also changed slightly depending on which of the two electrodes in the bipolar pair was used as the return electrode. For multi-site stimulation, activation patterns differed when using simultaneous stimulation vs. sequential (interleaved) pulses. Furthermore, it was possible to distribute the total current across multiple electrodes (instead of placing all current at one electrode) to achieve the same level of activation and reduce current spread.
Conclusions: Our findings confirm that ANIs offers the advantage of low thresholds and can be used for focused stimulation. Due to reduced channel interaction or greater channel independence, activation patterns are highly sensitive to the order and timing of stimulation when using multiple electrodes. This sensitivity must be considered when designing coding strategies for complex sounds. Additionally, the variation in frequency maps across animals highlights the need for individualized frequency mapping to optimize stimulation strategies.

SA93. Mechanical Influence of Acute Versus Chronic Cochlear Implantation in a Guinea Pig Model

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Category: Auditory Prostheses

Background: Residual hearing loss is a major issue impeding the effectiveness of the otherwise highly successful cochlear implant (CI). Hypothesized to be mechanical in origin, and due to the buildup of fibrosis and ossification in months to years following surgery, loss of residual hearing may be considered conductive in nature. However, the mechanical behavior of the site of residual hearing, the cochlear apex, has been studied little in the context of the CI.
In order to separate the influence of fibrosis and that of the CI alone, we compare the effects of acute and chronic cochlear implantation on the apical mechanical function of the guinea pig cochlear apex, using optical coherence tomography vibrometry (OCTV) and our minimally invasive surgical approach.

Methods: The cochleae of anesthetized guinea pigs were exposed, and a gold mirror was placed, permitting measurement of the transverse displacement of the apical 20% of the organ of Corti at three locations using a Thorlabs Telesto III spectral domain OCT microscope. For acute experiments, a non-functional CI (HL-08, Cochlear inc.) was introduced via an expanded round window approach, taking care not to move the preparation. For chronic experiments, a CI was introduced via the posterior bulla, and animals were housed for 8 weeks prior to OCTV experiments. A calibrated sound system was introduced into the ear canal and multitone stimuli were presented to produce iso-intensity displacement tuning curves for each location. Umbo responses were also collected. Measurements were made in living cochleae and following euthanasia. Measurements were made before and after the placement of the CI in the acute case, and after the CI was placed in the chronic case.
Results: The outcomes of acute and chronic implantation are varied, reflecting the clinical situation. A subset of chronically implanted animals showed conductive hearing loss, but preserved apical mechanical gain – the saturation of the amplifier was met at a higher sound pressure level than in unimplanted animals, and some implanted animals. However, amount of fibrosis was not necessarily predictive of apical function.

Preliminary analysis of mechanical data measured before, and immediately after CI surgery indicates that the CI alone can cause changes in apical organ of Corti mechanics. Unchanged, decreased and increased organ of Corti displacement was noted following CI. Middle ear function could also be influenced by the presence of the CI. Apical function was not dilapidated to the extent seen in some chronically implanted animals.

Conclusions: When compared to our prior analysis of amount of fibrosis versus middle ear function, the preliminary data from acutely implanted animals speaks to the hypothesis that residual hearing loss is primarily mechanical in nature and is mostly due to post-surgical scarring that increases intracochlear impedance.

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SA94. Quantifying Binaural Speech Fusion Using a Dichotic Formant, Vowel Identification Task in Children and Adults with Cochlear Implants

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Category: Auditory Prostheses

Background: The auditory system's ability to compare and integrate information across ears, known as binaural hearing, is critical for navigating complex acoustic environments. Binaural hearing allows a listener to distinguish a friend's voice from background noise in a crowded coffee shop, and identify where a car is coming from on a busy street. To access these functional benefits, the normal hearing (NH) auditory system analyzes the correlation of incoming signals at each ear to fuse sounds originating from the same source into a single percept; this is known as binaural fusion. Previous work has shown that cochlear implant (CI) users generally cannot access binaural hearing benefits to the same degree as NH listeners. This may be due to impaired binaural fusion stemming from pathological, surgical, and device related asymmetries that reduce interaural correlation. The goal of this study was to establish an ecologically valid measure of binaural speech fusion for CI users that is not dependent on their subjective perception of fusion.

Methods: To quantify binaural fusion, we employed a dichotic formant vowel identification task. Vowels are primarily identified by the first two formants, and previous studies have shown that NH listeners can identify vowels with alternate formants presented to each ear. This task requires effective binaural integration of formant information to correctly identify the vowel. Stimuli consisted of six vowels in an h-Vowel-d word context. Words were processed to preserve or remove specific formants (F), and participants were tested in five conditions: 1) Formants 1-4 presented bilaterally (Diotic F1-F4) to quantify baseline vowel recognition, 2) Formants 1-2 presented bilaterally (Diotic F1F2) to ensure that two formants were sufficient for good vowel recognition, 3) Formants 1 and 2 presented to opposite ears (Dichotic F1/F2), and 4/5) F1 or F2 presented bilaterally (Diotic F1 and Diotic F2) to ensure that vowels were not identifiable by a single formant alone. We hypothesized that, if listeners could effectively fuse formants 1 and 2 across ears, performance in condition three would be similar to condition two. Four participant groups were tested: NH adults, NH children, bilateral CI (BiCI) adults, and BiCI children.

Results: Results indicated good baseline vowel recognition in the Diotic F1-F4 condition for all groups. Performance in the Diotic F1F2 condition was similar to baseline, indicating that two formants were sufficient for good vowel recognition. Single formant conditions revealed that some vowels were identifiable by one formant alone; these were generally consistent across groups. Finally, all groups demonstrated some degree of fusion, but NH groups were able fully fuse and reach Diotic F1F2 performance, whereas BiCI groups did not. Confusion matrices and binaural unmasking results will also be presented.
Conclusions: Results indicate that reduced binaural hearing benefits in CI listeners may stem from impaired binaural fusion.

SA95. Biophysically Constrained Acoustic Models of Cochlear Implants Are Reliably Optimized Using Interactive Genetic Algorithms (iGA)

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Category: Auditory Prostheses

Background: Cochlear implant (CI) sound quality is distorted compared to normal hearing. This distortion may be related to cochleotopic mismatch in spectral tuning of healthy vs CI ears (Landsberger, Svirsky, Dorman), broad and asymmetrical tuning of CI electrodes (Arenberg et al. 2010), aberrant encoding of sound intensity (Zeng and Shannon 1994) and fine structure, and reduced spectral resolution. We are investigating the nature and extent of sound quality distortions by CIs by having single-sided deaf (SSD) cochlear implant users select an acoustic representation (delivered to their normal ear) that provides the best match to what they hear through the cochlear implant. In order to conduct these studies we also developed an interactive genetic algorithm for optimizing user-selected acoustic models.
Methods: An interactive graphical user interface and a genetic algorithm was developed for optimizing acoustic models. This combined software package was modified from a previous version developed for optimizing CI mapping (Lineaweaver and Wakefield 2011). Models of cochlear implant sound quality first replicated the sound processing of cochlear implant processors, and then output of each channel was used to modify several free parameters of output acoustic carriers. These parameters included cochleotopic mismatch, electrode tuning bandwidth, and type of acoustic carrier. The software initially randomizes a pool of acoustic models and the genetic algorithm uses a combination of principles of biological evolution such as gene crossover, selective insertion, and random mutation to evolve users' selections toward optimized ones. After optimizing acoustic models, double blind questionnaires were conducted to obtain ratings for the similarity between the acoustic model and the CI.
Results: We tested 18 SSD-CI human subjects and ran over 101 iterations of the GA. The average time to convergence for each model was less than 29 minutes. Acoustic models optimized with the GA were compared to a) models optimized using a method of adjustment procedure, and b) traditional acoustic models where the analysis and synthesis filters had the same center frequency and bandwidth. In all tested subjects, models optimized by the GA were deemed closer to the sound of the CI compared to both traditional and self-selected models optimized via method of

adjustment. Lastly, we found significant differences in acoustic models optimized across subjects in terms of cochleotopic mismatch, bandwidth, and acoustic carriers. Moreover, multiple repeated questionnaire ratings revealed significant preference for unique acoustic carriers over others.
Conclusions: Results reveal that acoustic models optimized across cochleotopic mismatch, bandwidth, and acoustic carrier closely match the sound of cochlear implants in SSD-CI subjects. Our interactive genetic algorithm enables a principled approach for optimizing acoustic models across a large parameter space. Similarly, modeling of single electrodes often enabled better rated models than those used in electroacoustic pitch matching (pure tones).

SA96. UmboMic: Fabrication, Design, and Fixation of an Implantable Middle Ear Microphone

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Category: Auditory Prostheses

Background: The UmboMic is a piezoelectric bimorph sensor implanted in the middle-ear to measure umbo motion. It serves as the microphone required to realize a totally-implantable cochlear implant (TICI). Here we present recent progress on its design, fabrication, testing and surgical fixation. This includes optimizing UmboMic geometry using analytical models, UmboMic fabrication from a polyvinylidene-fluoride (PVDF) substrate, testing UmboMic shielding against electromagnetic interference (EMI), and measuring UmboMic performance in a human cadaveric ear using the fixation system.
Methods: UmboMics are fabricated in the MIT.nano. Prototype fixation systems are manufactured by i.materialise and Sculpteo through laser powder-bed fusion of stainless steel and titanium. UmboMics are tested in electrically- and acoustically-isolated sound chambers at Mass Eye and Ear. During EMI testing, an UmboMic is exposed to a 500 Hz, 100 μT magnetic field, and a 500 Hz, 286 V/m electric field, per the World Health Organization's threshold for safe exposure. The ability of the fixation system to position the UmboMic is tested in human cadaveric temporal bones prepared via a mastoidectomy to access the middle-ear cavity. The UmboMic is modeled as an Euler-Bernoulli beam coupled to an umbo approximated as a spring-mass system. The analytical model is corroborated by finite-element analysis of the coupled UmboMic and umbo using COMSOL Multiphysics.
Results: Measurements of UmboMic linearity, equivalent input noise, and sensitivity are made with the UmboMic supported by the fixation system. The dynamic range of the UmboMic is between 35 dB SPL and 115 dB SPL. Experience with the current design indicates opportunities for improving the locking mechanism for ease of surgical insertion. Measurements show the portions of the arm closest to the sensor exhibit relative motion with respect to the anchor point of the fixation plate. EMI testing shows adequate shielding: 100 μT at 500 Hz leads to a 47 dB SPL sound while 286 V/m leads to a 52 dB SPL. UmboMic fabrication using a PVDF substrate is shown to be feasible, enabling completely-in-house fabrication. The analytical UmboMic model prescribes an optimal triangular UmboMic shape that maximizes UmboMic sensitivity and bandwidth when coupled to the umbo.

Conclusions: Future UmboMic development must consider different aspects of design and function. UmboMic design must consider how physiological differences between users impact UmboMic performance, and adjust dimensions accordingly. UmboMic fabrication must consider material choice to ensure biocompatibility. Once implanted, a well-designed fixation system should not degrade sensor performance. Finally, a thoroughly shielded UmboMic improves performance in the face of electric and magnetic fields. Next steps towards a viable microphone system for a TICI include studies in animal models, accelerated aging, and integration with a more robust fixation system.

SA97. The Relationship Between Self-Reported and Computer-Based Measures of Music Perception in Adult Cochlear Implant Users

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Category: Auditory Prostheses

Background: In recent years, the quality of music perception with cochlear implanted (CI) hearing has increasingly gained attention as a topic of research. Music, like speech, is a complex stimulus and therefore requires complex decoding mechanisms that can be challenging with a CI. However, measuring music perception is sometimes less straightforward than measuring speech perception. Because perceptual acuity and appraisal appear to be two distinct aspects of music perception, both should be assessed for a holistic clinical evaluation. Laboratory tests to evaluate music perception focus on objective sound properties that can be defined by the physical parameters of acoustic signals. Moreover, perceptual accuracy is not the only important aspect of music listening, and considering that music is often used for entertainment and mood enhancement, it is valuable to investigate to what extent the listener enjoys the music. While several studies have focused on the relationship between outcomes of laboratory musical tests and self-perceived music perception and enjoyment in CI users, the results have been variable, possibly due to factors such as the listener's musical experience, age, or other demographic characteristics that may affect music perception. Therefore, the aim of the current study was to examine the relationship between objective computer-based music perception measure and subjective measures of music perception and enjoyment in adult CI users.

Methods: Twenty-eight adult non-musician CI users participated as part of a larger training study (CIMUGAME). Objective computer-based tests were Melodic Contour Identification (MCI; organ target alone and organ target with the A5 piano masker), Instrument Identification Test (IDT) and Instrument Family Classification Test (ICT). Subjective measures of music perception and enjoyment were seven subsections of the Dutch Musical Background Questionnaire (DMBQ). Associations among the MCI, IDT, ICT and DBMQ subsections were assessed using a correlation table.

Results: Significant moderate correlation was found between IDT and ICT (R = .48, p LESS THAN .01). Within the DMBQ, several subsections correlated with each other, demonstrating internal consistency of the test (R's GREATER THAN .41, p's GREATER THAN .05). One subsection on the DMBQ — Elements of Music, which investigated the ability to perceive the rhythm, melody, and timbre components of music, differentiate vocalists, and to follow the lyrics of a song — correlated moderately with MCI (R = .43, p LESS THAN .05) and IDT (R = .42, p LESS THAN .05).
Conclusions: Two objective music perception metrics correlated with one subsection of the DMBQ. The fact that only subscores correlated, and not combined or total scores with other total scores, suggests that the relationship between computer-based and self-reported measures of music perception are highly nuanced. Therefore we recommend that future assessments of CI users' of holistic music perception and enjoyment be measured with both objective and subjective measures, and the results should be interpreted together.

SA98. The Opto-Electrical Cochlear Implant

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Category: Auditory Prostheses

Background: Hearing loss and deafness are widespread global challenges, currently affecting over 1.5 billion people. By 2050, nearly 2.5 billion individuals are expected to experience some degree of hearing loss, with at least 700 million requiring hearing rehabilitation. While cochlear implants (CIs) are widely used to assist those with hearing loss, the existing electrostimulation technology has its limitations. The main constrain lies in the low spatial precision caused by the spread of electrical currents within the cochlea, leading to interference between adjacent electrode contacts. This interference reduces the number of independent stimulation channels, which, in turn, negatively impacts sound perception.
Efforts to enhance the number of independent stimulation channels have included strategies such as positioning CI electrodes closer to the neurons and using multipolar stimulation to focus the electrical current. However, these approaches have not resulted in significant improvements in CI performance.

An innovative alternative involves the use of light for neurostimulation, which can selectively target small groups of neurons with a precision comparable to that of acoustic stimulation. Infrared neurostimulation (INS), a specific type of optical stimulation, presents a promising solution for improving CIs without requiring light-sensitive molecules known as opsins.

The integration of optical stimulation technology into CIs could represent a significant progress in auditory implants. Nevertheless, successful implementation requires careful consideration of factors such as the number of electrical and optical stimulation channels, energy efficiency, device size, coding strategies, and effective light delivery systems. This work presents the progress of a prototype of a fully implantable opto-electrical CI (oeCI) ready to be tested in a pre-clinical study in animals.

Methods: Our implant prototype has 24 electrical channels and 16 optical channels within a compact 6-layer PCB (3.5x3.5 cm^2). The design includes a top-layer microcontroller with Bluetooth, middle-layer electrical drivers with 24 current stimulators, and bottom-layer optical drivers that switch between constant and pulsatile modes.
We have built and tested multichannel optrodes using polyimide waveguides with ring electrodes, focusing on their mechanical properties and aging. Furthermore, we are assessing fluorinated waveguides known for their low propagation losses for the NIR spectrum.

Moreover, we have integrated a novel coding strategy into the oeCI to support key stimulation scenarios: using electrical stimulation to "prime" neurons and applying optical stimulation for small frequency bands while using electrical stimulation for broader bands. We have tested the coding timing performance across various FFT lengths and settings to enhance device efficiency and accuracy.

Results: The implant shows good performance and fluorinated waveguides provides a promising means for light delivery into the cochlea.
Conclusions: A preclinical study will be conducted verifying the prototype of the implant.

SA99. Optical Detection of Basilar Membrane Damage

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Category: Auditory Prostheses

Background: Hearing loss is a significant condition that impacts over 1.5 billion people worldwide, with 466 million experiencing severe impairment. While hearing aids manage mild to moderate cases, approximately 30 million individuals with severe to profound deafness could benefit from cochlear implants (CIs). Despite the success of CIs, with over 1 million users globally, the implantation process is not without risk. Inserting the CI electrode into the cochlea can potentially affect residual hearing, which may, in turn, impact the performance of the CI.
The basilar membrane, composed mainly of collagen fibers, plays a crucial role in preserving residual hearing and optimizing CI outcomes. Assessing the integrity of this delicate structure is fundamental, however no effective monitoring technique currently exists in the clinic to evaluate its health during or after CI implantation.

A promising approach involves birefringence, an intrinsic optical property of tissues exhibiting anisotropy structure. This phenomenon occurs when polarized light interacts with materials that have internal structural variations, leading to a change in the light's polarization state. Since the collagen fibers in the basilar membrane are anisotropic, measuring birefringence could offer a way to assess structural integrity and detect damage. In this study, we present a quantitative method for evaluating birefringence in mouse and human cochlea samples under varying damage conditions.

Methods: In our study, we performed quantitative birefringence measurements on unstained cochlear sections from both mice and humans, each with a thickness of 10 micrometres. We first obtained qualitative birefringence data using a conventional transmission-mode birefringence

microscope, followed by quantitative analysis with an LC-PolScope microscope. We analysed normal cochlear samples and those exposed to various damage conditions, including laser light exposure. Additionally, we assessed the damage after inserting a cochlear electrode array to verify the sensitivity of this technique in detecting damage to the cochlear sections.
Results: The technique demonstrated promising results in our study, indicating its potential for assessing cochlear damage. This method has been previously employed by other research groups to evaluate the resolution of normal cochlear sections. In this context, we have shown its implementation in a clinical scenario involving cochlear damage.
Conclusions: Further improvements will focus on evaluating these quantitative measurements in back-reflection mode. These outcomes will guide us as we build a custom cochlear probe specifically designed to measure birefringence in back-reflection mode.

SA100. Human Vs. Machine: Evaluation of a Novel Robotic Device for Electrode Insertion During Cochlear Implantation in a Large Animal Model

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Category: Auditory Prostheses

Background: A critical moment during cochlear implantation is the careful insertion of the electrode into the scala tympani. During this process, the delicate structures of the inner ear can be damaged if the insertion is performed too quickly or abruptly due to sudden increases of intracochlear pressure or penetration of the cochlear basilar membrane. Several modelling studies suggest that a very slow and careful insertion, without abrupt pressure fluctuations, is associated with less damage to the sensible cochlear structures and better postoperative hearing preservation. In order to enable a smooth and controlled insertion, various robotic systems have been developed, which are thought to be superior to manual insertion in terms of insertion speed and precision. In this study, the trauma elicited by manual insertion was compared to the one following insertion with a robotic device in a large animal model with human-like cochlear anatomy. The induced microtrauma and the postoperative hearing function were evaluated following cochlear implantation.
Methods: Twenty domestic pigs are going to be implanted with clinically-grade cochlear implants (Flex24 electrode, Synchrony 2, MED-EL, Innsbruck, Austria) either with the assistance of the OTODRIVE system (MED-EL, Innsbruck, Austria) or manually by an experienced otosurgeon. Pre- and postoperative auditory function is evaluated using ABR and ECochG measurements and the inner ears of the implanted animals are collected one week after implantation followed by histological analysis of the structural microtrauma.
Results: In our ongoing study, we have so far implanted five animals – four with robotic insertion and one via manual electrode insertion. Preliminary results show a tendency for reduced electrode impedances in pigs that were implanted with the help of the robotic insertion device compared to the impedances in the animal that was manually implanted. So far, ECochG measurements show no differences in the auditory function during and after electrode insertion in both groups. Further surgeries and analyses are planned until the presentation at the conference (expected n=8 per group).

Conclusions: The robot-assisted cochlear implantation appears to be a suitable, safe, and accessible alternative due to its easy operation and its presumable atraumatic mode of insertion. Our preliminary results show no inferiority of the robot-assisted insertion compared to manual electrode insertion. The automatically controlled insertion speed, set to 0.1 millimeter per second, may explain the mitigated microtrauma as observed in reduced electrode impedances. Therefore, we hypothesize that pressure peaks and involuntary movements can be prevented by using the robotic assisted insertion.

SA101. Study on the Method of Controlling the Vestibular Organ and Cochlea Using Bone Conduction Stimulation

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Category: Auditory Prostheses

Background: Bone conduction stimulation, when delivered to the cochlea and vestibular organ, can vary in both energy and direction due to the unique shape and properties of the skull. This variability complicates the ability to directly associate input forces from bone conduction devices with the motion of these organs. However, if the cochlea and vestibular organ could be directed to move in specific trajectories or rotations, it would greatly enhance the interpretation of bone conduction-based hearing and balance responses. This study investigates a method to precisely control the movement of the cochlea and vestibular organ using multiple bone conduction devices.
Methods: A comprehensive three-dimensional finite element model (FEM) of the head, including the auditory periphery, was developed for this study. Six transducers were integrated into the model: four at the bilateral mastoid and bone-anchored hearing aid (BAHA) positions, with two additional transducers positioned at the front and rear of the head. An optimization algorithm was applied to regulate the vibration amplitude and phase of the transducers, enabling control over the rotational and translational motion of the auditory periphery. A secondary optimization was performed using only four transducers, excluding those located at the mastoid positions.
Results: Using a genetic algorithm (GA), 50 iterations were conducted with 20 different input combinations per iteration, resulting in a total of 1000 simulations. Desired translations were set in the anterior, superior, and medial directions, with an additional arbitrary vector selected for comparison. Input force combinations were optimized for each of the four selected motions (translation and rotation). When all six transducers were utilized, the average error was 3%, whereas the use of only four transducers resulted in an average error of 5%.
Conclusions: This study presents a method for optimizing input force combinations to achieve specific movements of the cochlea and vestibular organ. With an average error rate of less than 5%, the findings indicate that precise control of these structures is possible, which may lead to improved understanding of bone conduction hearing and balance. This approach offers new possibilities for enhancing the design of auditory and vestibular devices.

SA102. Evaluating the Utility of Virtual-Channel-Based Sound Coding for Future Optogenetic Cochlear Implants

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Category: Auditory Prostheses

Background: Electrical cochlear implants (eCIs) are considered the most successful neuroprostheses and represent the state-of-the-art rehabilitation device for individuals with severe to profound hearing loss. While eCIs users typically achieve fair open-set speech perception in the quiet, their understanding of speech in daily situations with background noise and music perception remains limited. The reason is a large spread of electric current from each intracochlear electrode activating a tonotopically broad population of auditory neurons. This limits spectral selectivity of electrical sound encoding to less than 10 perceptually independent stimulation channels. Despite many efforts, this has not improved over a decade. These efforts also include current steering where two channels are simultaneously stimulated at different intensities to create intermediate virtual channels between them reaching a total of 120 channels in the Advanced Bionics HiRes Fidelity 120 strategy. Clinically, this did not robustly improve the outcome of eCI hearing rehabilitation. On the other hand, optogenetic cochlear implants (oCIs) hold the potential to overcome the eCI bottleneck as light can be better confined in the fluid-filled cochlea volume than electric current, promising enhanced spectral resolution.

Methods: In order to capitalize on the expected increased number of perceptually independent channels for sound coding in future oCI, we adapted the SpecRes strategy (research version of HiRes Fidelity 120) implemented in open-source generic MATLAB toolbox (GMT). The modified strategy is based on a non-uniform FFT (NUFFT) filter bank instead of FFT and a parallel (simultaneous) stimulation of multiple channels instead of interleaved stimulation. For stimulation, passive laser-diode-based oCI design was used. We used the strategy as a stimulation input to our recent in silico framework for sound coding optimisation (FraSCO). With sound-to-neuron information transmission index (SNITI) measure calculated within FraSCO we analysed the performance of the modified SpecRes strategy.

Results: Depending on selected opsin enabling optogenetic stimulation, as well as number of active channels and total stimulation channels, SNITI scores across 40 audio files show similar or better performance with higher number of available oCI stimulation channels comparing to state-of the-art eCI, given no laser diode energy consumption constraints. Also, no improvement of utilising 120 optical channels over 64 channels was observed. However, the superior spectral resolution of oCI allows to compensate for the considerably lower temporal resolution then that of eCIs.

Conclusions: Using an in silico framework for benchmarking hearing restoration, FraSCO, we demonstrate the potential and limitations of the modified SpecRes strategy as a starting point for further developing future oCI-tailored coding strategies.

SA103. The Effect of Neural Health on Azbio Sentence Scores Measured in Quiet and Noise in Postlingually Deafened Adult Cochlear Implant Users

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Category: Auditory Prostheses

Background: In cochlear implant (CI) users, two metrics derived from the electrically evoked compound action potential (eCAP) have been used to estimate neural health of the cochlear nerve (CN): the interphase gap (IPG) effect, and the phase locking value (PLV). IPG effect refers to the sensitivity of the eCAP to changes in IPG of a biphasic electrical pulse. IPG effect has been shown to be associated with the survival of spiral ganglion cells in pharmacologically deafened guinea pigs (Prado-Guitierrez et al., 2006; Ramekers et al., 2014, 2015; Schvartz-Leyzac et al., 2019), and indicative of the functional status of CN in human CI users (e.g., He et al., 2018; He et al., 2020a; Hughes, et al., 2018; Schvartz-Leyzac and Pfingst, 2018). PLV is an index quantifying neural synchrony in CN based on trial-to-trial phase coherence of eCAP sweeps (He et al., 2024). In adult CI users, PLV has been shown to be strongly correlated with temporal processing acuity and susceptibility to background noise (He et al., 2024). In this study, we evaluated the relationships between IPG effect, PLV, and AzBio sentence scores measured in quiet and noise in adult CI users.

Methods: Study participants include 24 postlingually deafened adult CI users (age range: 36.8 - 84.0 years; mean: 63.9 yrs, SD: 11.83 yrs). For each participant, AzBio sentence scores were measured both in a quiet condition and in two noise conditions, where 10-talker babbles were presented concurrently at +10- and +5-dB signal-to-noise ratios following standard clinical protocols. The IPG effect on the slope of the eCAP amplitude growth function (IPGEslope) and PLV were measured at four locations across the electrode array. For each participant, the averaged IPGEslope and PLV were calculated as the mean value across tested electrodes weighted by the frequency importance function of AzBio sentences (Lee and Mendel, 2017). Spearman's rank correlation tests were used to assess the correlations between age, IPGEslope and PLV. Multiple linear regression (MLR) was used to assess the amount of variance of AzBio sentence scores measured in different conditions that could be explained by age, PLV, and IPGEslope.

Results: Spearman's rank correlation tests showed that age was negatively correlated with IPGEslope but not with PLV. MLR results revealed a negative effect of the PLV on AzBio sentence scores measured in the two noise conditions. This effect was not observed for IPGEslope.

Conclusions: The IPG effect and PLV assess different aspects of neural health of the CN which affect speech perception outcomes in different ways. Further studies using animal models or computational modeling techniques are warranted to better understand the biological underpinnings of these two metrics.

SA104. Computational Modeling of the Electrode-Neuron Interface to Estimate the Electrode-modiolus Distance and Neuronal Density

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Category: Auditory Prostheses

Background: Performance outcomes with cochlear implants are highly variable among individuals. The quality of the electrode-neuron interface likely contributes to this variability. We previously developed a computational model to estimate the effects of changes in the distance between electrodes and spiral ganglion neurons, as well as the density of those spiral ganglion neurons, on monopolar and tripolar thresholds. The purpose of this study was to assess the accuracy of an inverted version of our model, using both monopolar and tripolar thresholds as inputs. We hypothesized that changes in electrode distance and neuronal density affect monopolar and tripolar thresholds differently, enabling unique model solutions for different scenarios and threshold profiles in cochlear implant recipients.
Methods: We used our previous cochlear model with simplified geometry and neuronal composition to investigate how the electrode-interface parameters of electrode distance and SGN density affects monopolar and tripolar detection thresholds, estimated by activation of 100 neurons. We then inverted the model to infer electrode distance and neuronal density from monopolar and tripolar threshold values obtained behaviorally in cochlear implant participants. We assessed the accuracy of fitting the threshold profiles, electrode distance and neuronal density for both known scenarios, and for 18 cochlear implant recipients for whom we had measured electrode distance with CT imaging.
Results: The inverted model reliably fit both the monopolar and tripolar thresholds to within an error of less than 1 dB. The model fits of electrode distance were accurate for some participants but not others. For about half the participants, the accuracy of distance estimates was less than 0.4 mm. The distance estimate errors depended on the estimated temporal bone resistivity, such that half the subjects had best fits using low resistivity while the others needed higher resistivity.
Conclusions: This model provides a simple, practical tool to better understand the electrode-neuron interface in cochlear implant listeners. Further exploration of the role of external resistivity is needed, which could involve using electrical field imaging data or human temporal bones where bone and tissue growth can be directly assessed.

SA105. Onset-Driven Dynamic Range Compression Resolves the Audibility/Sound-Quality Trade-Off

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Category: Auditory Prostheses

Background: State-of-the-art hearing aids use dynamic range compression (DRC) to compensate for loss of audibility and loudness recruitment resulting from sensorineural hearing loss, but choices of compression speed can differ depending on whether audibility or sound quality are prioritized. In this study, we explored three onset-driven DRC (OnDRC) strategies that aimed to both restore audibility and provide high sound quality by using an adaptive compression speed that switched from slow gain increments to fast gain increments whenever onsets, such as informative speech onsets, were detected in the audio input. The three OnDRC strategies differed in terms of onset-detection sensitivity, with OnDRC strategy 1, 2, and 3 requiring progressively more pronounced signal onsets to trigger fast gain increments. In addition, the three OnDRC strategies were compared with state-of-the-art slow- and fast-acting DRC strategies with fixed compression speeds, resulting in five DRC strategies that were compared overall.

Methods: Twenty participants with moderate-to-severe hearing losses took part in the study. The five DRC strategies were implemented in a software-based real-time simulation of hearing aids individually fit with Adaptive Phonak Digital gain calculations and presented over headphones in a double-walled sound booth. The five strategies were evaluated in terms of their efficacy in restoring audibility of soft speech components by measuring recognition of conversational-level CNC words preceded by a loud carrier. The five strategies were also compared via pairwise preference judgments using conversational-level speech stimuli in soft environmental backgrounds, based on recordings from the interior of a car, a quiet room with a ceiling fan, and an outdoor environment with a distant highway.
Results: Results demonstrate that the strategies with highest preference were OnDRC2, OnDRC3, and slow-acting compression. These three strategies showed similar preference. Furthermore, OnDRC2 and OnDRC3 were preferred over OnDRC1, and OnDRC1 was preferred over fast-acting DRC with fixed compression speed. In terms of CNC word recognition, all strategies showed similar performance except for slow-acting compression, which resulted in poorer recognition scores.
Conclusions: In general, the OnDRC strategies showed preference similar to slow-acting compression while providing speech intelligibility benefits similar to fast-acting compression, thus resolving the trade-off between sound quality and audibility. More specifically, OnDRC strategies 2 and 3 with more conservative onset detection were preferred over OnDRC strategy 1.

SA106. Predicting Hearing Performance With eCAP-Based Cochlear Health Measures in Cochlear Implant Users

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Category: Auditory Prostheses

Background: The electrically evoked compound action potential (eCAP) reflects the combined responsiveness to electrical stimulation of the spiral ganglion neurons (SGNs) that form the auditory nerve. In animal studies eCAP measures correlate strongly with numerical survival of SGNs, and with the health of the remaining SGNs. These correlations suggest that the eCAP is a potent predictor of hearing performance in human cochlear implant (CI) users, since it reflects the condition of the electrode-neural interface. However, correlations between eCAP measures and measures of hearing outcome are typically weak. Complicating factors in studies with human CI users include heterogeneity of the population (etiology, age, duration of implantation), low numbers, processing of clinically obtained data, and the involvement of cognitive ability in hearing outcome.
Methods: In the present study we examine the potential of the eCAP as a cochlear health measure in a population of GREATER THAN 80 MED-EL CI users. In this relatively large population the influence of etiology on eCAP measures can be studied. Effects of duration of implantation were assessed by comparing intra-operative with post-operative recordings using autoART in MED-EL's MAESTRO software. The influence of recording parameters was assessed by comparing the alternating polarity paradigm for artifact reduction with a forward-masking paradigm – for the latter, responses to anodic-leading and cathodic-leading stimulation were evaluated separately. Finally, in order to circumvent cognitive factors in the relation between eCAP measures and

hearing performance, a spectral ripple discrimination task was used in addition to conventional audiometry (speech in silence and in noise).
Results: Preliminary results show moderate correlations between several eCAP measures and patient characteristics, such as age or duration of hearing loss. Ripple discrimination scores appeared to be better correlated with eCAP measures of cochlear health than speech scores did. eCAPs recorded post-operatively were generally more stimulation-artifact-free and hence more reliable. Further analysis will reveal whether artifact reduction strategy plays a significant role in the predictive value of the eCAP for CI performance.
Conclusions: eCAP-based cochlear health measures are predictive of hearing performance with a CI. Occasionally reported weak correlations may be strengthened by optimizing both stimulation conditions and the choice for hearing outcome measure, and additionally by accounting for patient characteristics such as etiology and duration of hearing loss.

SA107. The Sound of a Cochlear Implant Longitudinally Investigated in Single-Sided Deaf Subjects

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Category: Auditory Prostheses

Background: The sound of a cochlear implant (CI) has intrigued hearing researchers since the early days of CI. Subjects with single-sided deafness (SSD) using a CI in their deaf ear can compare CI simulations presented to their normal ear with the sound perceived with their CI ear. Studies in these subjects (Peters et al., 2018; Karoui et al., 2019; Dorman et al., 2020) yielded a reasonable impression of CI sounds also showing great variations in CI percepts among and within SSD-CI participants. For instance, both sine and noise carriers, which sound quite differently, can be chosen by the same subject. The perception of sounds through a CI may change over the years, since the subject adapts to the sound. It is indeed qualitatively reported that with increasing use of the CI, it sounds more natural (Glennon et al., 2020). Therefore, we performed a longitudinal study in SSD CI users adding a natural-sound parameter in the set of vocoder simulations.
Methods: Twelve subjects with SSD were implanted with a Nucleus CI and CP920 or CP910 processor (Cochlear Ltd). Two, eight and twelve months after CI activation, participants performed the sound matching test themselves (with supervision of a researcher) using a graphical user interface on a laptop. Original stimuli were presented through a Cochlear CP910 audio cable for the CI ear, and vocoder simulations through an insert earphone to the better ear. Stimuli consisted of two Dutch sentences (one male, one female), one international speech test signal fragment and a piano music piece. The test consisted of 9 steps (per stimulus) including a choice between noise and sine carrier, a choice of number of channels, frequency bandwidth, a temporal and a spectral shift. To examine the extent of natural sound quality the subject was invited to blend the vocoder simulation with the original sound.
Results: Gradings significantly improved by mixing the original sound (median ratios 10% - 30%) and adding temporal (medians between 0 and 0.1 s) and spectral shifts (between -3 and + 4 semitones). Median gradings varied between 7 and 9 (out of perfect 10); the improvement with

time was not significant. While 11 out of 12 participants used the mixing with original sounds, the extent of mixing did not significantly increase with time.
Conclusions: Our quantitative measurements showed only marginal increases with time in natural sound ratio. CI users may think the CI sounds more natural after a year but when asked to carefully compare, it still sounds artificial. The adaptation to the CI sound may be much slower in SSD patients (if present) than in bilaterally deaf patients, because of the normal input they keep receiving through the acoustic ear.

SA108. Changes in Cochlear Microphonics and Electrode Impedances in Cochlear Implant Recipients in the First Year after Implantation

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Category: Auditory Prostheses

Background: Residual hearing preservation after cochlear implantation (CI) surgery is crucial to achieve combined acoustical and electrical hearing and thus improved hearing with CI. Besides, residual hair cells slow down degeneration of the auditory nerve. Electrocochleography (ECochG) has become increasingly valuable in CI surgery as it allows intra- and postoperative monitoring of effects of electrode array insertion on residual hearing. Additionally, intra- and postoperative electrode impedance measurements may provide information of intracochlear tissue growth after CI surgery. Here we examine postoperative changes in electrode impedances and the time course of residual hair cell function by performing ECochG in CI recipients intraoperatively and at several time points up to one year postoperatively.
Methods: Twenty-three patients with severe sensorineural hearing loss receiving a CI participated in a trial comparing electrode arrays (SlimJ and Mid-Scala of Advanced Bionics) and surgical approaches (Jwair et al., Trials, 2021). Here, the data are analyzed by investigators blinded to the randomization. Impedance measurements and ECochG were performed at 5 time points using active insertion monitoring (AIM) of Advanced Bionics: intraoperatively, at 4-6 weeks, 3-4 months, 6-7 months and 12-14 months postoperatively. Impedances were measured along all 16 available electrodes. ECochG responses to pure-tone stimuli with frequencies varying from 125 to 4000 Hz, at sound levels 100-115 dB HL, were also recorded at each of the 16 electrodes, in two opposite phases. The difference and sum of the recordings to the opposite phases were computed as estimates of cochlear microphonics (CM, reflecting hair cell potentials) and auditory nerve responses, respectively.
Results: Impedances gradually increased in the first year after cochlear implantation. Largest increases were observed between intraoperative and first postoperative recordings (4-6 weeks) and between second (3-4 months) and third (6-7 months) postoperative recordings. Significant CM responses (GREATER THAN 1.5 μV) were found in 11 out of 23 patients intraoperatively, and postoperatively in 8/22 at 4-6 weeks, 16/22 at 3-4 months, 13/20 at 6-7 months, and 7/12 at 12-14 months. Ten subjects showed large intraoperative responses, which decreased post-operatively. Notably, 7 intraoperative non-responders showed significant responses in the first postoperative session. No correlation was found between CM amplitudes and impedances intraoperatively and at 6 weeks postoperatively. A change in CM amplitudes between intraoperative and first

postoperative timepoints did not correlate with the increase in impedance between intraoperative and first postoperative measurements.
Conclusions: In most CI recipients CM amplitudes and thus residual hair cell function gradually declines over the course of one year. Notably, in several patients the CM increase in amplitude post-operatively, possibly due to fluid in the ear during intraoperative recordings and reduction of acute postoperative intracochlear inflammation. Increases in electrode impedance, which suggest intracochlear tissue growth, do not explain the CM changes.

SA109. Cochlear Health Assessments in Cochlear Implant Users Using Electrically Evoked Compound Action Potentials and Electrocochleography

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Category: Auditory Prostheses

Background: Hearing performance of cochlear implant (CI) users relies on the condition of the auditory nerve. Animal studies have shown that electrically evoked compound action potentials (eCAPs) can be applied to assess this condition. In particular, relative eCAP measures, obtained by comparing eCAPs to different stimuli, are strongly correlated with neural survival (Ramekers et al., JARO, 2014). Studies in humans demonstrated the value of such relative measures in their correlations with hearing performances (Zamaninezhad et al., Front Integr Neurosci, 2023). The presence of residual cochlear hair cells, which is also assumed to be beneficial for hearing performance, can be assessed with intracochlear electrocochleography (e.g., Giardina et al., Ear Hear, 2019). In an ongoing trial, we record eCAPs and ECochG in CI recipients to examine the predictive value for hearing performance using both linguistic and non-linguistic perception tasks.
Methods: Twelve subjects with severe sensorineural hearing loss, aged 60 to 80 years, received a CI (Flex28 arrays of MED-EL). Intraoperatively and approximately 4 months postoperatively, eCAPs were recorded to biphasic current pulses with varying interphase gaps (IPGs; 2.1 to 30 µs) and varying current levels up to saturation level, using an alternating polarity stimulus paradigm (AutoART, MED-EL). Also eCAPs to short pulse trains were recorded. Outcome measures obtained at each array electrode include amplitude and latency at maximum current levels, and the current level halfway the amplitude growth function, level50%. Relative eCAP measures were obtained by the difference between measures at IPG of 30 and 2.1 µs. ECochG was postoperatively performed to tones at 115 dB SPL at frequencies from 250 to 4000 Hz. Hearing performance was assessed by CVC in noise (+5 and +10 dB signal-to-noise ratio) perception and spectral ripple discrimination using the spectral-temporally modulated ripple test (SMRT; Aronoff and Landsberger, JASA, 2013).
Results: In all patients eCAPs could be recorded with amplitudes between 200 and 1000 µV. Absolute eCAP measures were generally similar for postoperative and intraoperative recordings. The postoperative IPG-difference measures aligned more with outcomes from animal studies than intraoperative measures. Pulse train responses varied from modulating to flat patterns, respectively indicating poor to good neural health. Cochlear microphonics could be recorded for a majority of participants with amplitudes up to 100 µV, not only at low frequencies but occasionally even for 4000 Hz. One notable correlation between hearing performance and eCAP outcome was observed:

the ripple discrimination score increased with decreasing eCAP latency (R2 = 0.3, p = 0.05). Speech perception scores did not significantly vary with absolute or relative eCAP measures.
Conclusions: In the present study, the eCAP latency appeared to have predictive value for a non-linguistic hearing outcome. We argue that speech perception is harder to predict from eCAP measures because of cognitive factors involved.

SA110. One Year Integrity and Biocompatibility Data of an Alginate Hydrogel Cochlear Implant Coating Under Simulated Inner Ear Conditions

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Category: Auditory Prostheses

Background: An atraumatic implantation of the Cochlear Implant (CI) electrode might be achieved by a flexible, smooth, and hydrophilic coating of the electrode array. Such a coating needs to be durable and must not swell or shrink to avoid micro-movements of the electrode. In parallel, it has to be biocompatible to ensure hair cell (HC) and spiral ganglion neuron (SGN) health. Given its inert and biocompatible properties, barium cross-linked ultra-high viscosity alginate shows promise as a candidate for this purpose. Its biocompatibility and mechanical, chemical and electro-chemical coating stability were tested under conditions simulating the inner ear.
Methods: CI dummies made of silicone were coated with alginate by using a cone-shaped semi-permeable membrane. Alginate sol was injected in these membrane cones, dummies inserted, and all covered with barium cross-linking solution for ionic gelation. Coated silicone dummies were incubated in artificial perilymph and observed for one year and coated CI-electrode arrays were electrically stimulated for 2 months in artificial perilymph with weekly medium change, photo documentation for assessing the coating integrity and impedance measurement. Additionally, beads of alginate hydrogel were co-cultivated with murine inner ear tissue for one week to test biocompatibility with SGN and HC.
Results: The alginate-coating stayed attached to the CI over the one-year observation period. Small variations in the diameter of coated dummies were detectable, likely due to rotation of the floating dummies, but no clear shrinking or swelling were seen. Two months of electrical stimulation did not affect the integrity of the coating nor did the coating affect the electrode impedances. Neither the number of SGN nor the number of HC were reduced by co-culture with alginate beads.
Conclusions: The study proved mechanical and long-term chemical and electro-chemical stability and biocompatibility of the alginate hydrogel in inner ear mimicking in vitro assays. These promising results support testing of alginate as lubricant-coating in vivo as a further step towards translation to clinical use for CI improvement.
Acknowledgement: This study is funded by the German Research Foundation (DFG), Project number 452405020

SA111. Towards Extracochlear Electric-Acoustic Stimulation of the Auditory System

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Category: Auditory Prostheses

Background: Combined electric-acoustic stimulation (EAS) has been shown to provide great benefits for CI-users with low-frequency residual hearing. However, cochlear implantation remains traumatic and carries the risk of causing high-frequency hearing loss after insertion of the electrode array. Extracochlear electrical stimulation has the potential to minimize cochlear trauma, thus providing benefits of electrical stimulation to a larger number of patients with substantial residual hearing. However, it is not known to what extent extracochlear electric stimulation can be used in conjunction with residual hearing. The purpose of this study is to investigate potential benefits of extracochlear electrical stimulation combined with acoustic stimulation.

Methods: Potential benefits of single electrode extracochlear electric stimulation were evaluated in 10 NH subjects using vocoder simulation as well as in a unique population of 10 CI users that retain acoustic hearing after partial cochlear implantation. We refer to these subjects as partial insertion CI users. In these subjects some electrodes of the CI are placed inside and some others outside the cochlea, so that it is possible to investigate benefits of electrical stimulation delivered through electrodes located close to the round window. Potential improvements in speech understanding and logatome identification using single electrode electric stimulation were evaluated. Low-frequency information of speech was delivered up to a cutoff frequency via acoustic stimulation. High-frequency information of speech above the cutoff frequency was delivered either acoustically using a single-channel vocoder (in NH subjects) or via electrical stimulation through single CI electrodes located close to the round window (in partial insertion EAS users).

Results: The results from the computational model show that a significant amount of current enters into the cochlea when electric stimulation is provided at the round window.

The results of the study in NH subjects show that speech reception thresholds obtained from low-frequency acoustic hearing significantly improved by approx. 11 dB SNR if combined with simulated single channel electrical stimulation ($p=0.005$). Moreover, a significant improvement in consonant identification (12% on average) was observed for the same listening condition. Results in partial insertion EAS subjects showed that extra-cochlear electric stimulation could elicit sound sensations. Loudness was in most cases scaled as soft and was usually accompanied by side effects. No improvement in speech reception was observed. However, a significant improvement was observed in the identification of consonants with different spectral ($p=0.042$) and perceptual ($p=0.031$) characteristics.

Conclusions: This study shows promising results for the benefit of extracochlear electric-acoustic stimulation. Based on these results a prototype for an extra-cochlear electric and acoustic stimulation device has been developed that will be used to reproduce the results in normal hearing subjects with non-invasive electric stimulation.

SA112. Behavioral and Electrophysiological Measurements of Vowel Integration

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Category: Auditory Prostheses

Background: Cochlear implant (CI) users with preserved hearing in the same (ipsilateral) or opposite (contralateral) ear often experience improved speech perception when combining electric and acoustic information. However, this improvement shows significant variability, which is associated with electric acoustic integration. The underlying mechanisms of this integration remain unclear. Moreover, it is not clear whether the integration mechanism differs with regard to having ipsilateral or contralateral acoustic hearing.
This study aims to investigate the process of vowel integration using both behavioral and electrophysiological measures in normal hearing participants. Vowel integration refers to a combination of different acoustic cues in order to recognize vowels, which plays a crucial role in speech perception.

Methods: Four synthetic German vowels were created, differing in their first (F1) and second (F2) formant frequencies, either presented individually or in combination. Five listening conditions were tested:
Control: Both F1 and F2 presented to both ears.

F1 alone: Only the F1 presented to one ear.

F2 alone: Only the F2 presented to the other ear.

F1+F2 monaural: F1 and F2 presented to the same ear, simulating CI users with ipsilateral acoustic hearing.

F1F2 binaural: F1 presented to one ear and F2 to the other, simulating CI users with contralateral acoustic hearing.

As there might be a temporal mismatch between electric and acoustic stimulation present in real subjects, the current study additionally included two conditions with a 15 ms delay between two formats, whereas F1 is delayed:

F1+F2 monaural 15 ms.

F1F2 binaural 15 ms.

For the behavioral assessment, participants completed a vowel identification task using a four-alternative forced choice paradigm. Electrophysiological data was collected using EEG to measure cortical auditory evoked potentials (CAEPs) in response to the vowels under each listening condition.

Results: The analysis of the behavioral results revealed distinct patterns in vowel recognition based on the contribution of formant cues. Introducing a 15 ms delay between F1 and F2 did not impact vowel recognition, regardless of whether the cues were presented binaurally or monaurally.

Further, the electrophysiological data indicated that the effect of the listening condition on the N1P2 amplitude varied depending on the frequency spacing of the vowels. For vowels with larger frequency spacings, the N1P2 amplitude remained relatively stable across different listening conditions. However, vowels with narrower frequency spacings showed a more pronounced sensitivity to the listening condition. The impact of the 15 ms delay was minimal across all vowels.

Conclusions: These findings suggest that the integration of electric and acoustic information is influenced by the specific vowel being processed and its associated formant frequencies. While the behavioral results obtained no effect of the contralateral or ipsilateral acoustic hearing on vowel identification, the N1P2 response differed across those conditions, pointing out a different mechanism for the information integration.

SA113. Temporal Bone Histopathology of a Malignant Peripheral Nerve Sheath Tumor

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Category: Clinical Otolaryngology & Pathology

Background: Malignant peripheral nerve sheath tumors (MPNSTs) are usually high-grade malignant tumors with a potential for hematogenous spread. They may arise from pre-existing neurofibromas, mostly the plexiform type, or sporadically with an approximately equal prevalence, or develop in a previously irradiated site.
Nuerofibromatosis type 1 (NF1) is characterized by an autosomal dominant loss of tumor suppressor genes predisposing to the formation of benign and malignant tumors, including its hallmark neurofibromas. Neurofibromas are benign tumors originating from Schwann cells and are comprised of additional components such as fibroblasts, mast cells, perineural-like cells, and residual axons. MPNSTS are diagnosed in 8-13% of patients with NF1 during their lives, contributing the shortened life expectancy of below 40 years.

The aim of our work is to provide for the first time a description of the histopathology of the temporal bones in two patients with MPNST in their IAC, one of which with NF1, and discuss their clinical implications.

Methods: The temporal bone (TB) collection at the Massachusetts Eye and Ear was searched for those involved with MPNST. The bones were processed in the commonly used technique: fixed in formalin and decalcified using ethylenediaminetetraacetic acid, followed by embedding in celloidin. A two-dimensional reconstruction of the cochlea was done. Cochlear and Vestibular neuronal cells were counted.
To facilitate the diagnosis suitable sections were stained for Ki67.

Results: The first case is of a 23-year-old man with NF1 and a spinal MPNST, with no documentation of audio-vestibular deficit. Histopathology demonstrated in both IACs a tumor most compatible with an MPNST. On the right side, the tumor was adjacent to the nerves and infiltrating them, and near the porous, it was superficially invading the dura. The tumor tissue had a strong Ki67 staining bilaterally, and in the right ear, it was specifically surrounding the tumor

necrosis area. The second case is of a 2.3 years old toddler diagnosed with C5-level spinal MPNST. Two months before he passed away, he had a right ear infection, followed by central facial nerve palsy and ataxia. Histopathology revealed in both IACs tumor tissue surrounding and invading the nerve trunks. In these two cases, the total cell count in the spiral ganglion was 59-74% of the age norm, and in Scarpa's ganglion around 100%. The strong Ki67 staining strengthens the diagnosis of MPNST by indicating high mitotic activity. The normal or near-normal cell count in Scarpa's and the spiral ganglions are likely attributed to the rapid-evolving nature of MPNST.
Conclusions: The normal or near-normal cell count in Scarpa's and the spiral ganglions are likely attributed to the rapid-evolving nature of MPNST. Further immunohistopathological characterization of neurofibromatoses syndromes may promote evolving diagnosis criteria and treatment. Early detection and clinical awareness are crucial for preventing fatal outcomes.

SA114. Transitioning to Personalized Care for Idiopathic Sudden Hearing Loss with Machine Learning

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Category: Clinical Otolaryngology & Pathology

Background: Idiopathic sudden sensorineural hearing loss (ISSNHL) is frequently encountered at clinic, with an annual incidence ranging from 5 to 27 per 100,000 people. The exact mechanism of ISSNHL remains unknown, leading to uncertainty treatment. The primary treatments include systemic or intratympanic steroid therapy. However, consensus on an intratympanic steroid injection protocol is lacking, and personalized management is not available. The aim of our study is to develop individualized management strategies for idiopathic sudden sensorineural hearing loss with machine learning.
Methods: This retrospective multicenter study recruited 1,790 patients who received intratympanic steroid injections from two medical centers in Taiwan. After excluding those not meeting the audiogram definitions of ISSNHL and those with known etiologies of hearing loss, 864 patients were eligible for analysis. Hearing outcomes were classified according to the last pure-tone audiogram, including complete recovery, partial recovery, and no recovery. The primary outcome was the area under the receiver operating characteristic curve for complete recovery. Modeling was conducted on the Quanta for Medical Care AI platform, with a train-test split of 4:1.
Results: The study included 864 (398 male and 466 female) patients, with a mean age of 56.6 years). Random forest classifier with 19 features outperformed other machine learning models in predicting hearing outcomes for patients with idiopathic sudden sensorineural hearing loss treated with intratympanic steroid injection, reaching an area under the receiver operating characteristic curve of 0.8286 for complete recovery. The time to treatment was the most critical factor, with intratympanic steroid injections administered within 8 days of hearing loss being associated with better outcomes.

Our model incorporates different contribution of each feature, thereby allowing obtaining personalized outcome predictions and timely treatment adjustments during the treatment course, optimizing the likelihood of complete recovery. The performance of our model with a trichotomy recovery status is comparable to that of the traditional binary model while retaining limited input features.

Conclusions: The machine-learning-based prediction model enables personalized strategies and timely adjustments during treatment for idiopathic sudden sensorineural hearing loss, optimizing the likelihood of complete recovery. Incorporating other adjuvant therapies into features may further enhance model performance.

SA115. The Exclusive Use of Local Anesthesia as an Alternative to General Anesthesia for Adolescent and Adult Patients Undergoing Cleft Lip Repair or Revision

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Category: Clinical Otolaryngology & Pathology

Background: Cleft lip and/or palate (CLP) is among the most common congenital anomalies worldwide, occurring in roughly 1 in every 1,000 live births. Surgical intervention remains the primary treatment for CLP, with the goal of restoring normal airway function, chewing, and swallowing. In the United States, cleft lip (CL) repair is typically performed in early childhood. However, in developing nations, limited access to healthcare often prevents timely intervention, leaving many children to reach adolescence or adulthood with unrepaired clefts, necessitating primary repairs and possible revisions later in life. Given this context, local anesthesia has been considered a viable option for adults undergoing CL surgery. This study aims to determine whether patients undergoing CL repair or revision under local anesthesia alone have a lower risk of post-operative complications compared to those who receive general anesthesia for the same procedures.

Methods: A comprehensive literature search was performed using PubMed, Google Scholar, and Embase in adherence to the PRISMA 2020 guidelines. The inclusion criteria focused on studies that exclusively examined the use of local anesthetic techniques in adolescent and adult patients undergoing CL surgeries. Excluded from the review were non-English studies, those involving patients younger than 10 years, and studies addressing cleft palate or other otolaryngological procedures. Key outcomes of interest included complications specifically related to local anesthesia in CL repair or revision, any reported general perioperative complications, instances requiring conversion to general anesthesia, patient-reported pain levels during surgery, wound dehiscence, wound infection, and the need for postoperative narcotics for pain management. To assess consistency between reviewers, interrater agreement was calculated using Stata18.

Results: The initial search identified 1,326 articles relevant to the topic. Of these articles, the authors selected 13 that satisfied the inclusion and exclusion criteria. Of these, eight studies

specifically gathered data on dehiscence and infection rates among patients undergoing CL repair with local anesthesia alone. None of these eight studies reported cases of wound dehiscence or infection. The remaining five articles focused on other metrics of surgical and anesthetic success, such as patient-reported pain levels and the need for post-operative opioids for pain management. Across the studies, the findings consistently supported the exclusive use of local anesthesia for CL repair and revision, with no reported cases of wound complications. Most patients experienced little to no pain and did not require general anesthesia during their procedures.
Conclusions: The results underscored the benefits of local anesthesia, notably its association with fewer complications and faster recovery times compared to general anesthesia. This approach presents a valuable option in resource-constrained regions where access to general anesthesia is frequently restricted.

SA116. Limited-English Proficiency and Its Impact on the Presentation and Management of Vestibular Schwannomas

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Category: Clinical Otolaryngology & Pathology

Background: Limited-English proficiency (LEP) and hearing loss have both been shown to be independent predictors of decreased access to clinical care and adverse health outcomes. Patients with both LEP and hearing loss face overlapping healthcare disparities, and it is increasingly important to identify potential diagnostic and treatment barriers raised by LEP status. Vestibular schwannomas are benign neoplasms that can cause hearing loss, tinnitus, and imbalance. Management is dependent on patient comorbidities, hearing status, and tumor characteristics. In this investigation, we evaluate whether and how limited-English proficiency (LEP) impacts vestibular schwannoma presentation and management.
Methods: A retrospective query of the University of Pennsylvania EMR database was performed utilizing ICD-10 codes to identify patients diagnosed with vestibular schwannoma between 2018 and 2023. This yielded 29 LEP patients and 1350 non-LEP patients. Data collected included patient demographics, tumor characteristics, audiometric data, management recommendations, and current status. The MatchIt package in R was utilized to match LEP patients with non-LEP patients using sex, age at diagnosis, insurance status, and median income of residential zip codes. Mann-Whitney U tests were used to compare continuous variables, and Chi-squared and Fisher's exact tests were used to evaluate categorical variables between groups. Analyses were also conducted replacing missing data with median values; this had no impact on results.
Results: There were no differences in symptoms at presentation, audiometric data, tumor characteristics, or initial management recommendation based on LEP status. However, concerning trends were noted that did not reach statistical significance. Mean tumor volume was 6072.5mm3 (SD 11,725) for LEP patients, 2.9 times greater than the mean tumor volume of non-LEP patients (2114 mm3, SD 4316; p=0.06). LEP patients were less likely to undergo word recognition testing (X2=35.2, p LESS THAN 0.0001) due to limited access to language-concordant testing materials. LEP patients that did undergo testing had lower mean scores than non-LEP patients [44.5% (SD

32.1) vs 61.9% (SD 31.5); p=0.1]. Lastly, time elapsed from symptom onset to diagnosis was greater in LEP patients [4949 days (SD 12953) vs 2609 days (SD 8371); p=0.58].
Conclusions: While there were no statistically significant differences in presentation and clinical management between LEP and non-LEP patients, our data reflect LEP patients' challenges in accessing and navigating the healthcare system, which has been demonstrated in the literature. We attempted to account for differences between groups through propensity score matching. Nonetheless, the combination of small sample size and missing data may reduce statistical power in our study. Multi-institutional studies are required to further understand how LEP status creates barriers beyond those which may be expected due to socioeconomic disparities alone.

SA117. Analysing a Large Clinical Dataset Using Linear and Non-Linear Data-Reduction Algorithms

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Category: Clinical Otolaryngology & Pathology

Background: The aetiology of hearing impairment is complex due to the likely involvement of several interconnected structures and cell types at the periphery of the auditory system and along the auditory pathway. Pure-tone audiometry is the gold standard test to assess hearing status in clinical practice. Previous work has attempted to classify patients into different subtypes of hearing, or auditory phenotypes. Researchers have proposed two different strategies: (1) starting from different cochlear pathological conditions based on histopathological examinations, both in animal models and in human cadavers and relating them to audiometric shapes; and (2) classifying large numbers of audiograms using unsupervised statistical tools to define auditory phenotypes. Both strategies have their strengths and weaknesses, but both have used only the audiogram as hearing measurement. Including additional hearing tests beyond audiometry may be beneficial in phenotypical analyses, since it is well known that the audiogram is not sensitive to all pathologies in the peripheral auditory system.
Methods: Here, a large clinical dataset collected from 1995 to 2022 at the Copenhagen Hearing and Balance Centre at Rigshospitalet hospital in Denmark, containing 84,280 unique adult patients and 288,295 audiograms, was analysed. Two methods were employed to facilitate comparison between a traditional approach to data reduction and a more complex one: principal component analysis (PCA), a linear data-reduction algorithm, and uniform manifold approximation and projection for dimension reduction (UMAP), a non-linear data-reduction algorithm. Finally, to compare the learned encodings with existing auditory phenotypes, individual datapoints from our dataset were classified based on the previously proposed auditory phenotypes from literature and projected back to our learned dataset spaces.
Results: From the PCA analysis, more than 90% of the audiometric data variance could be explained by the two first components. The first component represented the mean of the audiometric curve, while the second component indicated whether the audiogram is sloping towards high frequencies or towards low frequencies. The UMAP analysis showed the advantage of using a non-linear algorithm that can exploit both global and local data structures to represent the data in a 2D map. Classification of audiograms in our dataset without applying strict exclusion

criteria showed that up to 70-80% of the audiograms remained unclassified when using previously proposed phenotypes.
Conclusions: Audiometric measurements can be successfully represented in two dimensions, such that the encoding maintains a logical continuum of hearing loss severity and shape. The use of non-linear algorithms might allow for representing more complex data beyond audiometry in such two-dimensional space. Such space might be used to represent clinical hearing loss phenotypes and the main paths in the impairment progression. This might potentially represent a tool to counsel patients and evaluate new treatments for hearing loss.

This work was supported by the GN Foundation.

SA118. Histopathologic Assessment of Archival Temporal Bone Specimens With Pediatric Rickets

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Category: Clinical Otolaryngology & Pathology

Background: Children have increased vitamin D requirements since vitamin D is necessary for proper bone mineralization and growth of the developing skeleton. Vitamin D deficiency can result in rickets characterized by skeletal deformities and abnormal bone histology due to impaired bone maturation and calcium salt deposition. Vitamin D deficiency has been associated with several otologic disorders, such as sensorineural hearing loss, otosclerosis, cholesteatoma, and benign paroxysmal positional vertigo. The petromastoid part of the temporal bone undergoes endochondral ossification during the first years of life, which is highly dependent on available vitamin D. Today, clinical rickets due to nutritional vitamin D deficiency is rare in the United States but was common in industrialized countries until the 1920s-30s and remains prevalent in some parts of the world. This study analyzes histologic features of archival temporal bone specimens from pediatric rickets cases. We identify pathologic changes in the bony otic capsule and membranous structures.
Methods: Cases from our archival temporal bone collection were selected based on recorded clinical history. Hematoxylin and eosin-stained slides from pediatric rickets cases were included and matched with controls of the same age and similar co-morbidities. High-resolution images of the slides were acquired with a microscope and analyzed with ImageJ software. The width of each of the three layers of otic capsule bone and the opening and midpoint of the vestibular aqueduct were measured. The entrance and exit angles of the vestibular aqueduct were calculated. The vascular vs. total area of the stria vascularis and the globuli interossei vs. lamellar bone area was also measured. Qualitative comparisons were also made between rickets cases and controls.
Results: Rickets specimens (n= 10) showed differences in bone structure compared to controls (n= 12), including reduced total mature lamellar vs. partially ossified cartilaginous bone volume. The cartilaginous ossification pattern and woven bone quantity changed according to age in both groups. This could reflect the functional maturation of the inner ear in accordance with developmental motor and balance control milestones, such as unsupported walking around one year of age.

Conclusions: Temporal bone histology is altered in pediatric cases with clinically evident vitamin D deficiency compared to controls of the same age. Histopathologic changes could reflect cochlear dysfunction and predisposition to otologic disease.

SA119. Evaluating the Impact of COVID-19 Vaccination on Sudden Sensorineural Hearing Loss Prognosis

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Category: Clinical Otolaryngology & Pathology

Background: In 2022, the World Health Organization issued a safety signal regarding a potential link between Sudden Sensorineural Hearing Loss (SSNHL) and COVID-19 vaccination, yet their relationship remains unclear. Most cases of SSNHL are idiopathic, though viral infections are thought to be a primary cause of idiopathic SSNHL. Prior work investigating the relationship between SSNHL and COVID vaccination is inconclusive and lacks audiometric and clinical data. However, the consensus appears to be a low incidence of reported cases and no increased risk of post-vaccination SSNHL. Therefore, this study seeks to characterize treatment and recovery outcomes among temporally cases of COVID-19 vaccination related SSNHL.

Methods: 244 patients who experienced SSNHL and presented for treatment at the University of Miami Ear Institute were identified through retrospective chart review (#20230698). Demographics (including comorbidities), SSNHL history, COVID-19 vaccination, and audiometric records were reviewed. Hearing outcomes were determined by pure-tone averages (PTA) and word recognition scores (WRS) from initial and final audiograms. Recovery was categorized based on AAOHNS guidelines as no response (≤10 dB PTA recovery), partial (≥10 dB PTA recovery or ≥10% WRS recovery or return to serviceable hearing, defined as both ≤50 dB PTA and ≥50% WRS), and complete (≤10 dB PTA and ≤10% WRS compared to the unaffected ear). We also defined slight recovery as ≥10 dB PTA recovery but GREATER THAN 50 dB PTA or ≥10% WRS recovery but LESS THAN 50% WRS. Those who received COVID-19 vaccination within 54 days of SSNHL onset were classified as temporally related cases of SSNHL, and all others were considered non-temporally related cases.

Results: The temporally related SSNHL group received 2.14 (± 0.38) COVID-19 vaccines. Most patients were female across both groups, with a mean age of 53.1 years old (± 15.1) and 52.4 (± 12.1) for the temporally related and non-temporally related groups, respectively. Interestingly, for the temporally related group, initial PTA in the affected ear was higher (63.8 dB [± 37.7]), though final PTA in the affected ear was lower at 35.9 dB (± 27.2). Among temporally related SSNHL patients, 42.9% (n=3) had complete recovery, 28.6% (n=2) had slight recovery and 28.6% (n=2) had no response. Among non-temporally related patients, 27.4% (n=65) had complete recovery, 11.4% (n=27) had partial recovery, 14.8% (n=35) slight recovery, and 6.4% (n=110) had no response.

Conclusions: Among those who had SSNHL and underwent recent vaccination, we observed worse initial hearing loss but greater recovery. While both viral infections and vaccinations stimulate immune responses, vaccinations may cause more controlled inflammatory reactions that help to explain their extremely rare temporal association with SSNHL. Additional research is

necessary to clarify the specific mechanisms underlying a potential temporal link between vaccination and SSNHL, as well as recovery patterns. This will enhance our understanding of individual susceptibility and prognosis.

SA120. Cochlear Neural Degeneration in Ménière's Disease: A Temporal Bone Study

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Category: Clinical Otolaryngology & Pathology

Background: Analysis of audiological data from GREATER THAN 80,000 patients with sensorineural hearing loss (SNHL) showed that threshold-adjusted word recognition scores (WRS) in Ménière's disease (MD) were markedly worse than those from patients with presbycusis or any other SNHL etiology. Here, we ask if this difference is associated with an exceptional degree of cochlear neural degeneration in MD patients.

Methods: We analyzed 99 human temporal bones from the Mass. Eye and Ear archive, including 45 ears with MD, 9 contralateral ears from unilateral MD patients and 45 age-matched controls with age-related hearing loss (ARHL). Mean ages were 80, 79 and 81 yrs, respectively, with similar numbers of males (n=48) and females (n=51). All had recent audiograms and most (64/99) had WRS data. Each archive slide set included every 10th section, 20 μm thick, cut in the horizontal plane and stained with H and E. Fractional survival of inner (IHC) and outer (OHC) hair cells and spiral ganglion cells (SGC) was evaluated in all sections, auditory-nerve (AN) peripheral axons were fluorescently labeled and counted in each half-turn where the osseous spiral lamina is tangentially cut, and strial area measurements and spiral ligament fibrocyte counts were taken at 14 evenly spaced locations along the cochlea.

Results: Throughout the cochlea, the survival of IHCs, OHCs, AN peripheral axons and the stria in the MD ears was roughly half that of the ARHL controls, whereas the unaffected ears of MD cases were similar to the control. Fibrocyte survival was identical in all three groups at all cochlear locations. Primary neural degeneration, i.e. the ratio of AN loss to IHC loss, was greater in MD ears, especially with advancing age. Despite the massive pan-cochlear loss of ANs in MD cases, SGC loss was greater than controls only in the extreme apex. Throughout the rest of the MD cochlea, most SGCs survived despite loss of their peripheral axons.

Mean audiometric thresholds across all frequencies were significantly worse in the MD ears (72.3 vs 48.1 dB). Mean WRSs were also worse (49.41% vs 66.06%), but the differences were not statistically significant. Multivariable linear regression suggested that, once PTA has been factored in, AN survival dominates the prediction of WRS in MD cases, whereas IHC survival dominates in the ARHL cases.

Conclusions: As predicted, AN degeneration is significantly greater in MD cases than in ARHL cases. However, MD is also characterized by greater IHC, OHC and strial degeneration throughout the cochlea. Although all these factors contribute to the poor thresholds and speech discrimination, the AN degeneration is the major driver of poor word scores in MD patients.

SA121. The Effect of Undamping Feedback Force on Otoacoustic Emissions Derived From a Nonlinear Cochlear Model

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Category: Otoacoustic Emissions

Background: Otoacoustic emissions (OAEs) are an objective tool for diagnosis of hearing loss. It was recently suggested to combine OAEs generated by reflection and distortion to create a joint reflection-distortion OAE profile [Abdala and Kalluri J Acoust Soc Am. 142(2):812 (2017)], which should improve the potential of OAEs to detect hearing loss and distinguish between different etiologies. Here we show how the undamping feedback force and the position of the operating point (OP) in the nonlinear function transforming this force affects click-evoked OAEs (CEOAEs) and distortion-product OAEs (DPOAEs).
Methods: We use a cochlear model which simulates the transversal displacement of the basilar membrane (BM). In addition, the model contains a second array of oscillators whose displacement transformed by the second order Boltzmann function is directly proportional to the force acting on the BM. To generate OAEs due to reflection, roughness is introduced into this undamping feedback force. Two different variants of the model are used differing in the cochlear gain estimated form the simulated BM input/output (I/O) functions. The gain difference is about 20 dB. Manipulation with the OP in the Boltzmann function also affects (decreases) gain of the cochlear amplifier because the default position is in the inflection point. DPOAEs are simulated by using a smooth cochlear model, i.e. without roughness. CEOAEs are simulated by subtracting the response for the smooth model from the response for the model with roughness.
Results: Simulated CEOAE amplitude increases with increasing click level until it saturates. The click intensity at this saturation shifts towards higher intensities for the model with lower gain and CEOAE amplitude declines. Similar effect is shown for the model with shifted OP regardless of the direction. Simulations do not indicate that the gain and the OP shifts affect the CEOAE I/O slope. Also DPOAE amplitude saturates with increasing stimulus intensity. However in this case, the gain of the cochlear amplifier has much smaller effect on the position of the compression knee than for the CEOAEs. Similar result is achieved if the OP is shifted towards in the negative values. In contrast, the opposite OP shift changes the DPOAE I/O slope. This slope gets steeper such that the DPOAE amplitude strongly declines at the lowest stimulus intensities but at the highest intensities the DPOAE amplitude is almost the same as in the model with default OP position. We accompany the simulated results with OAEs measured in normally hearing subjects and OAEs taken from literature.
Conclusions: The presented simulations indicates that the joint reflection-distortion OAE profile should be able to detect the change in cochlear amplification. In addition, it may also be useful for the detection of the OP shift especially towards positive values.

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SA122. Predicting the Etiology of Hearing Loss With a Joint (Reflection-Distortion) OAE Profile

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Category: Otoacoustic Emissions

Background: The aim of this study was to examine whether the Joint-OAE profile, which is a combined analysis of both distortion- and reflection-type otoacoustic emissions (OAEs), can distinguish between ears with mild hearing loss due to noise-exposure vs presbycusis. Reflection and distortion emissions arise via different cochlear generation mechanisms and have shown distinct sensitivities to hearing loss (Abdala et al., 2024). By measuring both OAEs together in each ear we hope to exploit the two generation processes and in doing so, improve the differential diagnosis of hearing loss.

Methods: 122 individuals/ears with mild-moderate hearing loss served as subjects. 75 of these had hearing loss primarily due to aging and 47 had hearing loss due to noise-exposure. Rapidly swept tones (calibrated in forward pressure level, dB FPL) were presented to evoke distortion-product (DPOAEs) and stimulus-frequency OAEs (SFOAEs) in an interleaved fashion. DPOAE and SFOAE metrics included amplitude at fixed stimulus levels and measures of OAE growth slope and compression across five octaves and at multiple stimulus levels. Analysis was two-pronged: (1) Descriptive statistics and ANOVAs were applied to test for group differences in OAE metrics between etiologies and (2) Machine learning was applied to probe how accurately combined OAE factors (i.e. predictors) were able to classify each ear with the correct etiology. The Random Forest Decision Tree algorithm determined and applied the optimal combination of 11 predictors to classify each ear as either NIHL or presbycusis. Predictors included: audiometric configuration (i.e. high-frequency sloping, flat, notched, rising, etc.), age, and nine OAE metrics derived from the Joint-OAE profile.

Results: Significant differences were observed between etiologies on amplitude-based OAE metrics. In aging ears, the DPOAE was always more reduced in level than was the SFOAE; in ears with NIHL, both OAEs were equally reduced. Ears with NIHL showed more "SFOAE loss" (OAE level re: normative values) than did aging ears, in particular between 2-4 kHz; hence, the SFOAE appears to be selectively sensitive to NIHL. Neither OAE metrics alone nor audio config + age considered alone were able to classify etiologies well. However, optimized factors generated from combined OAE metrics and age/audio configuration increased classification accuracy substantially (hit rates of $\geq$ 80%).

Conclusions: The DPOAE and SFOAE show distinct sensitivities to the same hearing loss. Exploiting these differences (which likely reflect distinct generation mechanisms), appears to enhance the differential diagnosis of hearing loss, although it is likely that accuracy here was limited by our relatively small cohort. Using the Joint-OAE Profile in differential diagnosis of hearing loss warrants further development. Identifying the dominant etiology in impaired ears could guide targeted intervention and assist in the selection of candidates for future genetic and/or pharmaceutical therapies.

SA123. Characterizing Level Growth Functions of Distortion Product Otoacoustic Emissions Evoked by a Fixed L1 Swept L2 Paradigm

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Category: Otoacoustic Emissions

Background: Distortion product otoacoustic emissions (DPOAEs) are used to study cochlear mechanics and cochlear health. DPOAE amplitude level growth functions (LGFs) are measured using a range of stimulus levels that vary in discrete steps or are continuously swept. Primary tone levels can be varied together (e.g., the common "scissors" paradigm), or one can be held fixed while the other is varied (e.g., the "fixed L1" paradigm). LGFs are typically fitted with a mathematical function from which slopes and thresholds can be calculated. The mathematical form of the function fit to LGFs may be chosen based on some hypothesized model of DPOAE growth. Our recent work shows that the fixed L1 paradigm generates LGFs with different growth characteristics than the scissors paradigm, suggesting a different growth model is appropriate for fitting LGFs generated by the fixed L1 paradigm.
Methods: We compared three underlying models of growth applied to LGFs obtained using the fixed L1 paradigm with continuously swept L2 levels. The models were: (A) a straight line with data on a linear-log scale (DPOAE amplitudes in Pascals against L2 levels in dB), slope and intercept free to vary, (B) a straight line with data on a log-log scale, with a fixed slope of one and intercept free to vary (linear growth), and (C) a straight line with data on a log-log scale, both slope and intercept free to vary. The fittings focused on measurements below the LGF peak amplitudes. We employed a stochastic fitting method which modeled the underlying DPOAE growth along with additive random noise. LGFs were obtained from humans and guinea pigs. Human LGFs were from 112 ears of 56 participants (ages 20-81) with a broad range of audiometric hearing sensitivity. LGFs were measured with the f2 primary near 1.5, 3, 7, and 14 kHz. Data from guinea pigs treated with salicylate or furosemide included LGFs measured with the same paradigm. All LGFs were obtained using forward pressure level calibrated stimuli on ER10X probe systems.
Results: LGFs from the fixed L1 paradigm were often poorly fit by model A, which has been commonly applied to data obtained with the scissors paradigm. For ears with normal hearing sensitivity, LGFs were well characterized by linear growth (model B). For ears with hearing loss, models B and C were equally likely to provide the best fit.
Conclusions: Findings were consistent with the hypothesis that different etiologies of sensory hearing loss (e.g., metabolic damage to the lateral wall versus mechanical damage from noise) are associated with DPOAE LGFs having different slopes. Guinea pig and human LGFs were similar, providing a useful model for further addressing this hypothesis.
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SA124. Comparing Infant and Adult Electrophysiological Responses to Pitch Changes With and Without Variations in Brightness

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Category: Development: Human Subjects

Background: Our prior behavioral results showed that infants outperform adults without musical training on pitch discrimination in the presence of random variations in brightness, and vice versa. One possible interpretation of this finding is that adults have learned the statistical covariation between pitch and brightness in natural sounds, which may yield more efficient coding, but produce poorer performance when expectations of covariation are violated. We follow up this behavioral finding by recording the mismatch negativity (MMN) in response to pitch changes with and without random variations in brightness using electroencephalography (EEG) in both infants and adults. Our hypothesis is that the MMN amplitude will be more susceptible to random brightness variations in adults than in infants.
Methods: Infants of 7 months were chosen because their behavior suggests that they have yet to learn statistical regularities in speech and music but have relatively stable electrophysiological MMN morphology. Each condition (with and without random variations in brightness) consisted of 1500 trials with the standard trials composed of harmonic complex tones with a F0 of 200 Hz. Two deviant tones were randomly presented on 20% of the trials, with the first deviant corresponding to a small pitch change (212 Hz F0; 10% of tones) and the second deviant corresponding to a large pitch change (250 Hz F0; 10% of tones). Each infant was tested over two visits to the lab. Adult participants were tested in a single session.
Results: MMNs to pitch changes (i.e., deviant tones) are being recorded in both infant and adult participants. Preliminary data indicate that it is possible to measure an MMN in both infants and adults, although the data are still too preliminary to provide a test of the primary hypothesis.
Conclusions: Comparing MMN responses recorded in infants and adults is a feasible approach which may help elucidate infant-adult pitch perception differences because it does not require a task or sustained attention, and thus, can be measured in the same way for both age groups, independent of attentional or task-based constraints.

SA125. Developing a Personalized Hearing Framework with CARFAC v3 and ANN Based on Performance on the Categorical Loudness Scale and Quick-VC Tests

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Category: Psychoacoustics

Background: A key issue in generating an accurate and explainable machine-learning (ML) model of an individual's hearing profile is employing a suitable perceptual model to link the output of the underlying auditory model with the behavioural data on which the ML model is trained. Here we describe an artificial neural network (ANN) to make predictions of an individual's performance on suprathreshold, psychoacoustic tasks, based on the modelled neural activity pattern (NAP) generated by a computational model of the inner ear. (Cascade of Asymmetric Resonators with Fast-Acting Compression model (CARFAC v3)). The psychoacoustic tasks include the Categorical Loudness Scale (qCLS) test (Rasetshwane et al., 2015) and the Quick-VC (qVCV), a consonant confusion task (Hajcek et al., 2023). These tests provide a quick and highly

informative assessment of a range of hearing impairments, and so may help provide a generalizable ML framework for modelling individual hearing loss.
Methods: Using a normally hearing CARFAC v3 (including a middle ear (ME) filter tuned using pure-tone audiogram data), we have trained and validated the ANN using cochleagrams generated in response to either pure tone stimuli of varying intensity (qCLS task) or vowel-consonant-vowel (VCV) phonemes embedded in speech-shaped noise at different SNRs (qVCV task). The ANN predictions (e.g., loudness estimate or consonant confusion matrix) have been compared against human performance. Once the ANN was built for a normally hearing individual, we froze the ANN model and tune the CARFAC model parameters based on the performance of hearing impaired individuals in the qCLS and qVCV tasks. Both CARFAC and ANN in this study have been implemented using the JAX Python library.
Results: We present our initial data indicating the suitability of our ANN model to predict the normally hearing and hearing impaired behavioural results. We additionally cross-validate data from the ANN for hearing impaired behavioural results with the predicted pure-tone audiograms generated using a simple d' neurometric model of auditory perception that also employs CARFAC v3.
Conclusions: These results represent key steps towards the development of individualized CARFAC v3 models of the hearing impaired cochlea and are discussed alongside the potential etiologies that CARFAC can model. Furthermore, we describe a framework that incorporates an ANN-based signal processing model as a means of developing novel acoustic stimulation strategies in personalized hearing aids.

SA126. Speech Perception in Noise for Cochlear Impant Recipients With Forwardfocus

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Category: Speech Perception

Background: ForwardFocus is a advanced noise reduction program from Cochlear Nucleus® that can simultaneously attenuate noise from multiple sources behind the cochlear impalnt (CI) recipients. In this study, we analyzed effect of ForwardFocus on speech peception in noise.
Methods: A total of 12 CI recipients (21 ears) were enrolled in this study. All participants were post-lingual hearing impaired patients who were implanted with a Cochlear Nucleus® CI. Patients measured speech perception in noise with ForwardFocus turned on and off, and the two results were compared.
Results: The mean patient age was 29.5 ± 10.6 years and the mean duration of CI use was 86.0 ± 54.7 months. Improved speech perception in noise was confirmed with ForwardFocus On. Compared to ForwardFocus Off, statistically significant results were found for ling 6 (97.9 vs 74.3), consonants (85.3 vs 63.1), vowels (72.8 vs 46.3), monosyllables (68.1 vs 38.1), disyllables (73.1 vs 45.9), and sentences (64.0 vs 35.1) test (p LESS THAN 0.05).
Conclusions: ForwardFocus seems to provide significant benefits for speech perception in noise. This noise reduction program is likely to provide significant improve speech performance in real-world listening for CI recipients, and clinicians should consider and consult the use of ForwardFocus as a way to improve speech perception in noise.

SA127. Intracranial Electrophysiology of Cortical Responses to Self-Generated Speech in Delirium

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Category: Speech Perception

Background: Cortical activity exhibits differences in responses to self-generated vs. externally generated speech. These changes – 'speaker-induced suppression' (SIS) and 'speaker-induced enhancement' (SIE) – are partly driven by auditory predictive coding mechanisms. SIS can be reduced in neuropsychiatric populations compared to healthy individuals, corresponding to a reduced ability to accurately distinguish between self- and externally generated stimuli. Delirium is an acute disorder characterized by disorganized thinking, inattention, and fluctuating mental state and level of consciousness. Executive function impairment in delirium may interfere with top-down predictive coding mechanisms associated with SIS and SIE. This study examined changes in cortical activity in response to self-generated speech during delirium.

Methods: Participants were adult neurosurgical patients undergoing intracranial encephalography (iEEG) monitoring for medically refractory epilepsy. This patient population is at risk for post-operative and post-ictal delirium. Delirium assessments (Confusion Assessment Method for the Intensive Care Unit and the 3-Minute Diagnostic Interview for the Confusion Assessment Method) were performed twice daily between 24-72 hours following electrode implantation and within an hour following seizures. Audio recordings of the assessment interviews and iEEG data were recorded simultaneously. Activity was recorded from multiple brain regions, including the auditory cortex, surrounding auditory-related areas, prefrontal, and sensorimotor cortex. Analyses focused on the high gamma (70-150 Hz) frequency band. Suppression indices (SI) were calculated for each recording site as the difference between the average high gamma activity measured during listening to the interviewer and during speaking, divided by their sum. SIs were compared between delirium-negative and positive conditions.

Results: SIs derived from delirium-negative assessments were comparable to those measured in more extensive conversation-based paradigms in the same participants. Both the SIS and SIE patterns were broadly distributed throughout the cortex. Delirium was associated with more pronounced SIS and weaker SIE. Sites in the dorsolateral and ventral prefrontal cortex that exhibited SIS in delirium-negative condition displayed SIE during delirium. By contrast, sites in the middle temporal gyrus and ventrolateral prefrontal cortex that featured strong SIE in delirium-negative condition showed SIS during delirium. Finally, delirium was associated with a reduction in SIE within precentral gyrus.

Conclusions: Disproportionate cortical activity elicited by self-generated speech during delirium may reflect impairments in executive control. Results reveal a regional heterogeneity of responses to self- vs. externally generated speech and suggest distinct changes within the temporal and prefrontal cortex that occur in delirium.

SA128. Individual Spatial Auditory Cognitive Patterns and Self-Construal

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Category: Speech Perception

Background: This study investigates listeners' biological responses to speech under different masking noise conditions during a spatial selective attention task and the relationship between the responses and listeners' self-construal, which is the psychological index regarding individual cognition, emotion, and motivation. We hypothesized that self-construal can be one of the criteria for an individual's spatial auditory patterns.

Methods: 50 subjects (19 from the US and 31 from Japan) with normal hearing participated in the experiment, measuring both behavioral and biological responses during a speech identification task with varying noise streams. The target speech stream was played through a front-left speaker, while masking noise, consisting of music and non-intelligent speech, was presented through either the front-right or back-center speaker. The target speech was played 1.5 to 3.5 seconds after the Masker sound started. The experiment was conducted in each language, English or Japanese, as well as the target stimuli.

The neural responses were recorded from the participants using a 21-channel Wearable Sensing DSI-24 electroencephalography (EEG) system. The biological reactions were analyzed using Event-Related Potentials (ERPs) for four distinct conditions. These conditions included two signal-to-noise ratios (SNRs: -18 dB and -12 dB) and two spatial positions of the masking noise stream. The EEG averages for each condition were calculated after onset timing adjustment, and the peak amplitude of ERPs was calculated by referring to local maximums of EEGs around 300 ms after the masker or target onset to detect P300, considered one of the most fundamental responses to sound stimuli.

Results: Participants were divided into two self-construal groups—interdependent and independent - using an online-based self-construal survey. After excluding three outliers and three neutral self-construal people, the ANOVA result of ERP amplitudes revealed a difference only in the interdependent group's ERP response between the two masker positions, with lower mean peak values observed for the front masker (simple effects for masker position and self-construal interaction, $p=0.0005$ for Cz). Furthermore, there were no significant differences in interaction between their languages and groups ($p=0.3882$ for Cz).

Conclusions: This finding suggests that the masker position only affects listeners with interdependent self-construal, regardless of their native language or the language of the stimuli, while independent listeners are less concerned with the noise source. As the next step, we will use more practical speech, conversation-like continuous speech stimuli to examine individual spatial auditory cognitive patterns. The cognitive pattern analysis will be more detailed, incorporating a wider range of spatial patterns with these realistic speech stimuli.

SA129. Sensitivity to Amplitude and Spectrotemporal Risetimes Predicts Phonological Awareness and Literacy in Children

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[1]*University of Cambridge*

Category: Speech Perception

Background: The amplitude envelope is the overall energy profile, which in speech is dominated by slow-varying, i.e., low-frequency modulations, for example, at the 'prosodic rate' of ~2 Hz AM and the 'syllable rate' of ~5 Hz AM. Amplitude rise times, (ARTs) are key to neural speech encoding. Individual differences in children's sensitivity to ARTs has been related to the development of children's phonological processing across languages by the Temporal Sampling (TS) theory. However, different ART tasks have been employed in different studies, languages and ages.
Study hypotheses:

- ART thresholds would be elevated in children with dyslexia.
- Individual's ART sensitivity would be similar irrespective of ART format.
- Frequency rise thresholds would also be elevated in dyslexia
- Individual differences in ART would relate to individual differences in phonological processing and literacy.

Methods: This was a 5 year longitudinal study. 88 native English-speaking with and without dyslexia took part (age approx. 8 years; Dyslexic n=58; age matched typical readers n=30). Children were tested on a yearly basis. An adaptive 3I-2AFC paradigm was used. Three ART stimuli based on synthetic syllables (/ba/), sine tones and speech-shaped noise were used. Children's sensitivity to Spectro-temporal (rising frequency) rise time and intensity was also measured.
Results: Linear mixed effect models of the longitudinal data showed a significant main effect of group with higher (worse) thresholds for dyslexics for all risetime measures. For the control measure, Intensity, there was a main effect of time point but no main effect of group. ART discrimination in all three tasks was significantly inter-related. Varying relations to phonology and literacy were found for different ART tasks at different ages. In particular, the sine tone and speech-shaped noise ART tasks showed greater sensitivity in older children, while the synthetic syllable rise task showed greater sensitivity in younger children.
Conclusions: Latent variable analysis supported the presence of a unidimensional latent variable 'rise time sensitivity'. For the first time with English-speaking children, greater sensitivity to rising frequency in a sine tone task was shown to be associated with better phonology and literacy outcomes measured eighteen months and 2.5 years later.
These findings are consistent with TS theory. In particular, the data suggest that in addition to ART sensitivity, sensitivity to slow-rate spectral modulations are also predictive of outcomes regarding the acquisition of phonology and literacy.

SA130. Spatial and Spectro-Temporal Resolution in Spanish and English-Speaking Populations

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Category: Speech Perception

Background: There is a lack of culturally and linguistically appropriate test materials available for assessment of hearing in non-English speaking populations. This places individuals with limited-English proficiency are at increased risk for reduced access to hearing healthcare. Spatial and spectro-temporal resolution are underlying mechanisms contributing to the processing of complex acoustic signals. Hearing loss (HL) impairs these processes, which is expressed in difficulty understanding speech and localizing sound sources. Classification of these perceptual and processing mechanisms may provide alternative methods to characterize hearing function and provide a more complete profile of hearing without the reliance on speech itself. We aimed to examine the relationship between spatial and spectral resolution with speech perception in English and Spanish speakers with HL.

Methods: This cross-sectional study consisted of 10 normal hearing controls and 10 hearing-impaired listeners with 10 being native Spanish speakers and 10 English speakers to assess the correlation between speech perception and non-linguistic test measures, speech perception was evaluated in English and Spanish using the Word Intelligibility by Noise (WIN). Spectro-temporal resolution was assessed through Spectral-temporally Modulated Ripple Test (SMRT) and the Random Gap Detection Test (RGDT). Stimuli were presented to each ear at 15-30 dB sensation levels above their 2 kHz threshold (dB HL). Sound source localization was used to assess spatial resolution using broadband noise bursts (0.5 to 20 kHz) presented at 70 dB(A) from 47 speakers ranging ±90° in azimuth and ±30° in elevation.

Results: Preliminary data show that spatial and spectro-temporal resolution impairments worsen with increasing degrees of hearing loss. Spanish speakers demonstrated significantly better word understanding than English speakers, even when controlling for level of hearing loss. Spectral and temporal gaps increased with increasing hearing loss and were associated with decreases in speech perception. Although spatial resolution decreases in impaired subjects, preliminary data suggest that spatial hearing alone is not predictive of speech perception outcomes.

Conclusions: The findings suggest that non-linguistic markers of hearing function, such as spatial and spectro-temporal resolution, could offer an increased insights and objective assessment of auditory impairments, independent of language proficiency. Additionally, these results align with previous work from our lab, which demonstrated that Spanish speakers performed significantly better than English speakers on speech-in-noise tasks, even when controlling for hearing loss. This indicates that non-linguistic markers may help identify underlying auditory system deficits that traditional, language-based assessments may overlook, leading to more equitable and effective diagnosis and treatment across diverse linguistic groups.

SA131. Decoding Speech and Music From Listened and Imagined MEG Recordings

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Category: Speech Perception

Background: Decoding speech and music from brain responses provides valuable insights into how the brain processes these complex signals. Recent studies have focused on decoding speech from brain activity in response to listened stimuli (Defossez et al., 2023; Yang et al., 2024), but there is still limited understanding of how imagined speech can be decoded. This challenge arises from the difficulty of obtaining large datasets and recording accurate brain responses during imagination due to uncertain timing. However, research has shown that imagining music evokes neural responses similar to listening (Di Liberto et al., 2021; Marion et al., 2021). Building on this, we aim to explore how imagined speech, using short poems, relates to listened brain responses, and estimate listened responses from imagery. Then we intend to apply MEG/EEG-to-text models to decode speech from imagined responses.

Methods: We recorded MEG data from 17 musicians (11 males, 6 females) as they listened to and imagined two melodies and two poems. Participants memorized the stimuli in advance to facilitate the imagery task. A visual metronome was used during recording to ensure accurate timing, and its effects were removed during data pre-processing.

Results: To investigate the encoding of acoustic features in brain responses to both listened and imagined music, we performed Multivariate Temporal Response Function (mTRF) analysis. The studied acoustic features included the audio envelope and the onset vector of words (in poems) or notes (in melodies). The mTRF analysis revealed significant encoding of both features in imagined and listened responses.

We employed a convolutional neural network architecture to map imagined MEG responses to their listening counterparts, which significantly outperformed a null model trained on shuffled trials. The model generalized to unseen recordings and performed similarly across music and poem tasks, indicating cross-task generalization.

In MEG-to-text decoding experiments, we presented speech as short poems and attempted to decode words based on brain responses. We processed the MEG data using a brain module incorporating a convolutional neural network with spatial attention mechanisms to extract meaningful intermediate representations of brain activity. These were aligned with corresponding speech representations, establishing a direct mapping between neural signals and acoustic features. Additionally, we employed a multi-alignment encoder-decoder approach inspired by Yang et al. (2024), aligning brain activity with both speech and text representations. By integrating multiple loss functions, our model refines the MEG-to-text decoding process, ultimately outperforming a null model with shuffled brain response–word embedding pairs.

Conclusions: Our analysis indicates that imagined MEG responses reliably encode acoustic features and can be used to decode speech. The stable and generalizable mappings enable us to predict listening responses from imagined data. These predicted listening responses can then be used to decode speech using models trained on large databases of listened responses rather than imagined responses.

SA132. Neural Correlates of Spectro-Temporal Modulation Detection: Insights From Cross-Species EEG Studies

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Category: Speech Perception

Background: Sensitivity to spectro-temporal modulations (STM) has been demonstrated to correlate with speech-in-noise intelligibility, particularly in individuals with hearing impairments. At a mechanistic level, deficits in the perception of spectro-temporal modulations have been linked with the broadening of cochlear filters, suggesting that these impairments may serve as potential biomarkers for sensorineural hearing loss (SNHL).

Recent advancements have led to the development of a clinically viable STM test (Audible Contrast Threshold, ACT) that dynamically adjusts the STM modulation depth. This innovative approach has been utilized to evaluate STM sensitivity in both normal-hearing and hearing-impaired listeners, yielding valuable insights into auditory processing capabilities and potential clinical applications for diagnosing and treating SNHL. This STM stimulus is also language-independent, allowing it to be used on diverse patient populations.

While existing studies primarily focus on psychophysical measures, there is a pressing need to identify the underlying neural correlates of these perceptual phenomena. To address this gap, we have begun to investigate the neural underpinnings of STM using the well-documented cortical phenomenon known as the Acoustic Change Complex (ACC). ACCs are evoked potentials generated in response to a stimulus change and are also known to be a good predictor of speech intelligibility.

Methods: We conducted cross-species studies involving humans and chinchillas by recording electroencephalography (EEG) signals in both species in response to temporally varying STM stimulus blocks. The stimulus structure comprised blocks of STM stimuli presented alongside broadband noise, creating sequences of spectrally structured and unstructured components that maintained equivalent energy levels. For human participants, we utilized a standard 32-channel EEG cap to record responses to the STM stimulus train. In lightly anesthetized chinchillas, we employed a custom-designed mini-EEG cap featuring closely spaced 32 channels, which covered the majority of the frontal region of the skull. We characterized and compared the ACC responses observed across different EEG frequency bands, examining multiple combinations of modulation depth and STM direction. Additionally, we implemented machine-learning techniques to curate relevant stimulus parameters that influence the response properties.

Results: We conducted spectral analyses and observed that variations in the spectral and temporal characteristics of the stimulus are differentially represented across various EEG frequency bands. Notably, transitions between low and high signal-to-noise ratio (SNR) stimulus blocks are encoded more efficiently than transitions between blocks with similar SNR levels. Our findings also indicate that certain features of the neural signals within specific frequency bands remain consistent over extended time scales, while other features exhibit neural adaptation.

Conclusions: In conclusion, we identified distinct neural signatures corresponding to varying stimulus parameters in both humans and chinchillas. These results highlight the perceptual

significance of the spectro-temporal statistics embedded in the stimulus, underscoring their relevance in the investigation of neural mechanisms underlying SNHL.

SA133. AI-Driven Automatic Speech Perception Scoring

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Category: Speech Perception

Background: Speech perception testing is a cornerstone for evaluating outcomes in cochlear implant (CI) users, guiding clinical interventions and long-term care (Holden et al., 2013; Gifford et al., 2008). This process typically requires participants to listen to words or sentences and articulate their responses, which are manually scored against the original speech materials. However, manual scoring is labor-intensive, introduces variability, and can lead to inconsistent results (Kuk and Lad, 2010; Wolfe and Gurgel, 2019). We investigated the potential of using Whisper, a high-accuracy, open-source speech-to-text tool (Radford et al., 2023; Sinha and Azadpour, 2024), to automate CNC word and phoneme scoring in CI users.

Methods: Two experienced CI users completed word recognition tests with six CNC30 word list pairs (Holden et al., 2013). Audio recordings were transcribed using Whisper, supported by voice activity detection (VAD, Bain et al. 2023) and grapheme-to-phoneme (g2p) (Park and Kim, 2024) mapping. The g2p function allowed for phoneme-level comparisons for accurate speech perception scoring by addressing variations in spelling and pronunciation. Orthographic transcriptions provided by CI users served as the benchmark for evaluating scoring accuracy of AI-driven transcriptions and manual scoring by expert human scorers.

Results: Across CNC list pairs, Whisper-produced transcription errors averaged at -1.38% ± 3.35% for words and -1.00% ± 1.87% for phonemes. Expert human scorers had mean transcription errors of 2.38% ± 2.71% for words and 2.11% ± 1.43% for phonemes. Speech perception scores based on orthographic transcriptions averaged 73.67% ± 4.3% and 40.08% ± 4.72% for the two CI subjects. We found no measured difference in the absolute error rate between whisper and human scorers (paired t-test, $p=0.38$). We also found no measured difference in variance in the error rates, between Whisper and human scorers (Levene's test for equality of variances, $p = 0.451$; and F-test for variance, $p = 0.755$).

Conclusions: Whisper demonstrates strong potential as an AI-based tool for automating speech perception scoring in CI users, closely matching the benchmark orthographic transcriptions provided by CI users themselves. Notably, our findings reveal that expert human scorers consistently overshot the orthographic benchmark scores while Whisper undershot it. However, absolute error rates between Whisper and Human scoring were statistically indistinguishable. By automating speech scoring, Whisper could significantly streamline clinical workflows and enable more consistent CI outcome tracking, particularly in large-scale or remote settings. While minimal manual adjustments were required for audio file formatting in this study, the process holds the potential to be fully automated. Future work will focus on expanding the subject pool to ensure broader representation of CNC word and phoneme score performances, integrating AI confidence measures to trigger manual review when needed, and testing additional speech-to-text models like NeMo Canary (Kuchaiev et al., 2019) to further improve accuracy and robustness.

SA134. Improving Speech Intelligibility and Reducing Listening Effort With a Deep Neural Network Based Noise Reduction System

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Category: Speech Perception

Background: Understanding speech in background noise poses a significant challenge for individuals with hearing loss. To address this problem, modern hearing aids enhance the signal-to-noise ratio (SNR) with directional microphones and digital noise reduction, yet listeners still report difficulty understanding speech in noise. While effective, these techniques do have limitations, which can potentially be overcome by deep neural network (DNN) based noise reduction. For example, directional microphones are limited by the spatial distribution of sound sources while DNN noise reduction is not.

Methods: The efficacy of a DNN based noise reduction was evaluated in two independent studies following the same protocol by quantifying speech intelligibility and listening effort. An experimental hearing aid, equipped with conventional and DNN based noise reduction, was compared to two types of commercially available devices, as well as to the experimental hearing aid with the DNN based noise reduction deactivated. Speech intelligibility was assessed using a modified Coordinate Response Measure task presented at -3 dB SNR in a background of steady-state speech-shaped noise. Speech was presented randomly from one of four speakers placed at 60, 120, 240 and 300 degrees in azimuth while noise was presented from all four locations, as well as from 180 degrees azimuth, simultaneously. Listening effort was quantified using the Adaptive Categorical Listening Scaling task using English Matrix Test sentences presented from 0 degrees in azimuth in diffuse noise.

Results: The results of the initial study demonstrate a significant improvement in speech intelligibility and reduction in listening effort for the DNN based noise reduction compared to both of the two commercially available hearing aids and the experimental device with deactivated DNN based noise reduction.

Conclusions: These results indicate that the DNN based noise reduction is more effective at improving speech intelligibility and reducing listening effort for a complex speech in noise task than previous technologies. By providing an SNR benefit without requiring a specific spatial configuration of the listener relative to the signal, technologies like this DNN based noise reduction can improve the daily user-experience of hearing aid wearers.

SA135. Relationship Between Subcortical Speech Encoding, Sustained Auditory Attention, and the Neural Signal-To-Noise Ratio

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Category: Speech Perception

Background: Individual differences in speech perception ability in the presence of background noise should come from a combination of variability in peripheral and cognitive processing. The cortical measure of neural signal-to-noise ratio (SNR), the amplitude ratio of auditory-evoked responses to target speech relative to background noise, has been applied to gain insight into the variability in speech-in-noise performance reported in our recent studies in various hearing populations. However, the extent to which peripheral and cognitive processing influence the neural SNR remains unclear.
Methods: Electroencephalographic and behavioral responses were recorded from 30 young adults with normal hearing during the speech-in-noise task, which involved monosyllabic English words embedded in noise at 0 dB SNR. In the same participants, we also recorded speech-evoked brainstem responses in quiet and noise and evaluated their sustained auditory attention ability in competing noise. The present study examined how these measures were related to each other to better understand the individual variability.
Results: The results from this study indicate that neural SNR serves as a predictor of individuals' speech-in-noise performance, with the greater the neural SNR, the greater the performance. Subcortical speech encoding ability measured with the f0 amplitude ratios between quiet and noise conditions significantly correlated with the neural SNR and behavioral speech-in-noise performance, whereas sustained auditory attention performance did not correlate with those behavioral and cortical measures.
Conclusions: The present study examined how subcortical speech encoding and sustained auditory attention distinctly contribute to the neural SNR and behavioral performance, providing novel insights into the influence of bottom-up speech encoding and top-down attentional ability on behavioral and cortical measures of speech-in-noise processing.

SA136. Time Course of Attention During Word-In-Noise Recognition in Cochlear Implants

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Category: Speech Perception

Background: In the typical auditory system, the brain exhibits strong alpha (8-12 Hz) oscillations when not actively engaged in auditory processing. When an auditory stimulus is presented, alpha activity often decreases (known as alpha desynchronization) as the brain actively processes the incoming stimulus. This alpha desynchronization can serve as a potential index of attention or engagement with a task. In the present experiment, we explore the potential for using alpha desynchronization during a speech-in-noise task to index cochlear implantees' engagement during auditory processing when isolating a target word embedded in multi-talker babble.
Methods: Ninety-five adult cochlear implant (CI) users (with a variety of device configurations) participated in the Iowa Test of Consonant Perception (ITCP) while 64-channel EEG measured cortical responses during the task. The ITCP is a four-alternative forced choice task in which eight-talker babble is presented for two seconds on every trial. After one second of babble, a

monosyllabic CVC target word onset amid the babble. One hundred milliseconds after the babble ended the four response options appeared on the screen (labeled one through four) and the participant indicated which word they heard using a keypad. The ITCP consists of 120 target words grouped into 30 item sets. Within an item set, response options differ only in their initial consonant. Each target word was presented in each of two signal-to-noise ratios (SNRs): High SNR (+15 dB) and Low SNR (+7.5 dB). Time-frequency analyses were performed for each subject and for the grand average across all subjects to identify changes in alpha-band activity during the noise-only period (0 second – 1 second) and the target-word period (1 second – 2 seconds) relative to a pre-trial baseline.

Results: Participants' accuracy during the task was highly variable. The mean accuracy in the High SNR condition was 71% (range: 31-97%) while the mean accuracy in the Low SNR condition was 57% (range: 23-90%). This is consistent with many past findings regarding speech perception outcomes post-implantation for CI patients. In both conditions, alpha band activity showed the strongest change from baseline (compared to other frequency bands) with an increase as background noise onset and a decrease in activity shortly after the target word onset (event-related desynchronization). There was a substantial amount of variability in the pattern of alpha activity across participants. However, there was no relationship between alpha desynchronization and accuracy on the task.

Conclusions: Within this large cohort of CI users, alpha band activity showed large individual differences suggesting that some participants were engaging more cognitive resources to complete the task. The lack of relationship between alpha desynchronization and accuracy may suggest that these resources are deployed as a compensatory measure when auditory perception is a challenging for a particular individual.

SA137. Predictable and Periodic Rhythmic Cues Facilitate Concurrent Speech Perception at Nominal Speech Rate

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Category: Speech Perception

Background: Entrainment and predictive coding underlie accurate speech perception in both quiet and noisy environments. Isochronous, periodic auditory cues can facilitate entrainment and temporal expectations which benefit encoding and perception of target speech. However, it is not clear which acoustic cues drive this effect. Most studies using isochronous cues confound periodicity with predictability, which have different implications for understanding entrainment and its perceptual benefits. Additionally, it is not clear how presentation rate and target phase interact with the rhythmic cue to impact speech perception. To this end, we characterized how systematic changes in the acoustic dimensions of stimulus rate, target phase, periodicity, and predictably of an entraining sound precursor affects the subsequent identification of concurrent speech targets.

Methods: Healthy, young adults identified double-vowel tokens (/a/ + /e/, /i/ + /e/, /i/ + /a/) during a ~45 min behavioral task. Concurrent vowel mixtures were preceded by rhythmic woodblock cues which were either periodic and predictable (PP) (isochronous rhythm), aperiodic and predictable (AP) (accelerating rhythm), or aperiodic and unpredictable (AU) (random rhythm). Stimuli were

presented in separate blocks for each rate (2.5, 4.5, and 6.5 Hz). Target speech tokens were presented in-phase (0 degrees) or out of phase (90 degrees, 180 degrees) relative to the preceding entraining rhythm. We computed accuracy as the percentage of time both vowels in a mixture were identified correctly, and recorded reaction time. Participants were randomized to unique block orders using a Latin square design to avoid order effects.
Results: Speech identification accuracy was only weakly impacted by the three acoustic manipulations across all blocks. However, participants whose first block used a 4.5 Hz entraining stimulus showed subtle improvements when the preceding rhythm was periodic and predictable vs. aperiodic and unpredictable (PP GREATER THAN AU). Reaction time speeds showed an interaction between rate and condition where participants responded fastest at the 4.5 Hz rate with the PP compared to AP/AU conditions. Rate and condition interactions were not observed at the other rates. Target phase also impacted reaction time, with fastest reaction times for the anti-phase (180 degrees) target.
Conclusions: Our results support the notion that entraining rhythms can enhance subsequent speech processing and do so optimally for speech rates around 4.5 Hz. We conclude that while periodicity and predictability are both important for facilitation of speech learning, periodicity may drive temporal speech perception benefits.

SA138. Musicianship Modulates Cortical (but Not Brainstem) Effects of Attention on Processing Musical Triads

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Category: Auditory Cortex and Thalamus: Human Studies

Background: Many studies have demonstrated benefits of long-term music training (i.e., musicianship) on the neural processing of sound, including simple tones and speech. However, the effects of musicianship on the encoding of simultaneously presented pitches, in the form of complex musical chords, is less well-established. Presumably, musicians' stronger familiarity and active experience with tonal music might enhance harmonic pitch representations, perhaps in an attention-dependent manner. Additionally, attention might influence chordal encoding differently across the auditory system. To this end, we explored the effects of long-term music training and attention on processing of musical chords at brainstem and cortical levels.
Methods: Young adult participants were separated into musician and nonmusician groups based on extent of formal music training. While recording EEG, listeners heard isolated musical triads that differed only in the chordal third: major, minor, and detuned (4% sharper third from major). Participants were asked to correctly identify chords via key press during active stimulus blocks and watched a silent movie during passive blocks. We logged behavioral identification accuracy and reaction times and calculated information transfer based on the behavioral chord confusion patterns. EEG data were analyzed separately to distinguish between cortical (event-related potential, ERP) and subcortical (frequency-following response, FFR) evoked responses.
Results: We found musicians were (expectedly) more accurate, though not faster, than nonmusicians in chordal identification. For subcortical FFRs, responses showed stimulus chord effects but no group differences. However, for cortical ERPs, whereas musicians displayed P2 (~150 ms) responses that were invariant to attention, nonmusicians displayed earlier and reduced

P2 during passive listening. Listeners' degree of behavioral information transfer (i.e., success in distinguishing chords) was also better in musicians and correlated with their neural differentiation of chords, assessed via pairwise differences in the ERPs.
Conclusions: Our data suggest long-term music training strengthens even the passive cortical processing of musical sounds, supporting more automated brain processing of musical chords with less reliance on attention. Our results also suggest the degree to which listeners can behaviorally distinguish chordal triads is directly related to their neural specificity to musical sounds primarily at cortical rather than subcortical levels.

SA139. A Differentiable Model of Speech Processing Combining Neuroscience Models and Deep Learning

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Category: Speech Perception

Background: As processes in auditory neuroscience are multi-stage and highly nonlinear, models of cortical auditory processing based solely on linear processes lack the expressivity needed to capture the full range of cortical representations. On the other hand, deep learning architectures and optimization methods allow us to train large, nonlinear models efficiently. While generic deep learning models are not interpretable, combining deep learning with signal processing through differentiable programming allows for high-performing and explainable models.
Methods: We build on a classical model of mammalian hearing and make it differentiable, allowing us to integrate signal processing-based models of hearing with deep learning frameworks. The model contains a cochlear stage (including frequency decomposition through a filterbank, nonlinear power-law compression, lateral inhibition, and low-pass filtering/downsampling) and a cortical stage (spectrotemporal filters). This combination results in an expressive, explainable model that is easily trained on as few as a few hours of data. Particularly, our model is differentiable all the way from the cochlear to the cortex, allowing parameters to be jointly fitted alongside deep learning model parameters.
To showcase the power and explainability of the model, we applied this model to speech processing tasks, including phoneme categorization and the cocktail party scenario. In each case, the model takes in waveform input and performs cochlear and cortical analyses sequentially followed by a neural network module that produces the desired output (phoneme categories or speech of a target speaker).

Results: First, we evaluated the differentiable models on their performance. Results suggested that our models surpassed typical deep learning approaches which are "black-box", were more robust when generalizing to noisy distributions, and performed well with limited training data.
Secondly, we interpret the trained model parameters. In both the cortical spectrotemporal modulation filters and cochlear compression filters, the learned distributions differed between models trained in quiet and noise. This may be informative to the study of speech perception in noise as well as the effect of noise exposure to auditory development. Current work is underway

to ablate the models and inputs to study what information was used in cochlear and cortical processing, and compare between our model and animal data.

Conclusions: We contribute a differentiable model of auditory processing, which can be fitted to a variety of hearing-related tasks and enables the study of model parameter distributions. Our approach also has clinical potential for hearing aid fitting.

SA140. Oscillatory Activity Changes in Executive Attentional Networks Following Neurofeedback Training of Talker- And Space-Based Selective Attention

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Category: Speech Perception

Background: Selective attention enhances cortical responses to attended stimuli while suppressing others, aiding speech-in-noise (SiN) interpretation. This process, known as attentional modulation, is key in managing auditory inputs. Our previous research has demonstrated that neurofeedback training paradigms can improve attentional modulation of cortical auditory evoked responses, yet the mechanisms remain underexplored.

Methods: In this study, two neurofeedback training paradigms were designed to enhance control over cortical auditory evoked responses, focusing on talker identity and spatial selective attention. For the talker identity-based task, participants listened to a female voice repeating “Up” and a male voice repeating “Down” simultaneously from a central speaker. In the space-based paradigm, “Up” and “Down” were repeated from left and right speakers using male or female voices. During both paradigms, participants received gamified neurofeedback, where attention to the "Up" stream caused an on-screen object to move upward, and vice versa. Each participant underwent neurofeedback training on two separate occasions, typically spaced two weeks apart.

Results: In the space-based experiment, participants showed stronger alpha oscillation in the dorsal pathway during the post-cue, pre-sound period in the second session compared to the first, indicating enhanced spatial inhibition to block inputs from the left side. In both paradigms, increased attentional modulation of oscillatory activities in the ventral pathway (talker identity) and the dorsal pathway (space) was observed after training, suggesting improved brain activity for predicting the target sound. Furthermore, there was a general improvement in the strength of attentional modulation of sound-evoked cortical responses.

Conclusions: These findings indicate that neurofeedback training improves top-down processing of attentional circuitry, specifically within the dorsal and ventral pathways. This offers promising insights into the use of neurofeedback to improve attention-based auditory processing, with potential applications for individuals with difficulties in SiN interpretation.

SA141. Probing Vertical Sound Localization, and the Role of Ear Movements, in Head-Fixed Behaving Mice

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Category: Binaural Hearing & Sound Localization

Background: The localization of sound in the vertical plane relies on encoding in the dorsal cochlear nucleus (DCN) of location-dependent spectral distortions induced by the pinna and surrounding body. It has not been possible to determine the precise roles of DCN cell types in sound localization due to methodological limitations. To address this, we recently identified genetic markers and created cre-recombinase mouse lines to access DCN cell types (Jing et al., 2023). To begin to probe their function in vertical sound localization we have developed a behavioural system supporting two spatial discrimination paradigms and an approach to ear tracking and analysis, which are compatible with simultaneous recording in the DCN.
Methods: The first paradigm is passive vertical spatial oddball stimulation. Noise bursts are presented at regular intervals from either of two speakers, located 30 degrees to the right of center and either 0 or 60 degrees above the horizon. In each session, sounds come from the elevated speaker on 90, 50, or 10% of repetitions. Pupil dilation is used to read out acoustic saliency. Mice showed greater dilation to rare sounds from the elevated speaker compared to the horizon speaker; this effect was not observed when the horizon sounds were rare. Increased pupil dilation to sounds from the elevated speaker was also evident, but to a lesser extent, in the 50% condition, suggesting both an oddball and a looming effect in the pupil response. To assess the role of HRTFs, we tested mice with intact pinna and compared to those with ears taped back. Preliminary results suggest that the oddball response is absent with taped ears. In the second paradigm, mice are trained to respond to sound from one speaker, but not the other, by licking for sugar water reward in a standard go/no-go paradigm. Target speaker assignment is counterbalanced across mice. Preliminary results indicate that mice can selectively respond to the trained speaker. Taken together, these results suggest the presence of both a looming effect and an oddball effect that depends on normal pinna morphology, illustrating that mice discriminate vertical sound location using pinna cues.
Results: We have also developed a pose tracking algorithm for mouse ears based in 'Lighting Pose' software (Biderman et al., 2024). The trained network tracks 5 points on the ear concurrent with the behaviour and pupil tracking. Using tSNE and PCA-based dimensionality reduction, then unsupervised cluster analysis, we find a range of ear states that differ in extent of retraction and protraction.
Conclusions: In ongoing work, we are comparing pupil to the ear positional data to understand the relationship between state domains and performing manipulation and recording of DCN cell types in conjunction with ear morphology manipulations to better understand how DCN accomplishes vertical sound localization.

SA142. Hoo's There, a Ghost? Coding of Phantom Sounds in Barn Owls

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Category: Binaural Hearing & Sound Localization

Background: Barn owls (Tyto furcata) are sound localization specialists and utilize binaural cues of interaural time difference (ITD) and interaural level difference (ILD) to infer horizontal and vertical locations respectively in space. These cues construct a topographic map of space in the midbrain, but the readout of this map is corruptible to a dearth of frequency information. Potential ITDs are inferred by phase-locked frequency-specific neurons, and cross-correlation is performed across frequencies to determine the true individual ITD. However, pure tones and narrowband sounds (spatially ambiguous sounds, or SAS) lack additional frequency information, resulting in detection of multiple ITDs (true and phantom sound sources). Electrophysiological studies in response to SAS demonstrate minimal side peak suppression, suggesting roughly equal representation of true and phantom sound sources in the barn owl map of space in the optic tectum (OT), the avian homologue of the mammalian superior colliculus. However, behavioral studies in response to SAS have demonstrated barn owl head-turns to a single source (phantom or true) on a trial-by-trial basis, suggesting downstream suppression of additional ITDs. We suggest that this downstream suppression may be facilitated by both the midbrain stimulus selection network (MSSN) and spatial-frequency preferences of neurons in the midbrain map of space.
Methods: We performed single unit recordings at high-frequency and intermediate-frequency SAS in OT, whose responses over 100 trials suggested intermittent suppression, and now perform recordings using multi-electrode arrays (MEAs) to capture SAS responses of OT neurons located at true and phantom sources simultaneously.
Results: Responses from single units suggest intermittent suppression, MEA recordings to be performed soon after time of abstract submission.
Conclusions: The MSSN and spatial-frequency preferences of OT neurons may influence selection of one sound source amongst multiple, further work is forthcoming.

SA143. Dendrite-Based Delay Lines in Sound Localization Neurons of the Medial Superior Olive

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Category: Binaural Hearing & Sound Localization

Background: In mammals, neurons of the medial superior olive (MSO) encode interaural time differences (ITDs) of sounds originating from different horizontal locations. To accomplish this task, MSO neurons segregate ipsilateral and contralateral excitatory inputs onto lateral and medial dendritic branches and signal their temporal coincidence through variations in firing rate. This spatial tuning requires a cell or circuit-based delay to offset acoustic disparities. The source of this "internal delay", alternately assumed to arise from axonal delay lines or postsynaptic inhibition, remains controversial. Here we consider that the dendrites themselves form delay lines.
Methods: We used 2-photon fluorescence-guided patch recordings to measure propagation delays from distal dendrites of MSO neurons to the soma (gerbils aged 17-40; recording range up to 150 µm from the soma). Compartmental modeling was implemented in NEURON using reconstructions of 40 fully intact biocytin-labeled MSO neurons from Bondy et al., 2020. Channel models were implemented as in Matthews et al., 2010 and Khurana et al., 2011. Synaptic conductances were modeled as alpha functions (τ=0.27 ms), and either activated individually at

dendritic locations every 2 µm or were activated in clusters of 4, covering 28 µm (Callan et al., 2023), with a release probability of 0.45 (Couchman et al., 2011).
Results: In paired dendritic and somatic recordings we observed that delays of simulated EPSPs propagating from distal dendritic regions to the soma were diverse and correlated with local dendritic morphology and membrane time constant, the latter of which varied between 170 and 730 µs. Morphological analyses of reconstructed MSO neurons revealed striking variations in the degree of asymmetry between medial and lateral dendrites. Across neurons, the degree of dendritic asymmetry formed a continuum of medially and laterally biased cells, with up to ~2 fold differences between sides in dendritic surface area and length. Accordingly, in NEURON models of MSO neurons, these morphological asymmetries translated into branch-specific distortions in the rise time and duration of EPSPs after propagation to the soma. Across different dendritic locations, rise times of propagated EPSPs at the soma could vary by more than 300 µs. Finally, in simulations of binaural coincidence detection in our population of MSO models, cells with dendritic asymmetries exhibited differences in best delays (BDs) of up to 150 µs, exceeding the physiological range of interaural time differences (ITDs) reported for the gerbil.
Conclusions: We conclude that electrotonic asymmetry in the bipolar dendrites of MSO neurons produces shifts in optimal binaural EPSP summation and BDs that span the ecological range of ITDs. Much like axonal input delay lines in the avian sound localization circuitry, dendritically based delay lines in the mammalian MSO provide a stable, structural mechanism for tuning individual neurons to sounds from different azimuthal locations.

SA144. Developmental Impacts on Neural and Behavioral Measures of Binaural Acuity in Normal Hearing and Electroacoustic Stimulation (EAS) Listeners

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Category: Binaural Hearing & Sound Localization

Background: Binaural hearing is integral to real-world listening. Previous work has investigated the developmental trajectory of binaural perception; however, relationships between neural and perceptual binaural hearing acuity across the lifespan are not fully understood. Assessing how neural and perceptual binaural hearing acuity develop in normal hearing listeners is a critical step in understanding how electroacoustic stimulation (EAS) in CI patients impacts binaural development. EAS leverages the broadband auditory access from the CI and preserved binaural acoustic hearing to maximize access to ITD and ILD information in the low-to-mid frequencies. Although extensive research has documented EAS benefits in post-lingually deafened adults, research on EAS outcomes remains sparse, particularly in children.
This poster presents preliminary data from an ongoing multi-institutional study that will comprehensively analyze neural and behavioral binaural hearing measures across a broad age range (5–80 years old) in NH and EAS listeners. We addressed the following questions:

1. How do neural and perceptual measures of binaural hearing change as a function of age in NH and EAS listeners?

2. Do neural measures of sound detection (i.e., cortical "onset" responses) mature differently from neural measures of binaural hearing (i.e., cortical acoustic change responses to ITDs) in NH and EAS listeners?

3. Can neural measures of binaural hearing acuity predict binaural hearing perception in NH and EAS listeners adult and pediatric listeners?

Methods: NH and EAS listeners (5-80 y.o.) were recruited from Vanderbilt University and the University of Texas. Behavioral assessments include binaural intelligibility level difference (BILD) and interaural time and level difference (ITD, ILD) thresholds at 250 Hz. Cortical electrophysiological assays measured sound detection (i.e., P1-N1-P2 onsets) and ITD discrimination (i.e., the acoustic change complex) to dichotic stimuli. All assessments were completed via insert earphones or circumaural headphones with acoustic hearing only (i.e. CI off).
Results: Age effects were observed for neural responses to sound detection and ITD discrimination. While both responses demonstrate similar development early in life, neural measures of ITD discrimination mature slower than onset detection, beginning ~ 10 y.o. In the EAS group, this delay is more pronounced, suggesting that EAS listeners experience a more significant delay in processing binaural cues. Neural ITD sensitivity was predictive of behavioral ITD sensitivity in adult EAS listeners; more pediatric data are needed.
Conclusions: Preliminary findings demonstrate age-dependent differences in neural sound detection vs. ITD discrimination, with ITD discrimination developing slower than detection in NH and EAS listeners. The more pronounced delays observed in the EAS group suggest that binaural cue sensitivity is more significantly affected in these individuals, particularly during development.

SA145. Impact of Cochlear Implants for Single-Sided Deafness on Head Movement and Resulting of Interaural Level and Timing Difference Cues During a Novel Localization Task

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Category: Binaural Hearing & Sound Localization

Background: Interaural level difference (ILD) and interaural time difference (ITD) are critical for discerning spatial auditory perception. These acoustical cues may be static, as in the case of a very short stimulus or when an individual's head movement is restricted, or dynamic, when a listener moves their head during an ongoing stimulus resulting in changing ILD and ITD cues. It has been previously demonstrated that allowing head movement during localization tasks can improve accuracy, with increased benefit at the extreme lateral targets located within the cone of confusion (Pollack and Rose, 1967; Thurlow and Runge, 1967). Though this advantage of permissive movement has been well established, the impact of these movements on dynamic acoustical cues has not been well characterized. Additionally, individuals with single-sided deafness (SSD) may not employ head movement strategies in the same manner, and the available acoustical cues would be expected to vary. Furthermore, these individuals are increasingly rehabilitated with cochlear implants (CI) and understanding how the complex integration of acoustical and electrical stimulation impacts head movements and resultant dynamic ILD and ITD cues remains unknown.

Here, we investigate and explore the impact of head movement on dynamic acoustic cues in a novel combined localization and speech-in-noise task.
Methods: Subjects with normal hearing or SSD were tested in a dark, semi-anechoic chamber equipped with a 24-speaker array. An orienting stimulus followed by Harvard IEEE sentences were delivered by one of 12 randomly selected target speakers in a background of pink noise presented at seven signal-to-noise ratio levels ranging from -10 dB to +10 dB. Participants were encouraged to move their heads freely and instructed to repeat target sentences and indicate perceived stimulus location with a button press. Head movement was continuously monitored using a custom, head-worn, electromagnetic tracking system, and probe-tube in-ear microphones captured real-time, ear-level acoustic input to each ear.
Results: Data will be presented comparing head movement patterns between subject groups. We will quantify dynamic, ear-specific acoustic input by characterizing the interaural level and timing differences during stimulus presentation at multiple timepoints. We will assess for effects of stimulus location and signal-to-noise ratio on performance patterns.
Conclusions: Our study offers in-depth insights into sound localization behavior in individuals with SSD and how head movement strategies may change with cochlear implantation. This study will uncover acoustic cue adjustments employed by individuals with SSD and evaluate how use of CI may change behavior and result in additional cue changes. Data presented here will inform the ultimate goal of understanding the impact of binaural hearing deficits, as well as help elucidate the benefits and limitations of mixed modality stimulation offered by cochlear implantation for SSD.

SA146. Contribution of Diotic and Dichotic Speech-In-Noise Test in Hearing Loss Diagnosis

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Category: Binaural Hearing & Sound Localization

Background: This study investigates the diagnostic potential of diotic and dichotic antiphasic combinasion for speech-in-noise (SIN) test. Conventional SIN tests using headphones typically present stimuli monaurally or binaurally in a diotic manner (identical signal). Recently, dichotic antiphasic presentation (different signals delivered to each ear) has been developed for SIN tests, mainly for screening purposes. In this study we explored the binaural intelligibility level difference (BILD) in normal-hearing and hearing-impaired participants. We tested two ways of measuring BILD using either distinct diotic and dichotic tests, or a unique test combining both presentation modes. The goal was to define normative values of BILD and determine the expected results across various hearing impairment.
Methods: The study involved 739 adult participants including 565 normal-hearing and 174 hearing-impaired. Of these, 135 were classified as symmetrical sensorineural hearing loss and 39 as asymmetrical or unilateral hearing loss. Measurements were conducted using the SoNoise SIN tests, with an Android tablet (Samsung Galaxy Tab A8) paired with calibrated circumaural Bluetooth headphones (Orosound, Tilde Pro C). Each participant was tested with diotic and

dichotic SIN in one combined test, giving one speech reception threshold (SRT) for each presentation mode. The difference between the two SRT provides the BILD.
Results: : The established normative values (4f-PTA LESS THAN 20 dB HL, and age ≤ 25 years old) for diotic, dichotic antiphasic and BILD presentations were -10.6 dB SNR (SD = 1.7), -18.4 dB SNR (SD = 2.3) and 7.8 dB (SD = 2.2), respectively. Statistical analysis revealed no significant discrepancies between these values and those obtained from the individual tests (Wilcoxon test for independent samples). The average test duration was 311 seconds (SD = 58).
For participants with symmetrical sensorineural hearing loss, the results were -4.2 dB SNR (SD = 5.3), -8.9 dB SNR (SD = 6.8) and 5.5 dB (SD = 5) for diotic, dichotic antiphasic and BILD respectively. For participants with asymmetrical hearing loss, the results were -4.1 dB SNR (SD = 3.8), -5.3 dB SNR (SD = 6.4), 1.5 dB (SD = 2.7) respectively. For participants with unilateral hearing loss, the results were -6.3 dB SNR (SD = 3.2), -8.8 dB SNR (SD = 5.2), 2.5 dB (SD = 2.7) respectively.

Conclusions: Combined presentation of diotic and dichotic antiphasic modes is a promising test to enhance hearing loss types classification. The relevance of BILD for hearing impairment evaluation seems accurate to quantify the masking release capacity of the patient.

SA147. Confidence in Sound Localization Reflects Calibrated Uncertainty Estimation

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Category: Binaural Hearing & Sound Localization

Background: Humans localize sounds using a combination of binaural and monaural cues. However, a sound's location remains ambiguous in many conditions. Because sound localization is often used to guide behavior, representing the uncertainty of a sound's location is likely to be critical to decisions about where and when to act. However, little is known about whether humans represent the uncertainty associated with a sound's location and whether any such representations are calibrated to the actual uncertainty of localization. To study these issues, we developed a new class of stimulus-computable localization models to enable the representation of uncertainty. We optimized the model for sound localization in natural conditions and then compared its uncertainty estimates to those of humans.
Methods: The model estimates a probability distribution (represented as a bivariate von Mises mixture density) over spatial locations, inferring the parameters of the distribution from binaural audio input. The model was optimized to maximize the likelihood of the ground truth location of each of a large set of training examples (binaural audio from spatially rendering natural sounds in noise, using the same method as Francl and McDermott). If a sound's location is ambiguous, the model should learn to produce a wider estimated distribution (this distribution could potentially even be multi-modal, e.g., if there is front-back ambiguity). We conducted an experiment on human listeners to measure their confidence in localization in different conditions. Participants localized white noise and pure tone stimuli and placed a bet (from 1-5 cents) on their answer. To simulate the same experiment on the model, we took the model's confidence to be the entropy of the distribution it estimated for a sound's location.

Results: Human confidence judgments varied across conditions, with lower bets for stimuli producing higher average localization errors. Specifically, bets were lower for sounds at peripheral locations and for pure tones compared to noise. This variation in confidence across conditions was largely mirrored by the model.
Conclusions: Humans have internal estimates of the uncertainty of their localization percept for individual sounds. These confidence estimates are similar to those of a model whose uncertainty representations are optimized for accurate localization, indicating that human confidence is normatively appropriate in this domain. The modeling framework provides a way to investigate confidence in this and other domains.

SA148. Determining the Contributions of Sound Localization Cues to Spatial Tuning in the Auditory Midbrain Using Individualized Head-Related Transfer Functions

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Category: Binaural Hearing & Sound Localization

Background: Firing rates of neurons in the central nucleus of the inferior colliculus (ICC) are sensitive to changes in left and right monaural spectra, interaural time difference (ITD), and interaural level difference (ILD) of sound. These four acoustical cues covary with sound source azimuth within the front horizontal plane and underlie neural sensitivity to azimuth. In the present study, the contributions of the four cues to azimuth tuning in ICC neurons were determined and analyzed with respect to each neuron's characteristic frequency (CF).
Methods: Broadband noise stimuli were presented to awake rabbits over earphones either binaurally or monaurally via virtual acoustic space using the rabbit's own head-related transfer functions (HRTFs). HRTFs were digitally manipulated in several conditions to fix some cues while allowing the others to vary naturally with azimuth in order to assess the contributions of each cue to azimuth tuning.
Results: Average neural data followed a pattern approximately divided between neurons with CF above ("high-CF") or below ("low-CF") 3 kHz. Firing rates of high-CF neurons to sources at azimuths ipsilateral to the brain recording site were either largely or completely explained by neural sensitivity to ILD and monaural spectra, not ITD. Firing rates of the same neurons to contralateral azimuths were largely explained by sensitivity to the contralateral-ear spectrum only. The lack of a contribution of ITD to azimuth tuning of high-CF neurons was not due to neural insensitivity to ITD; for example, most neurons were sensitive to changes in ITD within the physiological range during a condition where ILD and monaural spectra were fixed to those for a sound source straight-ahead. Firing rates of a minority of low-CF neurons were exclusively explained by ITD, whereas the majority were not exclusively explained by any particular cue. Interestingly, most low-CF neurons showed more influence of ILD than ITD on their firing rates despite the range of ILD across azimuths being relatively small for frequencies below 3 kHz (±7 dB). At all CFs, binaural interaction increased the sensitivity of firing rates to sound source azimuth, as measured by the d-prime between maximum and minimum firing rates within the azimuth tuning curve. Increased sensitivity was due to an increase in maximum firing rates at some CFs and a decrease in minimum firing rates at other CFs.

Conclusions: Altogether, results of the present study demonstrate that the firing rates of ICC neurons to a simple broadband noise stimulus are determined by a complex interplay of acoustical cues that differs with respect to the CF of the neuron and the azimuth of the sound source.

SA149. Spectral Weighting of Interaural Time Differences Near the Low-Frequency "Dominant Region"

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Category: Binaural Hearing & Sound Localization

Background: To localize a complex sound, the auditory system must extract and combine spatial information from relevant acoustic cues–e.g., interaural time differences [ITD]–across the multiple frequency components comprising that sound. Because the component-specific cues are neither equally informative, nor equally reliable, their contributions must be weighted appropriately. Substantial evidence has demonstrated that such weights strongly favor fine-structure ITD cues in a narrow band of frequencies around 400-800 Hz–the so-called "ITD dominant region". The outsized impact of those cues may explain a variety of frequency-dependent binaural phenomena, including the rapid decline in human ITD sensitivity with pure-tone frequency above 700 Hz.
This study adapts existing methods to measure spectral weighting functions (SWF) for fine-structure ITD across components within and around the dominant region. The objective is to provide a high-resolution estimate of its spectral shape, especially its upper-frequency slope.

Methods: Complexes consisting of seven tonal components spanning 200-1600 or 240-1920 Hz (half-octave spacing) were presented over headphones at 30 dB HL/component with ITDs applied to the waveform fine-structure of each component. Individual components were assigned ITD values drawn from a uniform random distribution (±100 μs range) centered on a base value of -200 (left), 0 (center), or +200 (right) μs. The base value, and the independent component deviations varied randomly from trial to trial. Participants lateralized each stimulus using a touchscreen interface. SWFs were computed by multiple linear regression of the rank-transformed responses onto the component ITD values, resulting in seven weights (one per component) per condition. Normalized SWFs (i.e. component-relative weights) were computed for group-level analyses.
Results: Group-level SWFs revealed a region of enhanced weight spanning approximately 400 to 1000 Hz. Weights appeared relatively flat within this region, although SWFs for individual participants were peakier than the group average. The upper frequency segment of the SWF was marked by a consistent drop in weights above 1000 Hz, although some individual SWFs revealed significant weight even at the highest component frequencies (1600 and 1920 Hz).
Conclusions: SWFs reveal a ~1 octave region that dominates ITD-based lateralization. Its range (400-1000 Hz) is consistent with previous descriptions [e.g. 400-800 Hz reported by Folkerts and Stecker 2022, JASA 151:3409-25], although the group-level peak of the function appears lower (600-700 Hz) than in that study (800 Hz). Relatively flat weighting within this range suggests a uniform region of binaural input rather than a single peaked "filter" [Goupell et al. 2024, JARO 25:377-85]. However, it is unknown whether this aspect reflects flat weighting or peaked

weighting that differs across individuals. Similarly, it is not known whether variability in the upper slope of the SWF implies access to fine-structure ITD above 1400 Hz or is attributable to edge effects in SWF estimation [Ahrens et al. 2020, JARO 21:485-96].

SA150. Behavioral Strategies for Spatial Speech Perception under Monauralized Listening

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Category: Binaural Hearing & Sound Localization

Background: As the signal-to-noise (SNR) becomes poorer, listeners will adapt the positioning of the head to enhance their ability to perceive and understand speech. In increasingly noisy or complex environments the acoustic-head-shadow and pinna filtering cues can be used to leverage the better-ear effect. This study aimed to explore listening behavior to optimize speech perception on a dynamic speech perception-in-noise task. We hypothesize that under acute monauralization, binaural listeners would adapt to use head rotation to enhance their ability to detect and focus on target speech. Further, we hypothesized that even when hearing was restored via a hearing device on the monuaralized ear, that remnant asymmetries would result in a continued reliance on better-ear behavioral strategies in listeners.

Methods: Head-tracking was used to investigate listener behavior to optimize the SNR under binaural and monaural listening conditions. A within-subject repeated-measures design was used in 15 normal hearing listeners under the following conditions: normal binaural hearing, monauralized hearing (plug+muff), and aided-monaurlized hearing. The aided-monauralized condition was achieved by adding a bone conduction device on the mastoid of the monauarlized ear to "restore" binaural cues. Target sentences were randomly presented from 0° and ±60° azimuth in the presence of diffuse babble noise. Participants were tasked to repeat the sentence of the target talker at decreasing SNRs, and were allowed to freely move their head. Speech reception thresholds were calculated for each speaker location. Velocity of the head movement was also recorded to examine response variability and the promptness of the response.

Results: Speech presented from the front (0°) and to the better-ear led to lower speech reception thresholds, and was easier for participants compared to when presented to the monauralized ear. There was greater cumulative compensatory head-orientation (in degrees) and increase in errors for signals presented to the monauralized ear compared to 0° and speech better-ear (p LESS THAN 0.05). A reduction in errors was observed under the aided condition, although did not reach that of the better-ear or binaural hearing condition (p LESS THAN 0.05). Response promptness is improved under binaural hearing and when aided compared to the monauralized listening condition.

Conclusions: The magnitude and time course of head-orientation during spatialized speech perception is increased when binaural hearing is disrupted with listening behavior demonstrating an orienting behavior favoring the better-ear. High error rates and extended time to orient under monauralized listening conditions suggests and adaptive strategy reliant on acoustic head-shadow cues. These preliminary findings demonstrate the disorienting effect on listeners when binaural cues are disrupted, highlighting the critical role of accurate sound localization in improving the SNR and overall task performance in complex acoustic scenes.

SA151. Beta-Band Brain Activity in the EEG Associated With the McGurk Effect: A Comparison of Normal-Hearing and CI Users

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Category: Multisensory Processing/Interactions

Background: The McGurk effect is a well-known phenomenon in audiovisual integration where mismatched visual and auditory inputs create a perceptual illusion, causing the listener to hear a different phoneme than the one presented. This effect occurs when visual cues, like lip movements, interfere with auditory speech perception. In normal-hearing (NH) individuals, the McGurk effect is more likely in noisy environments, where the brain compensates for missing auditory information by relying on visual input. In contrast, individuals with hearing impairments, especially cochlear implant (CI) users, rely more on visual cues in daily communication, potentially increasing their susceptibility to the effect. Despite its importance, it is unclear whether the neural mechanisms behind the McGurk effect are the same in NH individuals and CI users. This study aims to clarify whether brain activity during the McGurk effect differs between NH and CI users.

Methods: The study included the NH group (n = 26) and the CI group (n = 23). The NH group was divided into respondents who frequently experienced the McGurk effect and non-responders (Res-NH and nonR-NH subgroups). A 32-channel EEG system measured brain activity during the McGurk task and compared the results between the groups. All CI participants showed a high occurrence of the effect and were further divided into three subgroups: CI users with congenital deafness who use auditory-verbal communication (prelingual-auditory subgroup), CI users with congenital deafness who use total communication (prelingual-total subgroup), and CI users with postlingual hearing loss (postlingual subgroup). EEG results were compared across subgroups.

Results: Preliminary analysis in the NH group revealed a larger beta-band activity at Cz and Oz in the Res-NH subgroup than in the nonR-subgroup when lip movements were initiated before the onset of auditory stimulation. In the CI group, a beta-band activity at Cz and Oz induced by visual stimuli was more evident in the prelingual-total subgroup than in the other two. Compared across the NH and CI groups, this beta-band activity significantly differed between the prelingual-total subgroup and NH subgroups.

Conclusions: The results of this study suggest that NH individuals and the prelingual-total subgroup of CI users engage different neural mechanisms when processing conflicting audiovisual information, particularly in the beta-band frequency range. The increase in beta-band activity observed in the prelingual-total subgroup during visual-only stimulation is greater than in the NH group, including the Res-NH subgroups. It may reflect an anticipatory mechanism of audiovisual integration specific to the CI group. The congenital deafness and visual-weighted language communication might develop a compensatory process, where visual cues prime the brain to optimize auditory processing and enhance cross-modal speech perception.

SA152. Refined Auditory-Visual Spatial Integration in Musicians is Accounted for by a Bayesian Prior Rather Than by Differences in Sensory Precision

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Category: Multisensory Processing/Interactions

Background: A long-standing question in psychology and neuroscience concerns whether long-term training affects basic perceptual abilities. Recent work suggests that musicians have refined cross-modal temporal integration, a process that underpins multisensory perception, as musicians are better than non-musicians at detecting asynchrony between flashes and tones. Given that playing an instrument requires flexible spatial attention and precise cross-modal integration, we investigated whether musicians exhibit refined spatial integration.
Methods: Musicians (≥ 7 years training, n = 22) and non-musicians (≤ 12 months training, n = 22) localised brief noise bursts presented from a speaker array along the azimuth, where unimodal and bimodal trials were interleaved. On bimodal trials, a white dot was flashed at the same location as the sound or 10º leftward or rightward. Bimodal trials elicit ventriloquism, a form of spatial integration where auditory localisation is strongly biased towards visual stimuli due to the greater precision of visual localisation. Unimodal trials elicit recalibration, an aftereffect of ventriloquism that biases unimodal auditory localisation, and which provides insight into the adaptability of multisensory perception.
Results: Musicians were significantly less susceptible to ventriloquism than non-musicians, which was replicated in an additional experiment with an independent sample of 24 musicians and 21 non-musicians. Across both experiments, auditory accuracy, auditory precision, and recalibration were not significantly different between the groups. We then conducted a third, preregistered experiment where 35 musicians and 35 non-musicians localised both the sound and flash across a greater range of auditory-visual spatial disparities, and we modelled participants' sensory precision and prior tendency to perceptually "bind" the stimuli, via Bayesian causal inference. As hypothesised, musicians exhibited less ventriloquism than non-musicians, which was captured by a reduction in binding tendency rather than by differences in sensory precision, although the effect seemed qualitatively smaller and less robust than in the previous experiments. Again, recalibration did not differ between the groups.
Conclusions: Our results suggest that multisensory spatial perception differs in musicians, which is accounted for by a Bayesian prior, and that spatial recalibration is driven by the physical rather than perceived spatial disparity between sensory signals.

SA153. Towards Characterizing the Biophysical Properties of Dimorphic Afferents From Central Zones of Mouse Vestibular Epithelia

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Category: Vestibular: Basic Research & Clinical

Background: The vestibular system encodes a wide range of head movements through two afferent pathways that are defined by the regularity of their spike patterns. The most irregular-

spiking afferents encode high-intensity head movements and innervate the central region of the vestibular epithelia. Regional differences in spike-timing regularity are thought to be driven in part by greater low-voltage gated K+ channel currents (IKL) in central region afferents. Similarly, isolated somata of vestibular afferents (VGN) with high IKL have transient-firing spike patterns that are thought to underlie the irregular spiking of central region afferents. To test if the biophysical properties of vestibular ganglion neurons are correlated to region of innervation, we characterized the innervation pattern and biophysical properties of vestibular ganglion neurons from VgluT1-Cre mice crossed with Ai14 TdTomato reporter mice.

Methods: We identified Vglut1-Cre positive (TdTomato positive) neurons in whole-mount immunolabeled samples of utricles, saccule, and cristae using confocal imaging. We used antibodies against calbindin, calretinin, myosin VII, and beta-3 tubulin to identify the central zone afferents, pure-calyx afferents, and hair cells, respectively. We used perforated-patch methods to record from disassociated, cultured, vestibular ganglion neurons collected from VgluT1-Cre * Ai14 mice at two age ranges: juvenile (P11 to P19) and adult (P60 to P100).

Results: TdTomato positive calyces and boutons were observed contacting exclusively in the central epithelial regions in the utricle, saccule and semi-circular canal cristae. TdTomato terminals co-labeled with calbindin but were largely (though not completely) non-overlapping with calretinin. Thus, fluorescence in the Vlgut1-Cre*Ai14 mice selectively marks central zone dimorphic afferents. Patch-clamp recordings revealed that fluorescently labeled (putative central-zone dimorphs) were diverse in somatic size and biophysical properties. Our preliminary data includes recordings of 21 and 22 VGN from juvenile and adult mice, respectively. Of these, 12 were fluorescent (6 from juvenile and 6 from adult) and the rest were non-fluorescent (15 from juvenile and 16 from adult). Fluorescent VGN were not distinguishable from non-fluorescent VGN in terms of cell size as measured by membrane capacitance (Cm fluorescent juvenile = 13.1 ± 3.1 pF, Cm fluorescent adult = 14.4 ± 4.3 pF, Cm non-fluorescent juvenile = 14.2 ± 1.2 pF, Cm non-fluorescent adult = 13.9 ± 1.6 pF). Fluorescent VGN also shared significant variability in their firing patterns, which ranged from sustained to transient.

Conclusions: Our preliminary findings underline the significant diversity amongst dimorphic vestibular ganglion neurons originating from in the central epithelial regions. Ongoing work will assess individual currents of central region dimorphs by administering IKL blockers such as dendrotoxin, linopirdine, and efferent receptor agonist oxotremorine-M. In addition, we plan to selectively record from central-zone dimorphs in a semi-intact preparation to test for correlations between the dendritic arbor, ion channels, and in vitro firing properties.

SA154. Calcium Puncta and Dynamics in Hair Bundles of the Mouse Crista Ampullaris

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Category: Vestibular: Basic Research & Clinical

Background: Sensory hair bundles in the adult mouse semicircular canal crista ampullaris can reach 80 microns in length extending deep into the cupula and placing the mechano-electrical transduction (MET) channels well above the hair cell body. The precise location of MET channels, kinetics of Ca2+ signaling to the cell body, and homeostasis of Ca2+ in vestibular hair bundles are

not well understood. Here, we use a relatively slow genetically encoded Ca2+ indicator to identify sites of high concentration along the bundle associated with MET channel locations, and examine [Ca2+] kinetics throughout the length of the bundle associated with Ca2+ signaling and homeostasis.
Methods: Mice with the genetically encoded calcium indicator, GCaMP5G (JAX 024477), were crossed with Gad2-Cre transgenic mice (JAX 010802) to generate Gad2+/GCaMP5G. Anterior and horizontal canals were micro-dissected (Leica, M165 FC) from adult mice of either sex, in accordance to IACUC protocol. Images were acquired at 5-50 frames-sec-1 for 10-200 sec using a Bruker swept field confocal microscope. Analysis of fluorescence modulation was determined pixel-by-pixel using ΔF/Fmin over the entire time sequence.
Results: First generation transgenic Gad2+/GCaMP5G mice showed GCaMP5 in a subpopulation of type I hair cells located in the intermediate zone of the crista, as well as in a variety of supporting cell types and to a lesser extent calyx terminals. Bundles were up to 80 microns long, and when perturbed by fluid motion moved in a stiff glass rod-like motion, pivoting near the hair cell apex. Fluorescent puncta were visible starting ~10 microns above the cuticular plate and extended in discrete sequences of 4-8 puncta up to 40-60 microns above the cell apex. Spontaneous Ca2+ puncta modulated intensity over time, possibly due to movement of hair bundles and MET gating arising from uncontrolled fluid movement. [Ca2+] transients along the bundle exhibited features that varied between hair cells. In some records, [Ca2+] modulation in the apical cell body was instantaneous with puncta in the bundle. In other records, [Ca2+] introduced at one point increased further down the bundle at a speed consistent with diffusion.
Conclusions: The locations of GCaMP puncta along type I hair cell bundles in the crista indicate MET channels are distributed at the tips of stereocilia and can be up to 60 microns from the cell body. Despite their long length, vestibular type I hair cell stereocilia in the crista are stiff and pivot at the base with modest bending. [Ca2+] transients indicate small increases in Ca2+ concentration near the bundle tip instantaneously release Ca2+ into the cell body, most likely due to electrostatic interactions in the bundle. In contrast, large increases in [Ca2+] in the bundle diffuse more slowly over time, possibly because [Ca2+] saturates the environment and transport becomes diffusion dominated.

SA155. Identifying the Vestibular Pathology in a Chick Model for Congenital Vestibular Disorders

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Category: Vestibular: Basic Research & Clinical

Background: Children with syndromic, congenital vestibular disorders (CVDs) form an abnormal inner ear early in development that results in postnatal challenges in maintaining posture, balance, walking, eye tracking, reading, hand-eye coordination, and language acquisition. The abnormal inner ear in CVD children with syndromic disorders most commonly forms a sac-like structure with the semicircular canals missing or truncated. It is unknown how the malformed, sac-like inner ear affects the development of the vestibular neural circuitry.

Methods: To study these CVDs, this lab implemented a chick animal model that forms a sac-like inner ear like that seen in CVD children. In two-day-old chick embryos (E2), surgical manipulation in ovo by Anterior-posterior Rotation 180° of the Otocyst results in a sac-like inner ear on the operated side, the ARO chick. In the first synaptic relay for central vestibular signal processing, the vestibular ganglion neurons (VG) transmit signals from the vestibular hair cells to vestibular nucleus neurons in the medulla oblongata. Previous work by others using CT scans suggests that the number of VG neurons are reduced in CVD children, so this lab focused on determining whether VG neuron number is reduced during embryonic development or shortly after birth in the chick model. Chick embryo gestation takes 21 days, so VG neurons from ARO and age-matched normal chicks were counted two-thirds of the way through gestation at embryonic day 13 (E13), an important stage when the orthogonal position of the canals is apparent. Two approaches were used: (1) transverse, serial, Nissl-stained tissue sections visualized by light microscopy and analyzed using QuPath software, and (2) biocytin Alexa Fluor-labeled VG neurons, fixed and cleared for whole-mount preparations that were imaged by confocal microscopy and analyzed using Imaris software.
Results: In both Nissl and biocytin-labeled preparations, VG neuron number on the rotated side of E13 ARO chicks was slightly, but consistently reduced compared to E13 normal chicks, but the reduction was not significant (Students' t-test; p GREATER THAN 0.05). In ARO chicks, the vestibular ganglion did not form a compact mass like normal E13 VG and ganglion neuron counts were highly variable, as reported in CVD pediatric cases. Counts of H5 normal chicks revealed that the VG neuron population is stable in E13 chicks compared to H5.
Conclusions: Since VG neuron number is maintained in the normal range before birth in the ARO chick model, the work suggests that the peripheral vestibular neural circuitry remains relatively intact before birth so that postnatal intervention may be useful to treat the pathology. Ongoing VG counts in H5 ARO chicks will determine what happens to VG neuron number during the perinatal period.

SA156. Prosthesis Stimulation and the Flocculus Activity for Compensatory Saccade During the Head Impulse Test in the Vestibular Impaired Monkey

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Category: Vestibular: Basic Research & Clinical

Background: The vestibulo-ocular reflex (VOR) plays a critical role in gaze stabilization. Patients with vestibular loss cannot maintain their gaze on a target during head movement because the gaze moves with the head movement away from the target. The head-impulse test (HIT) detects this unstable gaze. In this test, a patient is asked to fixate on a target and the head is rapidly and unexpectedly rotated to stimulate targeted semi-circular canals. Patients with vestibular loss make two types of compensatory saccades during HIT, covert saccades and overt saccades. Covert saccades occur during head rotation, whereas overt saccades occur after the head has stopped moving. A patient who has acquired covert saccades shows an improvement in the Dizziness Handicap Inventory score, higher visual acuity, and less oscillopsia. This may be because the displacement of the visual image is decreased by the saccade. Also, vision is greatly diminished during the saccade so the retinal slip is perceived less. Thus, learning to generate covert saccades

is important for improving the patient's quality of life. To study the neural basis of these movements, we investigated saccade behavior during HIT in vestibular impaired monkeys and demonstrated that the monkeys made covert saccades.
Methods: To investigate the neural mechanisms in inducing covert saccades, we recorded unit activity from the right flocculus (a cerebellar cortical region that encodes vestibular signals) during HIT. We also stimulated the right horizontal semicircular canal with a vestibular prosthesis to attempt to facilitate covert saccade occurrence.
Results: We found that flocculus neurons exhibited a burst of simple spike activity for ~200ms after HIT onset and a pause later during ipsiversive covert saccades. For ipsiversive overt saccades, the simple spike did not exhibit a burst. For contraversive covert and overt saccades, there was little phasic simple spike activity. Complex spikes discharged for both ipsi- and contraversive retinal slips. We also found that vestibular neuroprosthesis stimulation facilitated contraversive covert saccades but not ipsiversive covert saccades.
Conclusions: The results of the recording experiments suggest that the mechanisms to induce covert saccades are fundamentally different from those of overt saccades. We speculate that the burst of simple spikes suppresses the vestibular nucleus neuron's activity and, in turn, facilitates agonist activity and suppresses antagonist activity. Thus, flocculus neurons may be involved in assisting covert saccades. In addition, the pause of simple spikes suggests that the covert saccade command signal is created outside of the flocculus. The firing of the complex spikes during covert saccades was unexpected, since it fires for contraversive retinal slip predominantly in the normal monkey. The results of the neuroprosthesis experiments suggest that vestibular prosthetic stimulation may be able to enhance recovery through facilitation of covert saccades.

SA157. Kinematic Behavioral Differences Provide a Video-Based Method for Sorting SLC and LLC Startle Responses in Larval Zebrafish

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Category: Vestibular: Basic Research & Clinical

Background: The escape response in zebrafish (Danio rerio) begins with the C-start, a "C"-shaped body bend. The fast and accurate encoding for initiating C-starts provides a model for studying reflexes, excitation and inhibition circuits during locomotion, and prey-predator relationships in vertebrates. Categorizing the two primary types of C-starts with distinct initiation pathways—the Mauthner cell-mediated short latency C-starts (SLCs) and the non-Mauthner long latency C-starts (LLCs)—is essential for studying zebrafish C-starts. Studies typically rely on the bimodal distribution onset latency to sort the two types, yet some SLCs occur within LLC latency range and vice versa. Another method relies on whole-animal electrical recordings to measure field potential (FP) parameters of C-starts as there is a distinct difference between the two types. FP recordings require specialized equipment and are performed on individual larvae, thus we developed a rapid method for sorting C-starts based on differences in the kinematics of the two types of contractions.
Methods: Optical stimulation evoked startle responses in transgenic larvae Tg(myo6b:ChR2-EYFP) with Channelrhodopsin-2 (ChR2) expressed in both ear and lateral line (LL) hair cells (N=32, n=221) and Tg(lhfpl5b:ChR2-EYFP) with ChR2 expressed only in the LL (N=11, n=51).

Using open-source platforms FIJI and R, Video Analysis of Counterbend Kinematics (VACK) was developed and high-speed videos (1-kHz frame rate) time-locked with FP recordings were obtained for verifying VACKs ability to sort SLC and LLC events where its accuracy was benchmarked by FP-based determination of C-start type.
Results: VACK is a two-step categorization method that first processes each video by extraction of the six initial frames of a C-start to generate five difference frames through subtraction of intensities per pixel from each preceding frame. Pixel-by-pixel intensity difference values were then used to quantify larval movement. The second half of VACK uses R to analyze pixel intensity data from FIJI and capture the existence of a counter bend during LLC C-starts, a distinct feature of movement not seen in SLCs. We hypothesized that counter bends occur due to the delayed muscle activation along the anteroposterior axis during slower LLCs, where the loss of synchrony also results in loss of the large amplitude event observed in FP recordings of SLCs. VACK identified this counter bend feature by tracking a trough of pixel intensity changes across each difference frame with an accuracy of 97%. Latency-based sorting performed on the same population of C-starts was 84.3%.
Conclusions: VACK generates more accurate predictions than latency-based sorting, does not require an electrophysiology setup needed for FP-based sorting, and accommodates free-swimming escape responses potentially (using machine learning models) from multiple larvae simultaneously. Further, our findings from C-starts recording from lhfpl5b:ChR2 transgenics reveal that in addition to probably roles in directionality and latency, the lateral line alone can initiate both SLCs and LLCs.

SA158. Effects of Repeated Exposures to Low-Intensity Blast Overexposure via the Ear Canal on Vestibular Function in Rats

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Category: Vestibular: Basic Research & Clinical

Background: As air-filled structures, the ears are particularly vulnerable to the impacts of blast overpressure waves. Vestibular impairments are a common outcome of blast overexposure. Our previous studies have shown that medium to high-intensity blast waves directed into the external ear canal of rats can damage both peripheral and central vestibular pathways. In addition to the severe blast injuries seen in combat, dizziness has also been reported following repeated low-level blast exposure in operational and training environments. This study investigates the impact of repeated low-level blast exposure on vestibular function in rats by assessing the vestibulo-ocular reflexes (VOR) and vestibular afferent spontaneous discharge and responses to head rotation and translation.
Methods: Under isoflurane anesthesia, adult female Long Evans rats were exposed to multiple low-intensity (10 psi) blast exposures via the left ear. The VORs were tested before exposure, and 1 to 28 days post-exposure. Horizontal eye movement in response to head rotation (rVOR, 0.2-4Hz) and translation (tVOR, 0.2-2Hz) were recorded to assess the canal and otolith function, respectively. A separate group of rats underwent single-unit vestibular afferent nerve recording to

assess changes in spontaneous firing rate, discharge regularity, and sensitivity to head movements. Immunohistochemistry was performed on a third group of rats to examine neuroinflammation in the central vestibular nuclei.
Results: VOR gains showed no significant change 1 -28 days post exposure. However, preliminary analysis of single unit recordings revealed that one day post-exposure, 49% of the recorded afferents exhibited significant modulations in response to head rotation or translation, compared to 72% in the sham controls, and 64% at 14 days post-injury. Additionally, the irregular afferents showed increased sensitivities to head rotation and translation, and greater irregularity.
Conclusions: The preliminary results suggest that repeated low-level blast exposure cause damage to the peripheral vestibular function in rats.
Supported by NIDCD R01 DC018919

SA159. Effects of Noise Exposure on Vestibular Function in Tmc2KO Mice

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Category: Vestibular: Basic Research & Clinical

Background: The transmembrane channel-like 2 (Tmc2) gene encodes a protein shown to be a component of the mechanotransduction channel complex in auditory and vestibular hair cells (Kawashima et al. 2011; Pan et al, 2013; Ratzan et al. 2024). Our previous studies demonstrated that the loss of Tmc2 affects both canal and otolith function with progressive and accelerated aging effects on the canal function (Sit et al., 2024, Ratzan et al., 2024). In the present study, we further investigate whether noise exposure affects canal and otolith vestibular function in Tmc2 KO mice.
Methods: Wild-type (WT) mice (C57BL/6, N=11) and Tmc2 KO mice (N=6) were used in this study. At 3-4 months of age, the mice were exposed to broadband noise of 200Hz-20kHz at 103 dB SPL for 2 hours in a reverberant chamber. Vestibular function was assessed before and after noise exposure (1, 3, 7, 14, 28, 60 day(s)) by measuring the vestibulo-ocular reflexes (VOR) during head rotation (0.2-4Hz, rVOR) and translation (0.2-2Hz. tVOR), which assess the canal and otolith function, respectively. Eye movements were recorded using an ISCAN video-based eye tracking system. Gains and phases of the rVOR and tVOR were calculated by performing a fast Fourier transform (FFT) on the de-saccaded eye velocity and head velocity signals.
Results: WT mice exhibited little changes in the rVOR and tVOR gains following noise exposure. The Tmc2 KO mice, however, exhibited significant reductions in the rVOR gains at frequencies from 0.2 to 2 Hz at 3, 7, and 14 days post-noise exposure. At 28 days post noise exposure, the rVOR gains of Tmc2 KO mice returned to baseline. Similar to WT mice, the tVOR responses of the Tmc2 KO mice did not exhibit significant changes after noise exposure.
Conclusions: While preliminary, these results suggest that the loss of Tmc2 increases the canal hair cells' vulnerability to noise trauma. Ongoing studies, including single unit recordings of vestibular afferents and morphological analysis, will further elucidate the roles of Tmc2 in the vestibular function. Supported by NIDCD R01 DC018919 and R01 DC008853.

SA160. Testing the Requirement of Peripheral Type I Vestibular Hair Cells for Motor Behaviors in Adult Mice

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Category: Vestibular: Basic Research & Clinical

Background: Mammalian vestibular organs contain two types of sensory hair cells - type I and type II - which are distributed throughout each sensory epithelium. Although their precise functions are still poorly understood, their distinct morphological and physiological features suggest they have unique roles in vestibular sensation. Determining the specific contributions of each hair cell type to vestibular function is challenging because, in most cases, both hair cell types signal to the same afferent neurons. Here, we ablated type I hair cells specifically in the peripheral zones of all vestibular organs using available genetic tools to investigate the requirement for these cells in various vestibular functions.

Methods: We used adult Fbxo2-CreERT2:Rosa26-loxP-stop-loxP-DTA (Rosa26DTA) mice, in which exogenously delivered tamoxifen induces lethal expression of diphtheria toxin in most type l hair cells and a few type II hair cells in the peripheral zones of all vestibular organs (McGovern et al. 2022). At 1-8 weeks post-tamoxifen, we validated hair cell ablation in maculae and cristae using immunolabeling for hair cell markers, and we assessed the impact of hair cell ablation on vestibular function using various behavioral tests and by examining vestibular stimulus-evoked cFos expression in vestibular nucleus neurons after off-axis centrifugation.

Results: Immunolabeling of Fbxo2-CreERT2:Rosa26-DTA mice showed ablation of approximately 90% of peripheral type I hair cells in all organs by 1 week post-tamoxifen and at all other timepoints. We detected no significant change in numbers of type II hair cells or central type I hair cells in any sensory organ over 8 weeks. Vestibular stimulus-evoked cFos expression in vestibular nucleus neurons was lost by 1 week post-tamoxifen, coincident with the loss of peripheral type I hair cells. Video analysis of mice in a bright, clean cage up to 4 weeks post-ablation showed no differences in locomotion (e.g., distance traveled or average velocity) between mice with hair cell ablation and controls. However, at all times post-tamoxifen, mice with hair cell ablation could not remain on the balance beam for more than a few seconds and had significantly reduced horizontal vestibulo-ocular reflex (hVOR) gains compared to controls. While mice with hair cell ablation immediately fell off the rotarod (in light and dark conditions) at 1 week post-tamoxifen, their performance improved over time, approaching control levels by 4 weeks. Observations suggest mice learn to lean against the wall to facilitate remaining on the rotarod after hair cell ablation. However, mice may have instead regained coordinated limb movements while still experiencing balance impairment.

Conclusions: Our findings indicate that peripheral type I hair cells are essential to maintain hVOR gains and some behaviors requiring balance and motor coordination but not for locomotion. Future studies will address requirements for the other hair cell types using a similar approach.

SA161. The Effect of Genetic Background on Balance Behavior in an USH1C Mouse Model

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Category: Vestibular: Basic Research & Clinical

Background: Usher syndrome (USH) is a genetically and phenotypically heterogenous genetic disorder that causes sensorineural hearing loss, vestibular areflexia, and retinitis pigmentosa. There are three main clinical types (USH1, 2, 3). Due to a founder mutation in the Acadian population of Louisiana
and Canada, there is increased frequency of USH1C, a severe USH1 subtype. The Acadian USH1C c.216G GREATER THAN A mutation (216A) is a splicing mutation that results in a truncated harmonin protein. Harmonin is a scaffolding protein that is essential for cochlear hair cell development and photoreceptor function. To study vestibular disease mechanisms of the 216A mutation, balance behavior in USH1C knock-in mice carrying the 216A mutation on three different genetic backgrounds was assessed over the course of 1 year.

Methods: USH1C mice on a congenic B6, 129S6, or B6;129S6-mixed genetic background were evaluated for balance behavior over a 1-year time course. USH1C, wildtype (WT), and heterozygous (Het) littermate mice were assessed using open field, rotarod, and balance beam analyses at 1-, 3-, 6-, and 12-months of age.
Results: USH1C mice on all three genetic backgrounds (B6,129S6, and B6;129S6-mixed) have significantly increased circling behavior compared with WT/Het controls at all ages tested; however, USH1C mice on a 129S6 background have significantly reduced circling behavior compared with USH1C mice on the B6 or B6;129S6-mixed backgrounds. Additionally, USH1C mice on the 129S6 and B6;129S6-mixed backgrounds had significantly reduced rotarod latency-to-fall and increased time-to-traverse the balance beam compared with WT/Het littermates at all ages tested. Interestingly, USH1C mice on the 129S6 background had an increased rotarod latency-to-fall compared to USH1C mice of the B6;129S6-mixed background at all ages.
Conclusions: USH1C mice showed significant deficits in balance behavior compared with age-matched control mice at all ages, however the magnitude of the deficit significantly differed among the genetic backgrounds, suggesting that genetics may modify balance disorder phenotypes.

SA162. Investigating the Transcriptomic Landscape of the Vestibular Ganglion

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Category: Vestibular: Basic Research & Clinical

Background: The canonical classification of the vestibular ganglion neurons in mammals is based on their synaptic morphology – calyx-only, bouton-only, and dimorphic-only. However, the

molecular signatures that underlie the classification of the neurons and whether this is reflected in the neuronal subgroups remain to be fully understood. The immediate goal of this project is to use transcriptomics to interrogate the diversity of vestibular ganglion neurons. Eventually, we hope to understand how vestibular hair cells and afferent synapses are formed and maintained, and the key molecular pathways that underlie these processes.
Methods: We utilized single-cell RNA sequencing to investigate the transcriptomic profile of cells in the vestibular ganglion. Bilateral vestibular ganglia were isolated from three C57BL/6 mice at two months of age, with a single run, using the 10X single-cell RNA kit. We utilized the Seurat analysis pathway for scRNA data in the R software package. Using a combination of manual annotation of known cell markers and the automatic annotation program, scCATCH, we assigned identities to the cell types of the vestibular ganglion. We further divided our assigned clusters based on identified neural subsets and performed further analysis for gene expression.
Results: Our initial dataset consisted of 32,285 genes from 3,020 single cells and after quality control metrics were applied, 17,370 genes from 2,564 were included in our final analysis. We found distinct clusters of putative neurons, glia, fibroblasts, endothelial, and vascular cells. Further analysis of the putative neuronal and glial sub-groups revealed clusters with differential expression of known neuronal and glial markers.
Conclusions: We have successfully generated single-cell transcriptomic data from the vestibular ganglion of adult mice and have begun to define the transcriptomes of its cellular components. Our next goals are to perform at least one more biological replicate and to explore the existence of transcriptionally distinct subgroups of vestibular ganglion neurons. We will use in situ hybridization to validate putative markers for neurons and glia in our dataset and perform integrative analyses with a separate set of data from vestibular hair cells to identify genes that may control patterns of afferent innervation in the utricle.

SA163. Calyx-Only Afferents in the Vestibular Nuclear Complex Are Chemically Diverse

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Category: Vestibular: Basic Research & Clinical

Background: The peripheral vestibular end-organs are innervated by regular and irregular afferent fibers that slowly and rapidly adapt to stimuli, respectively. The range of rapid vestibular stimulation to which irregular fibers can adapt is relatively broad, with some irregular fibers responding to a narrower range than others. This functional heterogeneity may have discernable underlying molecular origins. To begin to address this gap, calyx-only afferents (irregular fibers) were assessed in the vestibular nuclear complex (VNC). Calcium buffering proteins (CBPs) have been used to distinguish amongst vestibular ganglion neurons. Calyx-only neurons are excitatory. Since vesicular glutamate transporters (vGluT1, vGluT2, and vGluT3) have been used to define functionally distinct subsets of excitatory neurons, colocalization of calretinin with vGluT in the VNC may be useful in discriminating classes of calyx-only afferents. The only vestibular ganglion neurons that produce the CBP calretinin are the calyx-only cells. Interestingly, the only source of calretinin-positive terminals in the VNC appears to be from calyx-only neurons. Therefore, we

have combined calretinin and vGluT labeling to address the question of diversity amongst calyx-only neurons.
Methods: Following transcardial perfusion (4% paraformaldehyde), Sprague-Dawley rat brains were collected, post-fixed, cryoprotected, and serially sectioned using a freezing sliding microtome. Immunohistochemistry was performed for calretinin and either vGluT1, vGluT2, or vGluT3 in free-floating sections of rostral and caudal VNC subdivisions.
Results: Puncta immunolabeled for vGluT1-3, but not calretinin were found throughout the VNC. As previously reported, calretinin labeling was punctate and was also observed throughout the VNC. Similarly, the distribution of vGluT1-3 was localized to terminals and had a distribution that correlated well with previous reports. Across rostral and caudal VNC subdivisions, less than 15% colocalization of calretinin with vGluT1-2 was observed. However, the percent colocalization increased to 30 - 50% in caudal VNC regions for vGluT3.
Conclusions: Our results indicate that subpopulations of irregular afferent fibers exist and may originate in Scarpa's ganglion neurons that are known to project to the VNC. The results also suggest that the predominant glutamate transporter for these afferents is vGluT3. This transporter is often co-localized with other classical neurotransmitters. In the auditory system, vGluT3 is important for encoding sound intensity and timing. Perhaps vGluT3 similarly encodes information related to rapid head movements and adaptation to stimuli. The rate of adaptation to stimuli may be end-organ specific (e.g., canal vs otolith). Given that vGluT1-2 isoforms differentially relate to synaptic plasticity and fidelity, future studies should use the biochemical signature of vGluTs to evaluate the origin and contributions of these irregular afferents to vestibular function centrally.

SA164. Signal and Noise Characteristics in Response Discharge of Semicircular Canal Afferent Neurons

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Category: Vestibular: Basic Research & Clinical

Background: Neuronal noise in sensory systems is defined as any variability in the neural response not accounted for by an applied stimulus. It has long been known that vestibular afferent neurons naturally exhibit noise in their response discharge during head motion. This noise arises from the stochastic processes that generate spikes in the peripheral vestibular epithelia and distal dendrites, giving rise to an ensemble of heterogeneous afferents typically, as a matter of convenience, classified as regular and irregular based on their spontaneous discharge. spontaneous discharge. It has been suggested that certain vestibular ailments (e.g. NF2-related schwannomatosis, demyelinating conditions), are associated with elevated peripheral vestibular noise. If this is true, then an analytical framework for characterizing peripheral vestibular noise is needed to disentangle that found naturally from that caused by disease and associated with vestibular hypofunction
Methods: The objective of this investigation was to characterize the neural noise in semicircular canal afferent spike trains recorded from healthy anesthetized chinchillas during sinusoidal and naturalistic head motion. Noise measures were derived from stimulus-response coherence, signal-to-noise ratio (SNR), total harmonic distortion (THD), inter-trial precision, and probabilistic deep learning. This allows quantification and comparisons of noise found naturally in the vestibular

periphery, and establishes a platform for comparable analyses in models of vestibular hypofunction (i.e. ototoxicity and NF2). In this analysis afferents were categorized as either regular or irregular on the basis of their spontaneous discharge CV* being less than (regular) or greater than (irregular) 0.1.

Results: Despite differences in sensitivity measures for individual afferents regular and irregular afferents exhibited very similar measures of coherence (0.9 for regular vs 0.82 for irregular, $p = 0.34$), SNR (17 for regular vs 9 for irregular, $p = 0.14$) and THD (0.05 for the regular vs 0.07, $p = 0.2$) for sinusoidal stimuli (i.e. 1.6 Hz, 15 °/s peak velocity). Response spiketrains to midband stimuli representing regular and irregular afferents were used as inputs to a neural network model trained to predict head velocity. This analysis revealed that, in some cases, predictions of head velocity derived from regular afferents were more precise than those derived from irregular afferents (e.g. R-squared=0.88 and 0.48, respectively), despite the overall lower magnitude of response sensitivity.

Conclusions: The results from this investigation show that, despite the dynamic response heterogeneity across the ensemble of afferents, signal and noise properties of regular and irregular afferents were similar. Furthermore, these analyses suggest that high signal-to-noise characteristics may be important in providing the CNS with high-fidelity estimates of head kinematics. This analytical framework can be readily applied to dynamic spike train recordings from animal models of vestibular hypofunction to isolate components of the peripheral vestibular system associated with increased noise.

SA165. Identifying Disparities in Saccade Testing Among Individuals With Mild Traumatic Brain Injury

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Category: Vestibular: Basic Research & Clinical

Background: High precision eye-tracking devices have been developed to establish normative values and provide objective insights into the effects of mild traumatic brain injury (mTBI) for various oculomotor and vestibular tests. These assessments provide objective insight into sequelae following injury, as the acquisition and processing of visual stimuli is performed by specific areas brain areas. A significant focus of this battery is comprised of saccades, including random and anti-saccade testing. Previous work has shown that various elements of saccades such as latency, accuracy, and gain are disrupted in mTBI. However, our current understanding of more granular metrics in these tests, such as centricity, is limited. Therefore, in this study we aim to quantify discrete differences in saccadic measures, (e.g., endpoint error [EPE]) during centric and eccentric saccades in individuals diagnosed with acute mTBI.

Methods: Adults with mTBI (11M/15F) between 18-65 years of age were recruited from the University of Miami Health System LESS THAN =14 days of injury. Control participants (9M/15F) were approximately age- and sex-matched (n=50). Following informed consent (#20220448), participants completed the test battery via the ADVISOR-II system. ADVISOR-II is a head-mounted display with integrated eye-tracking, delivering a complete battery of

oculomotor, vestibular, and reaction time testing. Saccade tests were presented in one of two paradigms, wherein the discrete location of the visual fixation target relative to the participant's point of fixation and eye movement were either eccentric (i.e., indicates a movement away from center), or centric (i.e., indicates eye movements towards center). Mixed models were generated to ascertain the prognostic effects of time and demographics for individual saccade tests.
Results: For random saccades, the mean EPE overall (i.e., both centricity measures) was 2.99° ((± 2.19) among mTBI participants and 2.20° (± 1.08) among controls, and the mean eccentric EPE was 2.83° (± 1.31) among mTBI and 2.19° (± 0.93) in control. Similarly, the mean centric EPE was 3.28° (± 2.69) for mTBI and 2.23° (± 1.16) for controls. Among those saccades, there was a significant difference between groups in the EPE for the trials collapsing across centricity measures (t(df=46): 2.711, p=0.01), centric-only trials (t(df=46): 3.016, p=0.005), and eccentric-only trials (t(df=45): 2.049, p=0.05). Similarly, during anti-saccades, we observed a mean EPE of 6.88° (± 4.50) and 5.42° (± 3.71) for overall EPE, and in centric-only trials a mean EPE of 4.24° (±2.09) and 3.23° (±1.37) in the mTBI and control groups, respectively. We also found significant differences in endpoint error for overall EPE during both centric and eccentric trials (t(df=47): 2.0711, p=0.04) as well as in centric-only trials (t(df=46): 2.541, p=0.01).
Conclusions: We found novel group differences in individuals with mTBI, particularly during centric saccade-based tasks. These findings may be utilized as prognosticators for recovery following injury and enhance clinical assessment and intervention strategies.

SA166. Application and Usefulness of Multimodal AI for Otological Examinations Including Nystagmus

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Category: Vestibular: Basic Research & Clinical

Background: Artificial intelligence (AI) has progressed remarkably, and its application is progressing in various fields throughout society. To date, efforts have been made in the field of otology to classify images using deep learning, and its usefulness in the diagnosis of middle ear diseases has been reported when used for tympanic membrane images. However, AI diagnostic imaging, which has been mainly verified so far, requires learning using a large amount of image data, and how to use it in clinical practice has been a problem. In recent years, generative AI has rapidly spread as one of the deep learning models, and it has become possible to generate responses to imperative statements. In addition, the Generative Pre-trained Transformer 4 Vision (GPT-4V) enables image recognition, enabling multimodal analysis in combination with linguistic information. We used GPT-4V, a large-scale language processing model, to verify the accuracy of the diagnosis of otological diseases by combining patient information with otological examination images (tympanic membrane images, nystagmus videos).
Methods: Using tympanic membrane images and nystagmus videos of various ear diseases, information such as the patient's age, gender, and chief complaint was given, and then the diagnosis of GPT-4V and the doctor was compared. As for doctors, in addition to otolaryngology specialists, general pediatricians were also asked to make judgments.

Results: The percentage of correct answers for GPT-4V on tympanic imaging was higher than that of general pediatricians and lower than that of ENT specialists. This was especially true for high-difficulty images. The nystagmus video is currently being analyzed.
Conclusions: Although the correct response rate of GPT-4V on tympanic membrane imaging was not as high as that of the ENT group, it exceeded that of the pediatric physician group, suggesting the usefulness of disease screening in clinical settings other than ENT. In the future, further advances in multimodal AI technology and prompt development can be expected to improve diagnostic accuracy.

SA167. Development and Characterization of Blast Apparatus for Preclinical Auditory and Vestibular Research

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Category: Vestibular: Basic Research & Clinical

Background: With the rise of improvised explosive devices, blast injuries have been increasing in incidence in combat and civilian environments. The resulting rapid pressure changes cause significant damage to the ear; the most common symptoms reported after blasts hearing loss, tinnitus, dizziness, vertigo, and balance issues. These injuries carry long-term consequences and impose a substantial financial burden on patients and the health system. Developing a reliable, well-characterized blast apparatus is crucial for establishing a preclinical model to study injury mechanisms and explore potential therapeutic interventions following blast exposure.
Methods: A blast apparatus was constructed using readily available materials by a local machine shop. A 1:4 acetylene to oxygen ratio was used for complete combustion. A 3-D printed mount was constructed to standardize placement of the rodent and protect its eyes. The blasts were characterized by peak pressure measurements using ICP® pressure sensors (PCB Piezotronics) positioned at the free end of the blast tube and lateral to rodent ears when placed in the mount. Ambient temperature and humidity were monitored using a thermometer and hygrometer (VWR®) and kept constant at 20-25 degrees Celsius and 50-55%, respectively. Distance from the blast tube to the rodent mount was kept constant at 14.5 inches. A simple linear regression analysis was performed to evaluate the relationship between the number of loading cycles and peak pressure. A Two-way Repeated Measures ANOVA was conducted to analyze the effects of the number of loading cycles and the experimental day on peak pressure. Tukey's Multiple Comparisons Tests were performed to assess specific differences between individual loading cycles.
Results: The blast apparatus was modulated to produce peak pressure between 0.8 and 20 psi based on number of loading cycles. The generated peak pressures showed a positive correlation (r2=0.96) with the number of loading cycles, with significant increases observed between each incrementing loading cycle except for between 2 and 3 (p LESS THAN 0.001). Furthermore, peak pressure did not significantly vary when blasts were performed on different days nor when different operators performed blasts.

Conclusions: We constructed a blast apparatus generating reproducible and reliable blasts that could be translated to varying intensities of blast injury, allowing for the standardization of blast delivery. Our team will use this apparatus to study the functional, behavioral, morphological effects and mechanisms of blast injury in the preclinical rodent model. The design and validation of this system offer a flexible setup that can be adapted to explore other physiological systems and more severe blast injuries. This is a vital tool that can be used by researchers in our field to not only study outcomes but also develop therapeutic interventions and protective strategies.

SA168. Advisor II: Rapid Oculomotor, Vestibular, and Reaction Time Testing for Mild Traumatic Brain Injury (mTBI)

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Category: Vestibular: Basic Research & Clinical

Background: Mild traumatic brain injury (mTBI) results from trauma to the brain through either direct or indirect injury. Such injuries are often only diagnosed through subjective symptomatic assessments. The "Assessment and Diagnosis of Vestibular Indicators of Soldier Readiness" system (ADVISOR II) utilizes oculomotor, vestibular, reaction time, and cognitive eye tracking tests (OVRT-C) to provide portable, quick, and accurate quantitative diagnostic method for mTBI. Studies have shown reaction time and reflexive saccade latencies to be an accurate prognosticator of mTBI, particularly when combined with other objective indicators for mTBI, such as the OVRT-C test battery utilized by ADVISOR. This work seeks to investigate whether saccade latency and manual reaction time were associated with mTBI with the ADVISOR II device.

Methods: This study (IRB#20220448) was conducted with the ADVISOR-II virtual reality goggles (Charles River Analytics [Cambridge, MA]), which was equipped with integrated eye tracking, inertial measurement units, headphones, and a wireless Bluetooth controller. A selection of OVRT-C tests, neurocognitive questionnaires, and a computerized test were performed on mTBI patients and healthy age and sex matched controls. The reaction times and reflex latency values investigated are a subset of the OVRT-C data collected. All sessions were ≤12 days of injury, and sessions ~ LESS THAN 65% complete were removed . Pre-habituated/habituated gaze latency was was classified during predictive saccades. Shapiro-Wilks normality and hypothesis testing was performed to investigate group differences. Bland-Altman plots were generated to assess quantitative agreement between OVRT-C and neurocognitive tests. All descriptive statistics are reported as mean(±SD).

Results: Among mTBI participants (n=26; 15 female and 11 male), the average age was 25.6(±8.94). They were primarily White (61%), though 36% identified as Hispanic. Similarly, the control group (n=24) was comprised of 15 females and 9 males at a mean age of 26.4(±6.66) years. Controls were also predominantly White, and 46% identified as Hispanic. During anti-saccades, gaze latency was not significantly different in individuals with mTBI vs. healthy controls (W-value=283, p=0.119). For predictive saccades, there were also no overall differences between groups (W: 281, p=0.58), but we did observe significant differences (W: 321; p=0.029) in pre-habituated latency and in habituated latency (t(df)=40.8; p=0.711). Lastly, for random saccades, we observed no group differences (W-value=269; p=0.222). There were no differences between mTBI and controls during visual reaction time testing (W-value=290; p=0.575). Lastly, response

times to auditory stimuli following button-press were significantly different in both the right ear (W-value=353; p=0.052), and the left ear (t(df)=40.217; p=0.039).
Conclusions: Generally, isolated measures in an OVRT-C battery are poor indicators for mTBI. Auditory response time and pre-habituated gaze latency should be pooled alongside other tests and neurocognitive metrics to identify symptom clustering and other trends among individuals with mTBI.

SA169. Evaluation of a Universal Canalith Repositioning Maneuver to Resolve 3-Canal Ipisilateral BPPV: Physical and Computational Models

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Category: Vestibular: Basic Research & Clinical

Background: Benign paroxysmal positional vertigo (BPPV) is the most common cause of episodic vertigo and is usually caused by dislodged utricular debris that is free to move in the endolymph (canalithiasis) of the long-arm (LA) or short-arm (SA), or resting/adhered to the cupula (cupulolithiasis). Canalith repositioning maneuvers (CRMs) have high rates of success LA posterior canal (PC) BPPV, but patient-specific morphological differences and complex BPPV where particles appear in numerous canals at the same time can make diagnosis and treatment challenging. We previously introduced a universal CRM (uCRM) that was predicted based on numerical modeling to clear all three ipsilateral canals simultaneously in a single movement. Here, we examine the efficacy of the uCRM using both mathematical and physical models of human labyrinths.
Methods: Physical Models: Enlarged three-dimensional physical models of human membranous labyrinths were printed in clear plastic resin based on reconstructions from micro-CT images of osmium tetroxide stained tissue published previously. The labyrinth lumen was filled with a glycerol solution, and heavy debris (spheres) were introduced into the lumen to mimic sedimentation times in humans. Debris was positioned in each canal to simulate BPPV in the long arm (LA) and short arms (SA) of each canal separately and in combinations. Epley, modified Semont-plus and the uCRM were performed by positioning the model labyrinth in space relative to gravity using a programable robotic arm. Debris sedimentation was recorded using cameras. Mathematical Models: CRMs and debris sedimentation were simulated using computational models based on the same subject-specific labyrinth morphologies used in the physical models. The computational model was validated by comparison of results to measurements in physical models. Robustness of each CRM was further evaluated by introducing noise into the clinical execution of the CRM and into the initial orientation of the labyrinth in the temporal bone.
Results: The uCRM was robust to errors in hold positions and labyrinth orientation in the temporal bone, successfully clearing LA posterior canal, LA anterior canal and LA horizontal canal, as well as SA-PC BPPV even in the presence of 12° standard deviation error in labyrinth hold positions (GREATER THAN 90% average clearance). The Epley and modified Sémont-plus CRMs were successful in clearing LA-PC BPPV in the absence of errors, but were outperformed by the uCRM clearing all 3 ipsilateral canals especially in the presence of errors in labyrinth hold positions.

Conclusions: The uCRM examined here has potential advantages over current standards of care in cases of complex multi-canal canalithiasis, or in cases where the initial position of the debris is uncertain. The uCRM is robust to errors in hold positions and does not require a priori diagnosis of initial canalith position, advantages that might combine to improve overall outcomes in patients experiencing BPPV especially when performed by non-specialists.

SA170. Vestibular Speed Advantage: New Insights Into Ultrafast Nonquantal Synaptic Transmission in Vivo

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Category: Vestibular: Basic Research & Clinical

Background: The inner ear vestibular organs of mammals evolved a unique calyceal postsynaptic terminal that surrounds mechanosensory hair cells and supports both quantal and non-quantal (NQ) transmission. The non-quantal component is of interest as it includes an ultrafast synaptic current from the type-I vestibular hair cell to their calyx partners, which are the dominate receptor subtypes in primates and presumably humans. It is currently unclear whether the synchronization of vestibular afferents and the generation of short latency vestibular potentials arises from quantal or NQ synaptic transmission, and what the interaction of these two transmission modes is. Understanding calyx synaptic physiology is important to understand vestibular health and disease and to uncover important ecological functions such as rapid compensatory reflexes important for terrestrial locomotion.

Methods: This work examined the hypothesis that synchronized vestibular action potentials are generated by NQ transmission in the mammalian inner ear balance system. Experiments were conducted in anaesthetized adult guinea pigs, with animal ethics approval from Macquarie University (#2023/001). Experiments compared cochlear and vestibular evoked potentials to determine differences in synaptic transmission modes in vivo. Evoked response measures from the inner ear included mechanical and electrical compound action potentials, nerve neurophonics, and hair cell microphonics. The sensitivity of the afferent system was further assessed using direct mechanical measures in vivo, such as laser interferometry.

Results: We present several lines of evidence supporting the hypothesis that NQ transmission is responsible for synchronized vestibular afferent responses in vivo. Unlike cochlear nerve responses, responses from the vestibular nerve were insensitive to several neurotransmitter (glutamate) blockers acting between the hair cells and postsynaptic afferent terminal. Latency comparisons between presynaptic vestibular hair cell activation and postsynaptic neural responses reveal that synchronized vestibular nerve responses occur without measurable synaptic delay and are over three times faster than auditory nerve counterparts. Using a paired-pulse interval paradigm designed to deplete the readily releasable pool of synaptic vesicles in inner ear hair cells, we reveal that synchronized vestibular nerve responses are unaffected by brief paired-pulse intervals, whilst cochlear nerve responses are significantly attenuated. Vestibular nerve neurophonics had larger relative amplitudes than cochlear afferents at higher frequencies, indicative of better phase locking performance. Vestibular nerve neurophonics also lacked pronounced adaptative features observed

in the auditory nerve neurophonic, which typically have two exponential time constants, indicative of neurotransmitter depletion.
Conclusions: Data demonstrate the most sensitive vestibular afferents are remarkably fast, much faster than their auditory nerve counterparts. Results reveal the vestibular speed advantage arises from ultrafast NQ electrical synaptic transmission from type I hair cells to their calyx partners. Our data support the hypothesis that the fast component of NQ transmission at calyceal synapses is indefatigable and responsible for ultrafast responses of vestibular organs evoked by transient stimulation.

SA171. Regeneration of Hair Cells and Restoration of Vestibular Function by Notch Inhibition

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Category: Vestibular: Basic Research & Clinical

Background: Notch signaling plays multiple roles in the development of inner ear hair cells (HCs) and guides the distinctive fates of cochlear and vestibular hair cell types. Here we assessed the role of Notch in cell fate determination of type I vs. type II hair cells within the vestibular sensory epithelia.
Methods: We employed genetic and pharmacological approaches to modulate Notch signaling in an adult mouse model of hair cell ablation. We conditionally deleted Notch1 from Plp1-expressing supporting cells in DT-ablated Pou4f3DTR/+ mice and performed lineage tracing and quantitative analysis to assess cell fate in response to altered Notch signaling. To compare Notch1 knockout to an alternative means of Notch inhibition, we quantified type I and type II hair cells and measured the vestibuloocular reflex (VOR) after administration of a gamma-secretase inhibitor locally via the round window membrane. Vestibular function was assessed by the vestibulo-ocular reflexes (VOR) and single vestibular afferent spontaneous discharge and responses to head rotation and translation.
Results: Our findings reveal that targeted manipulation of Notch leads to a significant increase in cells expressing hair cell marker, Myo7A. Conditional Notch1 knockout resulted in a complete replacement of type II HCs (Myo7a+TNC-) with a partial replacement of type I HCs ((Myo7a+TNC+). The changes in HC number were associated with a significant functional improvements as assessed by the VORs at 2- and 8-months post HC ablation. Single unit recording revealed that newly formed hair cells successfully re-established afferent connections. Similar results were observed following the local application of a gamma-secretase inhibitor, restoring near-normal vestibular function across all frequencies of rotational and translational VOR.
Conclusions: This work demonstrates a role for Notch signaling in fate determination of vestibular hair cells and may comprise a new approach to therapies for balance disorders related to loss of hair cells

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SA173. Socio-Demographic Barriers to Vestibular Rehabilitation Therapy for Schwannoma Patients in South Florida

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Category: Vestibular: Basic Research & Clinical

Background: Vestibular schwannomas (VS) are the most common benign peripheral nerve tumors in adults and a cause of vestibular dysfunction. Symptoms of VS may include dizziness and vertigo, which can be debilitating for patients. Management of VS may include vestibular rehabilitation therapy (VRT), which has been shown to alleviate symptoms and improve quality of life. Unfortunately, socio-demographic barriers to receiving VRT in individuals diagnosed with VS are currently unknown. Therefore, we investigated the relationship between various sociodemographic factors and VS patients' adherence to receiving therapy in order to improve future VRT compliance and post-treatment outcomes.

Methods: Through a retrospective chart review (#20230698), we examined a subset of VS patients (n=41) who were recommended VRT at the University of Miami Health System. Basic demographic data, including age, race/ethnicity, sex, insurance type, and prior treatments were documented. Descriptive statistics were generated for continuous variables (i.e., age, # of VRT visits) and categorical variables (i.e., race, sex, insurance type). Simple logistic regression was performed to ascertain whether independent predictors impacted the likelihood of VS patient participation in VRT.

Results: Overall, we analyzed the charts of n=41 patients who were diagnosed with vestibular schwannoma who had been recommended VRT. Most patients identified as White (48.7%) or Hispanic or Latino (40%). Patients were predominantly female (65.8%) and married (41%). The majority of patients receiving VRT had private insurance (63%), including BlueCross (34%), United (12%), Aetna (7%), and Cigna (5%). The remaining 37% of patients had public insurance (i.e., Medicare). About 37% of all patients who were recommended therapy participated in VRT; the number of VRT sessions attended ranged from 1 to 47, with an average of 11.4 (± 11.96) sessions. Among those who participated in VRT, 81% demonstrated improvement, which was corroborated by agreement among patient self-report and evaluations by their vestibular therapists. Following logistic regression, we found that neither insurance type (OR = 1.26, 95% [0.23, 1.16], p = 0.73), age (OR=1.00, 95% [0.95, 1.06], p =0.97), nor biological sex were significant predictors (OR=0.50, 95% [0.39, 5.72], p=0.55) of VRT attendance. Identifying as White was also not a significant predictor (OR=1.24, 95% [0.11, 28.22], p=0.87) for attendance.

Conclusions: Based on our analysis of n=41 patients, we found that insurance type, age, sex, and ethnicity were not significant predictors of VRT participation for those with vestibular schwannomas. While VRT patients receiving VRT in our retrospective cohort represent diverse cultural and economic backgrounds, the low overall compliance rate (36.6%) underscores the critical need for multi-disciplinary teamwork and clinical education regarding the benefits of VRT.

SA174. Cytomegalovirus Infection Effects on Vestibular System

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Category: Vestibular: Basic Research & Clinical

Background: Infection with congenital Cytomegalovirus (cCMV) is suspected to affect the vestibular system, which is essential for balance and spatial orientation. The vestibular system resides in the inner ear and includes the semicircular canals and otolith organs: the utricle and saccule. The semicircular canals detect rotational movements, while the otolith organs sense linear acceleration and head position relative to gravity. The aim of this study is to characterize the phenotype of CMV- induced vestibular dysfunction.

Methods: BALB/c mice were used to model congenital CMV infection. Mice were divided into two groups: a treatment group that received an intracranial injection of 2000 plaque-forming units (pfu) of murine CMV on postnatal day 3, and a control group injected with saline.

Vestibular and auditory function were evaluated as the mice matured, using various methods such as the Forced Swim Test (FST), vestibular sensory evoked potentials (VsEP), auditory brainstem response (ABR) testing, scanning electron microscopy (SEM), and immunohistochemistry analysis.

FST assessed depressive-like behavior in mice by measuring immobility time during a six-minute swim, with increased immobility indicating behavioral despair. Vestibular function was evaluated using VsEP to assess responses in the utricle and saccule to linear acceleration. SEM and Immunohistochemistry were used to visualize structures in the temporal bone and vestibular system. ABR testing was used to evaluate hearing sensitivity.

Results: At around six weeks of age, CMV-infected mice exhibited longer periods of immobility in the Forced Swim Test (FST), indicating depressive-like behavior compared to control mice. However, motor coordination, assessed through the rotor rod test, did not differ significantly between the two groups. Vestibular sensory evoked potentials (VsEP) testing revealed significantly higher thresholds in CMV-infected mice, elevated by approximately 7 dB, along with delayed peripheral peak latencies, both of which indicate vestibular dysfunction. Auditory brainstem response (ABR) testing showed elevated thresholds at 8 kHz and 16 kHz, suggesting hearing loss in CMV-infected mice.

Scanning electron microscopy (SEM) further demonstrated structural abnormalities in the lateral and anterior cristae of the semicircular canals in CMV-infected mice, which contrasted with the normal morphology observed in controls. Notably, this damage persisted even in mice co-treated with D-methionine, a compound hypothesized to protect inner ear structures. Despite this damage to the cristae, immunohistochemistry did not reveal significant harm to the utricular hair cells, suggesting selective damage to specific vestibular structures within the inner ear.

Conclusions: These results indicate that CMV infection disrupts the vestibular system, as evidenced by both functional impairments and structural abnormalities in infected mice. This study provides a basis for future research to better understand how cCMV affects vestibular function, which could help inform treatments for the balance and hearing deficits associated with congenital CMV infection.

SA175. Cumulative Vestibular Dysfunction and Changes in Walking Speed Associated With Repeated Noise Exposure

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Category: Vestibular: Basic Research & Clinical

Background: It has been established that the vestibular apparatus is susceptible to noise-induced damage. It has been demonstrated that intense noise exposure can exert a rapid and persistent effect on the inner ear within hours, causing damage to vestibular sensory epithelia, abnormal vestibular short-latency evoked potential (VsEP) responses, and slower walking speed in a balance beam crossing task. It is unclear if the same effect will be observed using repeated exposures to a sound level that has previously been shown to produce only a transient effect with single exposure.
Methods: In the current study, rats were trained to cross a balance beam and then subjected to 6-hour, 110 decibel sound pressure level (dB SPL) noise exposures every other week for eight weeks, for four exposures total. Rats were assessed between noise exposures and after the final noise exposure in order to observe immediate and long-term effects of noise on balance beam crossing speed. Although our previous work has only reported balance beam crossing performance in a well-lit testing space, the present work adds a dark condition to assess the importance of vision during the balance beam crossing task. A separate group of rats was used for VsEP testing during and after noise exposure to determine if there was a relationship between impaired vestibular function and slower walking speed.
Results: Results of this work indicate that lesser noise exposures produce cumulative changes in balance beam crossing speed in the "lights on" condition. After one noise exposure, crossing times are slower. After two and three exposures, crossing times improve slightly but do not return to baseline. After a fourth exposure, rats slow down further. In addition to the changes noise exposure causes versus baseline, noise exposed rats are also affected to a greater extent than age-matched controls that were not noise exposed. After recovering for 1-month from a series of 4, 110dB SPL noise exposures, rats appear to compensate under light conditions. This is consistent with our previously published data using a single 120dB SPL noise exposure. Next, noise-exposed and control rats were tested in a dark condition, which was expected to reduce compensation. In this condition, slower crossing times were observed in noise-exposed, but not age-matched control rats. This large increase in balance beam crossing time under dark conditions suggests that an underlying deficit is still present. In addition to the changes observed in balance beam crossing, VsEP responses also worsen progressively during repeated noise exposures. Age-matched controls' VsEP responses are relatively stable over the eight-week period and the weeks following noise exposure.
Conclusions: Results of this work build on prior studies to demonstrate that moderate-intensity noise is capable of producing cumulative damage resulting in impaired vestibular function and slower walking speed in rats.

SA176. Role of LRP2 in Cellular Uptake and Efflux of Aminoglycosides in Vitro

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Category: Vestibular: Basic Research & Clinical

Background: Aminoglycosides are a class of broad-spectrum antibiotics widely used to treat life-threatening bacterial infections. Yet, they are toxic to the kidney and inner ear, resulting in reversible kidney damage, as well as permanent hearing loss and vestibular deficits. Over 35% of US adults aged 40 years and older (GREATER THAN 69 million residents) experience vestibular dysfunction during their lives. Vestibular disorders can be caused by disease, ototoxic drugs, or aging, and are often treated with medication (including aminoglycosides), lifestyle changes (e.g., diet, exercise) or surgery, yet few are efficacious. In the US, medical care for chronic balance disorders exceeds $1 billion per year. Aminoglycosides, such as gentamicin, interact and bind with low-density lipoprotein receptors (LRP). LRP2 is an endocytic receptor that facilitates gentamicin-induced nephrotoxicity, predominantly in the brush border of the renal proximal tubule epithelial cells. Our primary objective is to elucidate the uptake and efflux kinetics of fluorescently labeled gentamicin (GTTR) in cell lines.
Methods: qRT-PCR and immunofluorescence assays identified both LRP2-positive (LRP2+) and LRP2-negative (LRP2-) cell lines. We examined the influx and efflux kinetics of purified GTTR using confocal microscopy (time course study).
Results: Confocal microscopy revealed that both LRP2+ and LRP2- cell lines take up and clear GTTR, suggestive of multiple mechanisms of cellular uptake and clearance of GTTR. We next tested whether cilastatin, a blocker of LRP2 activity, modulated aminoglycoside pharmacodynamics. Cellular influx of GTTR was not visibly affected by cilastatin in either LRP2+ or LRP2- cell lines. In LRP2+ cells, the degree of GTTR efflux was visibly reduced by cilastatin, suggestive of partial inhibition of efflux kinetics. In LRP2- cells, the efflux of GTTR did not appear affected.
Conclusions: Although prior studies have shown multiple mechanisms in the cellular uptake of GTTR, our data also revealed multiple mechanisms in the cellular efflux of GTTR. Cilastatin has been shown to ameliorate aminoglycoside-induced ototoxicity and nephrotoxicity. Although cilastatin can partially attenuate GTTR clearance from cells, our data also suggest that the cellular efflux of GTTR is predominantly independent of cilastatin inhibition in both LRP+ and LRP2- cell lines. We will use HPLC-fluorescence to quantify gentamicin uptake and efflux over time to verify these observations, determine how cilastatin inhibits GTTR efflux, and define cilastatin-independent mechanisms in the cellular efflux of GTTR.

SA177. Hypoxia and Radiation Reduce Viability of Normal Schwann Cells but Not NF2-Mutant Schwann Cells

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Category: Vestibular: Basic Research & Clinical

Background: Vestibular schwannomas (VS) are intracranial tumors that arise from the Schwann cells of the vestibulocochlear nerve and cause hearing loss, dizziness, and other neurological sequelae. These tumors develop after mutations in the NF2 gene that normally encodes the tumor suppressor protein, merlin. Although radiation is a common treatment modality for VS, approximately 75% of patients with good hearing who receive radiation treatment will develop unserviceable hearing loss long-term. In addition, about 30-35% of rapidly growing tumors will continue to progress with time. Tumor hypoxia occurs when tumors outgrow the ability of the vasculature to provide sufficient oxygen and nutrients to support cell proliferation. Although tumor hypoxia is associated with radiation resistance and poor clinical outcomes in various cancers, the role of tumor hypoxia on tumor progression, radiation response, and hearing loss in patients with VS are unknown.
Methods: Normal Schwann cells (HS11) and NF2-mutant human Schwann cells (HS01) were seeded onto 96-well plates at 10,000 cells per well in Schwann proliferative media. Cells were cultivated in either normal oxygen conditions (normoxia) or hypoxia for 24 hours prior to treatment with radiation (0 or 18 Gy). Viability was measured 96 hours post-irradiation using cell-based assays and analyzed using two-way analysis of variance. Immunocytochemistry (ICC) was performed at 6 hours post-irradiation for p21 (cell cycle arrest marker), HIF-1α (angiogenesis marker), and γH2AX (DNA damage marker).
Results: Hypoxia caused a significant reduction in the viability of normal Schwann cells (p LESS THAN 0.05). Although radiation caused a significant decrease in the viability of normal Schwann cells under normal oxygen tension (p LESS THAN 0.05), radiation did not affect the viability of normal Schwann cells in hypoxic conditions (p GREATER THAN 0.05). In contrast, hypoxia alone, radiation alone, and the combination of hypoxia + radiation did not cause significant changes in the viability of NF2-mutant Schwann cells when compared to control (p GREATER THAN 0.05). Expression patterns in p21, HIF-1α, and γH2AX are described per condition.
Conclusions: Although hypoxia caused cell death of normal Schwann cells, hypoxia did not affect the viability of NF2-mutant Schwann cells. Because oxygen is a potent radiosensitizer, it was not surprising to find that radiation treatment did not significantly reduce viability of either normal or NF2-mutant Schwann cells in hypoxic conditions. These findings suggest that: (1) tumor hypoxia may promote radiation resistance in VS, and (2) hypoxia may impair hearing by reducing survival of cochlear nerve Schwann cells. Further investigations are warranted to understand how NF2 mutations can alter cell proliferation of NF2-mutant Schwann cells in hypoxic conditions and measure the effects of hypoxia on cell proliferation and radiation response in primary VS cells. Understanding the molecular mechanisms responsible would enable identification of novel therapies for VS patients.

SA178. Functional Consequences of Selective Ablation of Type I Hair Cells in the Striolae and Central Zones of the Vestibular System

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Category: Vestibular: Basic Research & Clinical

Background: The vestibular system of the inner ear functions to mediate our sense of balance and spatial orientation. Each of the five vestibular sensory organs in mammals can be divided into two morphologically distinct regions: striola and extrastriola of the otolithic organs and central and peripheral zones of the cristae. Previously, we showed that converting striolar and central zones to extrastriolar and peripheral zones identities during development causes deficits in mice such as absence of vestibular evoked potential (VsEP), head tremor and difficulty in traversing a narrow balance beam. Most features of striolar/central zones are affected in these mutants including the otoconia, type I hair cells (HCs) and afferent nerve endings. To decipher the contribution of individual components of striolar and central zones in vestibular functions, we selectively ablated type I HCs in these regions using cre-mediated expression of diphtheria toxin (DTA).
Methods: To investigate the role of type I HCs in striolar/central zones, we generated an OcmCreER/+ strain, in which only type I HCs in striolar/central zones express cre. We administered tamoxifen to OcmCreER/+; RosaDTA/+ newborn mice at postnatal day 0 (P0) and P1 to induce Cre-mediated type I HC ablation. The number of type I and type II HCs within the striola of the utricle at P16 were quantified using anti-oncomodulin and anti-SOX2 antibodies, respectively. The number of calyces was quantified using anti-calbindin antibodies. Additionally, vestibular functional tests such as head tremor was conducted at P8 and VsEP and balance beam tests were conducted between 2-4 months of age.
Results: Comparing to RosaDTA/+ controls, there was a 79% reduction in the number of oncomodulin-positive type I HCs in the striolar region of OcmCreER/+; RosaDTA/+ mutant utricles by P16. To determine possible off-target ablation by DTA, we quantified the number of type II HCs in the striola. Surprisingly, there was a 70% increase in the number of SOX2-positive type II HCs in the striola. This increase in HCs is presumably due to a regenerative response of the vestibular organs to the loss of type I HCs. Striolar-specific calyces based on anti-calbindin staining was reduced in the mutant utricles, compared to RosaDTA/+ controls. The otoconia formation, however, was normal.
The OcmCreER/+; RosaDTA/+ mutant mice exhibit head tremor and reduced or non-detectable VsEP, similar to the previous mutant with global loss of striolar/central zone identities. In contrast, the OcmCreER/+; RosaDTA/+ mutants differ from the previous mutant in that they exhibit no deficit in traversing a narrow balance beam.

Conclusions: These results validated this striolar type I HC deletion mouse model and underpinned the importance of striolar type I HCs in mediating VsEP and head stability. We are currently optimizing the condition for ablating striolar type I HCs in mature animals.

SA179. An Advanced Rule-Based Mobile Classifier for the Automated Diagnosis of Vestibular Disorders

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Category: Vestibular: Basic Research & Clinical

Background: Several disorders, including vestibular migraine, probable Meniere's disease, and probable benign paroxysmal positional vertigo, can be diagnosed solely based on patient-reported symptoms. Diagnosing dizziness is challenging due to the extensive history taking required and the need to organize this information into recognized diagnostic patterns. The task involves covering numerous questions for multiple disorders defined by the ICVD (International Classification of Vestibular Disorders) of the Barany Society, potentially supplemented by additional questions for migraine symptoms. This process demands considerable time and effort, compounded by the overlapping symptoms across vestibular disorders and the lack of a standardized, efficient diagnostic tool. To address these challenges, our research aims to develop a mobile application for assessing six key vestibular conditions: Meniere's disease (MD), benign paroxysmal positional vertigo (BPPV), vestibulopathy (VEST), orthostatic dizziness (OH), vestibular migraine (VM), and persistent postural-perceptual dizziness (PPPD). The system also screens for vascular vertigo. We hypothesize that this mobile classifier can enhance diagnostic precision by collecting, organizing, and processing information, while initially screening for critical conditions. The assessment uses a 47-question survey based on the ICVD criteria.
To evaluate the effectiveness of a mobile application as a preliminary screening tool for six common vestibular disorders and compare its diagnostic accuracy with clinical diagnoses made by experienced physicians.

Methods: This prospective, comparative effectiveness research included 86 participants that completed a 47-item questionnaire using a tablet device and underwent a comprehensive evaluation by an experienced otologist. The precision of the mobile application was measured by comparing its top two diagnostic outcomes against the clinician's diagnoses. The study included 86 participants (65 females, mean age 59.9 ± 15.7 years) presenting with vestibular symptoms at a specialized clinic.
Results: The mobile application had an overall classification accuracy of 82.1%, with 51.2% of the diagnoses completely correct and 30.9% partially correct. For vascular vertigo, the application showed an accuracy of 82%. The average time taken by participants to complete the questionnaire was 7.24 minutes.
Conclusions: The mobile application is a feasible and accurate tool for the initial screening of prevalent vestibular disorders, demonstrating high overall classification accuracy and specific accuracy for vascular vertigo. Its implementation could streamline the diagnostic process and improve patient care, particularly in settings with limited access to vestibular specialists.

SA180. Towards a Vestibular Epithelium Model: Modeling Quantal and Nonquantal Transmission in Dimorphic Afferent Neurons

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Category: Vestibular: Basic Research & Clinical

Background: Vestibular epithelia in amniotes have two main varieties of sensory cells: type I hair cells and type II hair cells. Following transduction of head motion, type I hair cells transmit to cup-

like (calyx) terminals and type II hair cells transmit to compact (bouton) terminals of afferent neurons. By building a computational model of a single type I hair cell-calyx synapse, we previously showed that changes in synaptic cleft potassium ion concentration '[K+]' and electrical potential 'ϕ', drive post-synaptic currents during type I hair cell stimulation (Govindaraju et al. 2023) and underlie non-quantal transmission (NQT). Most commonly, however, mammalian vestibular afferent neurons contact multiple type I and type II hair cells. Such "dimorphic" afferents with both calyx and bouton terminals receive both NQT and quantal transmission (QT) from their synaptic contacts. We do not yet know the functional significance of how quantal and non-quantal signals are integrated by these neurons. We have built a new model to study how these signals are integrated in dimorphic neurons.

Methods: To simulate type II cells, our latest model incorporates new data on major ion conductances in the mouse utricle (Martin et al. 2024) and a phenomenological representation of QT. Our QT implementation simulates voltage-gated calcium channel (CaV) channel activation in hair cells, glutamate release into the synaptic cleft at discrete locations representing ribbon synapses, glutamate diffusion, and the kinetics of post-synaptic AMPA receptors as a function of glutamate concentration. Quantal release was modeled as a stochastic process dependent on the level of hair cell CaV channel activation. Boundary conditions (ion concentrations and electrical potential) assumed a perilymph bath where the afferent fiber exits the basement membrane of the epithelium. The model predicts EPSCs and EPSPs in the afferent as functions of hair bundle deflection and can integrate signals from multiple hair cells.

Results: We present our implementation of QT in both type I and type II hair cells, interactions with NQT, and simulations of the afferent response for a varying number of terminals contacting multiple hair cells. Despite the presence of ribbon synapses, quantal release in type I hair cells was less frequent due to their hyperpolarized resting potentials and smaller receptor potentials relative to type II cells. The innervation of multiple type II cells by a single afferent improves the chances of EPSC summation and leads to modulation of spike rate. In cases where an afferent innervates both type I and type II hair cells, it is possible for the first action potential to be driven by non-quantal transmission alone.

Conclusions: The ability to simulate the activity of a dimorphic afferent neuron is a step towards creating a model of how the entire vestibular epithelium works in concert to encode and represent head motion.

SA181. Protective Effects of Non-Invasive Mild Therapeutic Hypothermia in Preclinical Models of Blast-induced Hearing and Vestibular Loss

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Category: Vestibular: Basic Research & Clinical

Background: Blast-induced vestibular loss (BIVL) is a prevalent yet under-recognized consequence of blast exposure, particularly in military personnel and civilians exposed to explosive devices. The vestibular system, responsible for balance and spatial orientation, is highly susceptible to blast waves, leading to debilitating symptoms such as dizziness, imbalance, and chronic disequilibrium. While blast injuries are often associated with traumatic brain injury (TBI)

and blast-induced hearing loss (BIHL), vestibular deficits are frequently overlooked, contributing to prolonged recovery times and reduced quality of life. The underlying mechanisms of BIVL involve both direct mechanical damage to peripheral vestibular organs, such as the otoliths and semicircular canals, and secondary effects resulting from vascular compromise and inflammation. Given the complexity of these mechanisms, therapeutic approaches to mitigate vestibular damage are of great interest. Recently, mild therapeutic hypothermia (MTH) has emerged as potential intervention in models of noise-induced hearing loss, where it decreases metabolic demand, inhibits inflammatory responses, and reduces oxidative stress. Given these protective effects against noise overexposure, MTH may represent a promising approach for treating BIVL.
Methods: We investigated the long-term functional effects of MTH on hearing and balance in ecologically valid models of mild to moderate blast trauma (n=4/group, male Brown Norway rats, 14-16 weeks). None of the subjects presented with tympanic membrane perforation following blast exposure. Non-invasive MTH was administered using a cooling probe inserted into the ear canals near the eardrum. To determine the optimal MTH therapeutic window, the left ear of each animal in the MTH+Blast group was treated 30 minutes after blast exposure, the right ear 24 hours after trauma. Auditory and vestibular responses were assessed prior to injury, and at 7- and 28-days post-blast. The metrics considered included ABR (4, 8, 16, and 24 kHz) and cVEMP (1 and 8 kHz) thresholds, as well as P1-N1 amplitude and latency.
Results: The blast paradigm (9-12 psi) caused significant changes in auditory and vestibular function in all subjects. We observed a significant increase in ABR and cVEMP thresholds, along with a significant decrease in P1-N1 amplitude. In this cohort, blast exposure had no effect on P1-N1 latency. Our preliminary data revealed no protective effect of any MTH protocol on auditory function 7 days post-blast. However, we observed complete recovery of ABR thresholds by day 28 in the ear where treatment was administered 24 hours after injury. Additionally, a detailed analysis of vestibular responses demonstrated the effectiveness of delayed treatment in reducing blast-induced shifts in cVEMP thresholds and P1-N1 amplitudes. Improved outcomes were observed as early as 7 days post-blast, with full recovery by day 28.
Conclusions: Delayed MTH has proven particularly effective in preserving vestibular and auditory function following blast exposure, underscoring its potential as a viable therapeutic intervention for mitigating both BIVL and BIHL.

SA182. Effects of the Middle Ear Muscle Reflex on the Cochlear Microphonic Evoked by Swept Tones

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Category: Middle & External Ear

Background: The mechanisms that facilitate robust speech understanding in background noise are poorly understood. One potential mechanism is the middle ear muscle (MEM) reflex. This reflex stiffens the ossicular chain, resulting in less sound reaching the cochlea, especially low frequency (LESS THAN 1000 Hz) sounds that are common in noisy environments. For clinical and research applications in humans, the activity of the MEM reflex is inferred by measuring ear-canal sound pressure. This measurement approach involves complex calibration procedures, a constant air-tight seal, and indirect inference of middle ear transmission based on several

assumptions regarding forward and reverse pressure waves in the ear canal. He we adopt a more direct approach to measuring middle ear transmission by assessing a signal generated in the cochlea – the cochlear microphonic (CM) potential. This research has the potential to support or improve clinical measurements of the MEM reflex that are based on sound pressure.
Methods: The CM was measured from an electrode placed on the tympanic membrane. Ear-canal sound pressure was measured simultaneously in the probe ear. The MEM reflex was evoked by contralateral acoustic stimulation (CAS, broadband noise, 90 dB SPL), which was presented simultaneously with an ipsilateral tone (probe, 90 dB SPL) swept upward or downward in frequency from 100 to 6000 Hz. Experimental conditions with and without CAS were interleaved. Participants completed a sweep in each direction in a randomized order. Changes in magnitude and phase from the presentation of CAS were calculated for probe frequencies from 100-6000 Hz and compared with minimum detectable changes to identify significant CAS effects.
Results: Preliminary data reveal that CM amplitude decreased in the presence of CAS for probe frequencies less than 1000 Hz and this decrease was accompanied by phase leads for frequencies between 500-2000 Hz. Magnitude and phase changes were larger for the downward-sweeping compared to upward-sweeping probe consistent with hysteresis.
Conclusions: These results support the interpretation that CAS evoked the MEM reflex, thereby reducing transmission of low-frequency energy into the cochlea. CM results will be compared with ear-canal sound pressure measurements to assess similarities and differences among input (ear-canal sound pressure) and output (CM) approaches to evaluating MEM reflex function. Further, results from this study will be compared to a previous study that used a lower-level CAS to evoke the medial olivocochlear (MOC) reflex to describe potential across-frequency effects that may improve differentiation of activity from these two reflexes.

SA183. Identification of Conductive Hearing Loss Using a Sweep Frequency Impedance (SFI) Meter

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Category: Middle & External Ear

Background: The middle ear function is typically evaluated by subjective tests such as pure-tone audiometry, as well as objective tests like CT scan and tympanometry. However, it is considered difficult to detect subtle abnormalities in the ossicular chain joints using CT scan, and diagnostic accuracy of tympanometry is considered to be low. Since the 1970s, although various attempts have been made to assess middle ear function using acoustic methods similar to tympanometry, none have been implemented for practical use to date. We previously developed a sweep frequency impedance (SFI) meter, which utilizes a 10-second sinusoidal sweeping frequency sound as the stimulus, and demonstrated its effectiveness. In the present study, we explored the practical application of a new SFI meter that shortens the measurement time and incorporates machine learning for classifying measurement results.
Methods: A 0.5-second sweep sound (0.1–2.0 kHz) was transmitted into the ear canal through earphones, and changes in sound pressure at the entrance of the ear canal were measured using a microphone. The impulse response was then calculated from the acquired signals, and sound pressure changes in response to the input frequency (SPL curve) were obtained by fast Fourier

transform (FFT). Measurements were performed on 42 ears of 25 adults with normal middle ear function, as well as 28 ears of 24 patients with conductive hearing loss (18 ears with ossicular fixation and 10 ears with ossicular discontinuity). From the resulting SPL curves, two features were extracted, i.e., the middle ear resonance frequency (RF) and the change in sound pressure level (ΔSPL). By classifying the distribution of these features on a 2D map using machine learning, we attempted to distinguish between ossicular fixation and ossicular discontinuity.
Results: The SPL curves obtained from the normal ears, the ears with ossicular discontinuity or the ears with ossicular fixation exhibited similar trends to those observed in our past measurement data. The RF showed significant differences between normal, fixation and discontinuity cases, while the ΔSPL showed significant differences between normal and discontinuity. When these features were classified based on their distribution positions on a 2D map, the diagnostic accuracy for ossicular fixation and discontinuity was 0.80 and 1.0, respectively. By contrast, diagnostic accuracy for ossicular fixation and discontinuity using tympanometry was 0.47 and 0.92, respectively, indicating the effectiveness of the proposed method.
Conclusions: SFI measurements using short sweep sounds were conducted on adults with normal middle ear function and patients with conductive hearing loss. The newly proposed method demonstrated a testing time 20 times shorter than the conventional SFI meter, while achieving higher diagnostic accuracy for detecting ossicular fixation and discontinuity compared to tympanometry.

SA184. Exploring Geometrical Variations in Middle Ear Models for Conductive Pathology Analysis

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Category: Middle & External Ear

Background: Traditional 3D finite-element (FE) models of the auditory system rely on manual or (semi-)automated segmentation of CT and micro-CT datasets, which are often limited by intersubject anatomical variations and subjective segmentation. These variations affect key structural features such as tympanic membrane (TM) and ligament thickness and positioning. While current sensitivity analyses primarily focus on material properties, geometrical variations remain underexplored, limiting the use of machine learning for simulation-based inference (SBI) in diagnosing conductive pathologies from wideband tympanometry. By parameterizing anatomical geometry in these models, patient-specific diagnostic accuracy and surgical planning could be significantly enhanced.
Methods: This study introduces a novel yet simple and easily applicable approach that incorporates controlled geometrical variations within a single baseline FE model. Mechanical and thermal stresses, along with anisotropic pseudo-mechanical properties (e.g., directional thermal expansion coefficients and elastic moduli), simulate anatomical variability and control directional deformations while maintaining continuity between middle ear components, focusing on features like TM and ligament thickness, as well as ossicle size and orientation. These modified geometries are then subjected to frequency-domain analysis to evaluate middle-ear (ME) transfer functions, including input impedance, absorbance, and stapes velocity.

Randomized variations of these geometrical parameters form a dataset used to train a simulation-based inference (SBI) neural network (NN), extending our previous NN trained solely on material properties. The NN predicts both anatomical and material parameter distributions for reference (e.g., clinical) datasets, facilitating objective inference from patient-specific data.

Results: We investigated the effects of geometrical variations, such as TM thickness (±20% from baseline), on ME transfer functions. Despite keeping material properties consistent across all models, changes in TM thickness caused significant shifts in ME resonance frequency, reduced stapes velocity at lower frequencies, and altered impedance and absorbance, particularly below 2 kHz. A thicker TM increased overall stiffness, raising the resonance frequency, while a thinner TM lowered it.
The inferential NN, trained on the baseline geometry, estimated the equivalent Young's modulus (YM) of the TM, predicting a 10% reduction for a 20% thinner TM and a 22% increase for a 20% thicker TM. The NN also adjusted other material parameters, such as ligament stiffness, to compensate for changes in stapes velocity, demonstrating that geometrical variations influence overall ME behavior and interact with material properties.

Conclusions: The NN, trained on both anatomical and mechanical characteristics of the ME, enhances diagnostic capabilities for conductive hearing pathologies using wideband tympanometry and enables personalized surgical planning. This approach reduces the labor and computational cost of generating multiple models while expanding the scope for sensitivity analysis and synthetic data generation, supporting machine learning applications in auditory diagnostics.

SA185. Three-Dimensional Vibration of the Human Tympanic Membrane Under Pathologic Conditions

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Category: Middle & External Ear

Background: The tympanic membrane (TM) plays important roles in middle-ear sound transmission, i.e., converting sound waves in air into mechanical vibration of the middle-ear structures. When the structures of the TM are damaged/altered by congenital and/or non-congenital causes, vibrational patterns of the TM become different, affecting middle-ear sound transmission. This study aims to measure three-dimensional vibration of the human TM under pathological conditions.
Methods: TMs with malleus and surrounding annular bone were isolated from cadaveric temporal bones and an artificial ear canal was attached to the medial-side bony rim of the TM. Acoustic stimuli of single sines (0.5-8 kHz) were provided and recorded through the artificial ear canal. Three-dimensional vibrational components and coordinates of approximately 1,500 points on the lateral surface of the TM were measured using a novel three-dimensional scanning laser Doppler vibrometry (3D SLDV) system (PSV QTec 3D Scanning vibrometer, Polytec GmbH), where three SLDV systems, each using QTec® multi-path interferometer, are installed with three different

angular positions. The vibration of the intact TM was measured, and the measurements were repeated with pathological conditions of the TM mimicked. The simulated pathological conditions of the TM include local and full thickenings, small and medium perforations of the TM, and closure of the perforation. Based on the surface profile of the TM, vibrational components normal and tangential to the TM were calculated from the measurements.
Results: With the intact TM, the vibrational component normal to the TM surface was dominant at low frequencies whereas vibrational component tangential to the TM surface became considerable at high frequencies. With the local thickening of the TM simulated, decrease of the vibrational magnitude was observed around the thickened area. When the thickening was applied to the total area of the TM, the vibrational magnitude decreased through the entire TM, and the decrease became severe at high frequencies. Small- and medium-sized perforations affected the vibrational pattern around the perforated area rather than the total area of the TM. With closure of the perforations, vibrational patterns of the TM were recovered to the vibrational patterns of the intact TM at low frequencies, but the vibrational patterns of the closed area were considerably different from the corresponding area of the intact TM at high frequencies.
Conclusions: A new 3D-LDV system with QTec® multi-path interferometer allows for reliable measurements of three-dimensional motion of the TM with its surface profile. The local thickening and perforation of the TM have local effects on vibration of the damaged area rather than the vibrational pattern of the total TM area.

SA186. An Open-Science Cross-Species Data Resource for Standardized Hearing Assessments

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Category: Other

Background: Open Hearing is a platform being developed by Creare (Hanover, NH) to accelerate translation of auditory research and algorithms into patient care. Creare's open-source Tympan Audio Processing hardware and TabSINT software application form the foundation of this platform. This accessible software/hardware platform is intended to help standardize multiple-measure assessments that can be collected consistently across subjects, species, and laboratories. As part of Open Hearing, we are implementing modern data-science approaches to develop a harmonized data resource that allows researchers to contribute their data to an online hub that can be openly accessed by anyone to directly explore auditory phenomena within and across species and identifying patterns and profiles of hearing that are important in dissecting subtypes of SNHL.
Methods: Building on Purdue's hub technologies, we are developing a datahub that uses a range of open standards and software, which include a MongoDB data backend, cluster management tools for orchestrating workspaces (Kubernetes and MetalLB), OAuth for open authentication, and JupyterHub for user analytics. We are building a custom Application Programming Interface (API) for interactions between TabSINT and the datahub. Processed data will be available on the datahub with complete provenance information and workflow specification for reproducibility. Ensuring the datasets are annotated by default using open data-type schemas will streamline data harvesting by machine learning (ML) and artificial intelligence (AI) algorithms as contributions to the dataset

are made. The open science datahub will enable collaborative networks by greatly lowering the barrier of access to shared data repositories, which enables cutting-edge data-science approaches that can mine data from new perspectives.
Results: Expanded Tympan/TabSINT system functionality will include audiometry, otoacoustic emissions, wideband acoustic immittance, middle-ear-muscle reflexes, speech recognition, surveys, and eventually evoked potentials. The Open Hearing system will be validated in the Audiology Research Diagnostics Core (ARDC) at Purdue, which includes these same measures collected in a double-walled booth with clinical equipment. In parallel, we have piloted the implementation of ontologies and data organization in our chinchilla work based on ARDC standards, which has facilitated both across-group (e.g., across species) and within-subject (e.g., pre vs. post noise-exposure) comparisons. We plan to refine these ontologies and data standards to best meet the needs of the hearing-science community.
Conclusions: Open Hearing will provide improved transparency, reproducibility, and standardization of human and animal diagnostic studies by providing easy access to an open-science repository that encourages collaboration across diverse research disciplines. This open framework will facilitate data aggregation from large enough populations to achieve the statistical power the hearing-science community requires. Our harmonized data framework will facilitate the development of quantitative causal models powerful enough to capture the relation between underlying pathophysiology and real-world speech intelligibility in humans, ultimately enabling innovation in the development of diagnostics, treatments, and interventions for SNHL.

SA187. Biocompatible 3D Printing Platform With Integrated Miniaturized Optical Systems for Otolaryngology Applications

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Category: Other

Background: 3D printing has emerged as a transformative technology in biomedical engineering, driven by advancements in engineering materials that have enabled the development of biocompatible devices, ranging from medical-grade resins to metals. Numerous leading 3D printing platforms, such as those offered by FormLabs, EnvisionTEC, 3D Systems, Stratasys, and others, now facilitate the manufacturing of medical-grade materials that meet strict biocompatibility standards set by the FDA and adhere to ISO 13485 requirements for medical device production.
These advanced 3D printing platforms support the creation of highly specialized and customizable tools for both clinical and research applications, offering significant cost reductions and increased production scalability. This makes such devices more accessible and widely available for various medical and scientific needs.

Building on these capabilities, the integration of miniaturized optical systems represents a significant advancement, particularly in the field of otolaryngology, where space within the ear, nose, and throat is often limited. With the advent of microfabrication techniques, it is now possible to develop compact, high-precision optical systems that can be seamlessly incorporated into

biocompatible 3D-printed devices. In this work, we present a 3D platform and showcase examples of how these integrated devices can enhance research and clinical applications in otolaryngology.

Methods: In this study, we used a 3D printing platform from FormLabs with biocompatible resins offering various mechanical properties and tissue exposure suitability. For optical systems, we employed microcameras from Omnivision, selecting miniaturized sizes based on application needs, ensuring high-resolution imaging for clinical and research purposes.
We developed and patented several innovative devices, functional sleeves for standard endoscopes, as well as other devices for research and clinical applications

Additionally, we created a series of miniaturized surgery tools of varying sizes, equipped with optical systems tailored for different animal models, from gerbils, guinea pigs to mice. These devices are designed for research, allowing precise visualization during drug delivery and other procedures, providing valuable insights in preclinical ear, nose and throat (ENT) studies.

Results: The devices showed excellent performance during bench testing, confirming their potential for research and clinical applications.
Conclusions: Several of these devices are currently undergoing further testing to assess their suitability for translation to the clinic, indicating promising advancements in their development for practical use in ENT procedures.

SA188. High BMI and Tuberculosis Impair Central Auditory Processing in Children and Adults Living With HIV in Dar es Salaam, Tanzania

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Category: Immunology

Background: High body-mass index (BMI) and tuberculosis (TB) infection significantly influence health outcomes, particularly in individuals living with HIV. The interaction between BMI, a history of TB, and central auditory function has not been studied, and has significance in understanding the influence of HIV on central auditory function. This study explores the effect of BMI status and TB on central auditory processing in a longitudinal cohort of children and adults living with and without HIV in Dar es Salaam, Tanzania.
Methods: 400 children (3–10 years; 204 children living with HIV [CLWH] and 196 children living without HIV [CLWOH]) and 500 adults participated in this study.
Socio-demographic questionnaires collected data on nutritional status, TB infection history, socio-economic status (SES), and health history. Central auditory function was assessed using the Triple Digit Test (TDT), Staggered Spondaic Word Test (SSW), and Hearing In Noise Test (HINT) in Kiswahili. Data analysis was conducted using multiple linear regression models evaluating the effects of nutritional status and TB infection on each CAT. HIV status, age, and SES were included as covariates in each model.

Results: High BMI was significantly associated with poorer performance on the SSW and HINT (p LESS THAN 0.01). TB infection history also significantly predicted impaired central auditory

processing. The interaction of a positive TB history and high BMI showed a significant impact on TDT scores (p LESS THAN 0.01) suggesting those with high BMI and a history of TB had diminished central auditory function. These effects were independent of HIV status, age, and SES. **Conclusions:** High BMI and TB infection are significantly associated with impaired central auditory processing in children, independent of HIV status, age, and SES. The interaction of high BMI and TB infection show a strong link between health and auditory outcomes in children. Future research should explore the possible explanations for these findings, including the effect of HIV and TB treatment drugs on BMI.

SA189. Effect of Inhalation Exposure of Mn Fumes on Hearing Loss

Vijaya Muthaiah*[1], Supriya Mahajan[1], Ignacio Novoa Cornejo[1], Athika Kandaswamy[1], Calista-Mehitabel Okine[1], Sakina Bhapuranwala[1]

[1]*State University of New York, Buffalo*

Category: Hearing Loss: Consequences and Adaptation

Background: Occupation-related inhalation of the toxic element manganese (Mn) results in serious health effects, including the neurological symptoms of Manganism, which is similar to Parkinsonism and is caused by Mn accumulation in the extrapyramidal system of the brain. Despite the reports of Mn-induced neurotoxicity in humans, such as welding-related Parkinsonism, there is not much clinical evidence about manganese-induced ototoxicity.
Methods: We evaluated the effect of 90 days of Mn fumes inhalation alone on the functional integrity of the auditory system using Auditory brainstem response (ABR) and distortion product otoacoustic emissions (DPOAE) measures.
Results: The paired t-test indicates that the ABR thresholds were significantly elevated across frequencies (2, 4, 8, 16, 32, and 64 kHz) in Long-Evans (n=4) that were exposed to 90 days of Mn fumes when compared to the ABR threshold levels before Mn fumes exposure. The mean of differences was 6.25 ± SEM 1.44 (Paired t (5) = 4.330, p=0.0075) with a 95% CI of 2.54 to 9.96. This indicates that Mn fumes exposure in rats reduces auditory sensitivity across frequencies. We assessed the latency changes of ABR Wave I (summed response of a mixed population of SGN fibers) as a function of stimulus intensity in pre-exposure and post-90 days Mn fumes exposure. We found that wave I latency was increased significantly in Mn-exposed rats. The significant changes were consistently at an alpha level of 0.001 i.e 2, 4, 8, 16, and 32 kHz. These results indicate that Mn exposure results in abnormal action potential propagation along AN and demyelination and/or degeneration of AN fibers. Next, we compared the otoacoustic emissions before and after post-90 days exposure. We found that otoacoustic emissions were significantly reduced across frequencies. We found that the significance was as follows: in 2 kHz, 4 kHz, 6 kHz, 8 kHz, and 16 kHz. In Low frequencies, the Mn fumes induced decrease of otoacoustic emission was significant at an alpha level of 0.05 at 2 kHz, at 0.005 at 4 and 16 kHz, and 0.001 in 6, 8, and 20 kHz. Even, in higher frequencies, DPOAE amplitudes were reduced at the following frequencies 20, 24, 30, 35, 40 and 45 kHz. In high frequencies, the Mn fumes induced decrease of otoacoustic emission was significant at an alpha level of 0.05 at 35 kHz, and at 0.001 in 20, 24, 30, 40, and 45 kHz.
Conclusions: Overall, the 90 days of exposure to Mn fumes alone reduced the integrity of outer hair cells and reduced the amplification function.

SA190. Comparative Micro-Ct Analysis of Inner Ear Anatomy in Adult Mouse, Chicken, and Human: Insights for Surgical Access and Drug Delivery

Jing Wang*[1], Fabian Blanc[1], Nesrine Benkafadar[2], Michel Mondain[3]

[1]*Institute for Neurosciences of Montpellier (INM), University Montpellier, INSERM U1298, Montpellier, France,* [2]*Stanford University School of Medicine* [3]*Montepellier - Hospital St. Charles*

Category: Gene Therapy

Background: The semicircular canal injection is now commonly recognized as an effective approach for gene delivery into the inner ear to ensure reliable transduction efficiency in targeted cell types while minimizing any associated hearing loss in both mice and chickens. While mice are commonly employed in inner ear research due to available genetic tools, their small size limits the translational relevance to human application. In contrast, the chicken inner ear is notably larger, potentially offering more relevant insights for surgical approaches, and semicircular canal injection is a validated approach in this model. This study aims to provide a comparative analysis of inner ear anatomy in mice, chickens, and humans, to optimize surgical techniques.

Methods: Temporal bones from a 21-day-old male FVB mouse and a 7-day-old chicken were extracted, fixed in 4% paraformaldehyde, and stained with phosphotungstic acid for one week. This preparation enabled detailed analysis of the membranous labyrinth and surrounding structures. Non-destructive 3D imaging was performed using µ-Computed Tomography (EasyTom 150), capturing 1601 shadow images with a pixel size of 3.8 µm. 3D reconstructions of the perilymphatic and endolymphatic spaces, as well as the bony and membranous labyrinths of the semicircular canals, were generated and compared across species, alongside human data from the OpenEar library.

Results: We present a comprehensive comparison of the bony and membranous labyrinths across mice, chickens, and humans. The mouse inner ear mirrors human cochlear and vestibular segmentation and cochlear coiling but represents only 1% of the human inner ear volume. Its perilymphatic space connects to the cerebral fossa via a broad cochlear aqueduct. In contrast, the chicken inner ear is five times larger than the mouse's, lacks cochlear coiling, and has no direct perilymphatic connection to the cerebral fossa. Measurements indicate that the mouse bony semicircular canals are 20% of the human size, with the membranous labyrinth occupying 75% of the canal's axial section (compared to 25% in humans). The chicken's canals are 60% of the human size, with a membranous labyrinth ratio of 15%.

Conclusions: Effective preclinical models are essential for advancing inner ear drug delivery methods. This study underscores key anatomical differences between humans, mice, and chickens, with important implications for translational research in drug and gene delivery. While mice remain valuable for genetic studies, their anatomical limitations must be acknowledged. Chickens, with their larger and more human-like semicircular canal anatomy, provide a promising alternative for inner ear therapies. Furthermore, chickens are easier to maintain than larger mammals like pigs, or non-human primates, which present ethical challenges. This comparative anatomical study provides key insights for improving surgical access techniques in translational inner ear research.

SA191. Evaluation of Variability of Otoacoustic Emissions When Measured by Different Persons

W. Wiktor Jedrzejczak*[1], Krzysztof Kochanek[1], Joanna Krubnik[2], Henryk Skarzynski[1]

[1]*Institute of Physiology and Pathology of Hearing, Warsaw, Poland,* [2]*The University of Maria Curie-Sklodowska in Lublin*

Category: Otoacoustic Emissions

Background: This study aimed to evaluate the repeatability of transient evoked otoacoustic emissions (TEOAE) when the probe is placed in the ear by different individuals. Specifically, the research sought to compare reference measurements (REF) performed by an audiologist with measurements performed by trained, inexperienced individuals (SELF). The findings could inform the feasibility of utilizing minimally trained individuals for OAE testing, with potential applications in teleaudiology services.

Methods: Three female subjects, each aged 23 years, were tested. A total of 72 students with no prior experience in otoacoustic emissions were trained to place the probe in the ear canal and conduct the measurements. TEOAEs were recorded using a non-linear protocol with a stimulus intensity level of 80 dB SPL, and 250 responses were averaged for each test. Measurements were evaluated at frequencies of 1 kHz, 1.4 kHz, 2 kHz, 2.8 kHz, and 4 kHz. For each subject, two REF measurements were conducted in both ears by an audiologist, followed by two SELF measurements after the probe was placed by the trained individuals. Statistical analysis was conducted to assess consistency between REF and SELF measurements, including mean differences, standard deviations, and concordance based on signal-to-noise ratios (SNR) of GREATER THAN 6 dB and GREATER THAN 3 dB.

Results: No statistically significant differences were observed between the REF and SELF measurements for any of the subjects. The percentage of SELF measurements with a difference moduli ≥1 dB was 3%, which was not statistically significant. Using the SNR GREATER THAN 6 dB criterion, agreement between REF and SELF measurements ranged from 87% to 97%. In subject 2, the right ear SELF measurements exhibited concordance, but did not meet the SNR GREATER THAN 6 dB criterion, necessitating the use of the SNR GREATER THAN 3 dB criterion to assess agreement.

Conclusions: The study indicates that reliable and repeatable TEOAE measurements can be achieved even when the probe is placed by individuals with minimal training. This supports the possibility of expanding teleaudiology services by utilizing trained individuals to perform OAE testing in remote settings.

SA192. Travel Booth to Assess Hearing in Rodents

Jordan Villa*[1], Joaquin Cury[1], Claus-Peter Richter[1]

[1]*Feinberg School of Medicine, Northwestern University*

Category: Other

Background: Recording the auditory brainstem response (ABR) is the conventional way researchers and clinicians assess the auditory pathway's functionality and sensitivity. In clinical

research, ABRs allow researchers to detect differences in audiometric thresholds before and after potential treatments have been applied to both human and animal models. Clinically, the ABR is considered in the evaluation of schwannomas, demyelinating diseases, genetic disorders, and psychiatric conditions. Given its broad use, having access to recording an ABR can be crucial in both diagnosis and expanding our knowledge, particularly in auditory neuroscience.
Auditory researchers are consistently looking for ways to enhance the conventional sound booths used when measuring ABRs. A standard sound booth used is capable of decreasing sound intensities by roughly 25-30 dB. Optimally, it will be able to suppress both acoustic and electrical background noise. With the need to suppress more noise may come the need for more materials. Recording a quality ABR within the laboratory minimally requires a sound booth, surface electrodes, a speaker, an amplifier, and a computer for analysis. These conventional sound booths and the necessary supplies require a considerable amount of space in the laboratory and a substantial financial commitment. This not only limits its use for other researchers but also limits collaboration with those looking for such a system. Nonetheless, when available, the current systems provide reliable measurements with minimal background noise, and thus a sufficient means of measuring auditory function A system that occupies less space, costs less, fully portable, and offers the same quality of acoustic testing could encourage more researchers to invest in it, recognizing the valuable insights it can provide for their studies.

Methods: We introduce a fully transportable sound booth able to provide an enhanced acoustic environment of current standard supplies. This study describes the fabrication of such a device and initial acoustic testing. The booth is made of a 2 ft x 2 ft travel box with two layers of acoustic soundproofing panels. Copper tubing is utilized alongside warm water pumps for maintaining proper body temperature of animal models being studied. Noise suppression tests were completed to determine noise floor of the travel booth.
Results: ABRs have been successfully recorded with minimal background noise. Furthermore, our novel portable acoustic chamber (30 – 35 dB) has been shown to outperform traditional sound booths used in auditory research in terms of noise suppression.
Conclusions: The initial results discussed promote its use in auditory neuroscience research and support its further development to expand its use beyond measuring ABRs including measuring distortion product otoacoustic emissions (DPOAEs).

Special Session 1: Deep Neural Networks and AI for Auditory Modeling

Chair: Malcolm Slaney*, Stanford University*

2:30 p.m. - 4:30 p.m.

Ocean Ballroom 1 - 4

Deep-Neural-Network Models of Peripheral Auditory Processing and Their Applications for Hearing Research

Sarah Verhulst[1], Chuan Wen[2], Fotios Drakopoulos[2], Deepak Baby[2], Arthur Van Den Broucke[2], Marjoleen Wouters[2], Sarineh Keshishzadeh[2]

[1]*Ghent University,* [2]*Hearing Technology @ WAVES, Ghent University*

Individual Abstract: In classical computational neuroscience, analytical model descriptions are derived from neuronal recordings to mimic the underlying biological system. These neuronal models are typically slow to compute and cannot be integrated within large-scale neuronal simulation frameworks. Furthermore, to fully exploit the capabilities of neural-network and GPU computing, the model equations should be of differentiable nature to allow for backpropagation. Application examples of this approach are optimizing the characteristics of an auditory stimulus in yielding a desired neural response, or the development of auditory signal processing algorithms that modify the speech such to minimize the difference between a normal and hearing-impaired neural response.
To this end, we present a hybrid, machine-learning and computational-neuroscience approach (CoNNear) that transforms analytical models of cochlear mechanics, sensory neurons and synapses into deep-neural-network neuronal units with the same biophysical properties. We discuss which architectures and hyperparameters (layer numbers, activation functions, skip connections) are suitable to match the nonlinear and adaptation characteristics of the respective cochlear structures and we show how the receptive field can be used in this process. Secondly, we present a method of how human auditory physiology measures can be adopted to determine the parameters of a hearing-impaired CoNNear unit that can be used within a closed-loop network to develop novel hearing diagnostic methods or hearing-loss compensation algorithms. Taken together, the presented approach to cast deterministic cochlear and neuronal models into CNN versions thereof, leads in a new era for numerical approaches that can help accelerate auditory applications in diagnostics or signal processing.

This work was supported by FWO Machine Hearing 2.0 (G063821N) and EIC Transition Grant EarDiTech (101058278).

Modeling the Central Auditory System With Machine Learning

Josh McDermott[1]

[1]*Massachusetts Institute of Technology*

Individual Abstract: Decades of experimental and theoretical work have led to models of the cochlea and auditory nerve that are widely used throughout our field. Comparably accurate models of the central auditory system could be similarly transformative. This talk will describe my lab's latest efforts to leverage contemporary machine learning to build neural network models of our auditory abilities and their instantiation in the brain. Such models have enabled a qualitative step forward in our ability to account for real-world auditory behavior and illuminate function within auditory cortex. They also open the door to new approaches for designing auditory prosthetics and understanding their effect on behavioral abilities.

Developing Personalized Hearing Models in Jax to Train Novel Machine Learning Strategies for Cochlear Implant Stimulation and Hearing Aid Processing.

Maryam Hosseini[1], Jason Mikiel-Hunter[1], Nima Salimi[1], Alan Kan[1], Jorg Buchholz[1], Rob Schonberger[2], Honglin Yu[2], Tim Brochier[3], Zachary Smith[3], Simon Carlile[2], Dick Lyon[2]

[1]*Macquarie University,* [2]*Google Australia,* [3]*Cochlear Limited*

Individual Abstract: BACKGROUND

The use of machine learning (ML) and Deep Neural Networks (DNNs) to develop novel hearing assistive devices offers a potential paradigm shift beyond the current state of the art. To arrive at these benefits efficiently, however, it is important that we leverage our knowledge of the auditory system to take a feature-based approach based on physiologically accurate models (as opposed to an end-to-end "black-box" ML approach). This can not only compensate for the paucity of data available for training models but also better account for the need to parametrize a personalized solution to fit specific functional needs of an individual user. Given the range of potential causes of hearing loss and the different listening needs of users, the personalization of hearing assistive devices may prove especially important for both cochlear implants (CIs) and hearing aids (HAs). By either circumventing "first-order" audibility strategies of current HA's amplification or helping to overcome the technical limitations of CIs, an ML-developed processing/stimulation strategy that is personalized may not only lead to auditory nerve activity patterns more closely resembling those observed in "normal-hearing" individuals, but also bring us closer to the desired goal of improving speech intelligibility in challenging acoustic environments.

METHODS + RESULTS

Here we present our implementation of two hearing models, the Cascade of Asymmetric Resonators with Fast-Acting Compression model of the human cochlea (CARFAC; Lyon et al., 2024) and the electrical hearing (EH) model (Brochier et al., 2022), in the ML framework JAX. We show how JAX's efficient compilation across different devices, and its auto-differentiability make it ideal for training personalized solutions for CIs and HAs. Furthermore, we demonstrate how our training loops for both CI and HA solutions are based on loss functions that compare the neurogram outputs of the personalized CARFAC and EH models of "hearing loss" with the target neurogram output from the "normal-hearing" CARFAC model. To calculate this loss, we approximate processing by subcortical auditory nuclei, using a combination of the Stabilized Auditory Image (SAI), an autocorrelation-based analysis using strobed temporal integration, and the Structural Similarity Index Measure, a measure of similarity between SAI images. Finally, we discuss the current state of our DNN-based solutions for HAs/CIs, highlighting the advances we have made in developing novel, biomimetically inspired strategies for CI stimulation and HA amplification.

A Neural Network Approximation of Cochlear Filtering and Hair-Cell Transduction for Outer and Inner Hair Cell Hearing Impairments

Anil Nagathil[1], Ian Bruce[2]

[1]*Ruhr-Universität Bochum,* [2]*McMaster University*

Individual Abstract: Computational auditory models emulate cochlear processing and neural transduction in the hair cells and auditory nerve. They contribute to a deeper understanding of hearing mechanisms and can be utilized for the development of bio-inspired speech and audio enhancement algorithms in hearing devices. However, accurate models of auditory processing entail a high computational complexity, which prevents their application in real-time signal processing algorithms or machine-learning tasks. To circumvent such restrictions, several approaches for approximating auditory models by deep neural networks (DNNs) have been proposed recently, which learn the non-linear and time-varying relationship between an input signal and its neural response (e.g., Drakopoulos et al., Commun. Biol. 4(1), 2021; Nagathil et al., JASA 154(1), 2023; Leer et al., IEEE/ACM TASLP 32, 2024). Advantages of such approximations are accelerated execution and full differentiability, making them applicable in the context of DNN-based speech and audio enhancement.

In this work, we extend a recently proposed normal-hearing WaveNet approximation (Nagathil and Bruce, JASA 154(1), 2023) of a widely used cochlear filtering and hair-cell transduction model (Zilany and Bruce, JASA 120(3), 2006) to the case of hair-cell hearing impairment. The WaveNet model was trained with a large speech and music data set at a wide range of sound pressure levels using various audiogram settings for frequency-dependent, mild-to-severe hearing losses as conditioning variables. To deal with the large dynamic range of the inner hair cell potential we applied a scalable compression/expansion approach during training. The approximation model was evaluated with previously unseen speech and music signals and, additionally, with pure tones and click sounds for a set of standardized audiograms (Bisgaard et al., Trends Amplif., 14(2), 2010) and a set of randomized audiograms to encompass some uncommon patterns of hearing loss.

To provide an estimate of the required DNN approximation accuracy, the outcomes of the original model and its DNN counterpart were further processed with a neural transduction model (Bruce et al., Hear. Res., 360, 2018) and the resulting neurogram representations were compared in a statistical analysis.

The WaveNet model exhibits accurate approximations for the considered test signals and different audiogram settings. It is computationally efficient and fully differentiable, making it deployable for the development of future DNN-based hearing loss compensation algorithms in hearing devices.

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Deep-Learning Based Hearing-Loss Compensation Using an Auditory-Nerve Model

Peter Leer[1], Lars Bramsløw[1]

[1]*Eriksholm Research Centre*

Individual Abstract: Computational models of hearing have been used for research in normal and impaired hearing in academia and studied in industry to inspire hearing aid design. But the potential of applying them directly in hearing aid design for e.g. addressing the 'cocktail party' problem has never been fulfilled. The present project aims at applying these auditory models directly for training a deep learning (DL) algorithm to provide individualized hearing-loss compensation (HLC) and noise reduction (NR), thus paving the way towards fully neural- and AI-based hearing devices. In this study, we propose a DL-based closed loop approach for hearing loss compensation, which is trained on the outputs of hearing-impaired and normal hearing auditory models in response to speech signals.
This project encompasses three components for the creation of such a hearing device:

An auditory model emulator. For reasons of computational speed, an arbitrary auditory model cannot be used directly in the closed loop training. Hence, a deep neural network (DNN) was trained to emulate an auditory nerve model (Zilany et al, 2014). A new cost function was designed to accommodate for the very large dynamic range across input levels, frequencies, and hearing losses.

A DNN for hearing loss compensation. The proposed DNN was added in front of the hearing-impaired auditory model emulator and the emulator outputs from the normal and impaired branches were compared and trained to minimize the difference in a dedicated cost function.

A noise reduction system using trained with a noise-free reference; hence a perceptually relevant noise reduction may be obtained. The noise reduction and hearing loss compensation can be combined via joint training.

The performance of the system has been evaluated by inspecting gain and output. Furthermore, listening tests with 13 hearing impaired listeners have been conducted: word recognition in noise using the Danish Hearing In Noise Test (HINT) test and sound quality rating using a variant of the Multiple Scaling with Hidden Reference and Anchor (MUSHRA) method.

The results demonstrate that the proposed approach results in feasible hearing loss compensation strategies. Our proposed approach was shown to provide an increase in speech intelligibility and was found to outperform the conventional approach in terms of perceived speech quality. Thus, the proposed DNN-based approach might hold great potential in improving the quality of life for people with hearing loss.

Hearing Aid Speech Intelligibility Enhancement Using Speech Foundation Models
Jonathan Barker[1]

[1]*University of Sheffield*

Individual Abstract: In recent years, a new breed of DNN-based speech models has emerged that use self-supervised learning approaches to leverage huge amounts of unlabelled speech data. These 'foundation models' are revolutionising performance in many downstream tasks (speech recognition, enhancement, conversation diarisation). However, despite their promise, their large sizes make them impractical for direct use in low-power, low-memory, and low-latency devices such as hearing aids.

This talk will explore emerging approaches that aim to bridge the gap between big models and small devices. Specifically, we will discuss how speech foundation models have been used for hearing aid speech intelligibility enhancement. Recent work has demonstrated that representations extracted from these models (e.g., embeddings from hidden layers) can be used to construct loss functions for training smaller, hearing-aid compatible speech enhancement models. This approach aims to offload the computational cost to the training stage, thereby minimising latency, model size, and computational cost during inference. We will present results from employing these techniques in the Clarity Challenges (claritychallenge.org) — an ongoing series of open machine learning challenges focused on speech intelligibility enhancement evaluated with hearing-impaired listeners.

Podium 1: Accessing the Inner Ear: Advances in Cochlear Drug Delivery

Moderators: Thore Schade-Mann and Athanasia Warnecke

2:30 p.m. - 4:30 p.m.

Ocean Ballroom 5 - 8

Perilymph and Tissue Distribution of the Novel Drug AC102 After Intratympanic Drug Delivery in a Large Animal Model

Anselm Gadenstaetter*[1], Michael Nieratschker[1], Matthias Gerlitz[1], Erdem Yildiz[1], Till Buschhorn[1], Caroline Sesztak[1], Reimar Schlingensiepen[2], Clemens Honeder[1], Christoph Arnoldner[1]

[1]*Vienna General Hospital, Medical University of Vienna,* [2]*AudioCure Pharma GmbH*

Background: In inner ear therapy, there is an evident lack and unmet need of approved and effective pharmacotherapeutics. The novel drug, AC102, is a promising candidate to treat various inner ear diseases with preclinical successes in noise-induced hearing loss and cochlear implantation models and an ongoing clinical trial investigating its use in idiopathic sudden sensorineural hearing loss. Moreover, the pyridoindole-derviate AC102's small and lipophilic structure suggests that it can quickly diffuse into the cochlea and evenly distribute within its tissue. To confirm this hypothesis, we applied AC102 intratympanically in pigs followed by apical perilymph sampling and tissue collection quantifying AC102's concentration along and within the cochlea.

Methods: AC102 formulated in a thermoreversible hydrogel was surgically applied into the middle ear of domestic pigs. After predefined timepoints of 1, 4, or 24 hours (n = 3 per group), perilymph was sequentially sampled from the cochlear apex and samples from different tissues (sensory tissue, modiolus, and cochlear nerve) and fluids (CSF, and plasma) were collected intraoperatively. Subsequently, the individual samples' concentration of AC102 was determined via high-performance liquid chromatography.

Results: One hour after intratympanic application, notably high concentrations of AC102 were found evenly distributed along the cochlea with a mean concentration of 40.67±15.62 ng/mL.

AC102 concentrations within perilymph peaked at 4 hours after administration with mean levels of 95.09±84.18 ng/mL. After 24 hours, AC102 perilymph levels decreased below the lower detection limit. At all timepoints, AC102 levels in cochlear tissues were considerably higher compared to perilymph levels.
Conclusions: Due to its small structure and high lipophilicity, AC102 possesses the abilities to quickly penetrate into the cochlea and evenly distribute along the cochlear length, thus also rapidly accumulate in the cochlear apex. Moreover, AC102 swiftly enters different cochlear sensory and neural tissues, which resemble the actual targets of inner ear pharmacotherapy. Due to the pig cochlea's human-like size and physiology, a highly similar situation can be assumed in humans, which would enable efficient local drug delivery of AC102 via intratympanic delivery.

Porcine Ex-Vivo Chamber for Quantitative Assessment of Drug Permeability Through the Round and Oval Window Membranes

Farimah Moazzam*[1], Adele Moatti[1], Samuel Holdsclaw[1], Alon Greenbaum[1]

[1]*North Carolina State University*

Background: Background: Effectively delivering drugs to the inner ear for treating Sudden Sensorineural Hearing Loss (SSHL) presents significant challenges. Systemic treatments often prove ineffective, with only a minimal amount of the therapeutic agent reaching the cochlea. Consequently, localized methods such as intratympanic (IT) injections are being explored. During IT injections, the middle ear cavity is filled with a drug solution, which can either pass into the vestibular system via the oval window or reach the scala tympani in the cochlea through the Round Window Membrane (RWM). Since pharmacokinetic measurements of the inner ear and vestibular system are difficult to obtain both clinically and in animal models, the precise distribution of the drug after IT injection between these two systems remains largely unknown. It is generally assumed that most of the drug penetrates through the RWM. However, contrary to this belief, a magnetic resonance imaging (MRI) study by King et al., using the MRI contrast agent Gadolinium (Gd) in guinea pigs, showed that 90% of the Gd entered the vestibule directly through the stapes, i.e., through the oval window. Complicating the understanding of drug distribution, permeability varies between drugs, meaning that each drug may distribute differently following IT injection.
Methods: To address this issue, we developed a modular ex-vivo porcine chamber that incorporates either the RWM, the oval window, or both. The chamber consists of two liquid compartments separated by these membranes, with a structure resembling a transwell system. Tissues are extracted from fresh pig cadavers, and membrane viability is confirmed using Alamar Blue. The drug being tested is placed in the top chamber, and its passage through the membranes is assessed by sampling the liquid from the bottom chamber.
We selected to utilize the pig model for its anatomical similarity to humans, especially in the size and thickness of key barriers. Our study aims to validate these drug delivery pathways, ensuring that IT injected therapeutics effectively reach the cochlea, rather than being diverted to the vestibular system, which could compromise treatment efficacy.

Results: Preliminary data from our ex-vivo porcine chambers show that the tissues remain viable for at least several days, even when the oval window is incorporated. We also observed differences in drug permeability between chambers containing only the RWM and those that also include the

oval window. Specifically, after 22 and 24 hours, we noted an increase in drug permeability, suggesting that drug passage through the oval window may not be negligible.
Ongoing analysis will further refine these observations and evaluate their impact on treatment efficacy.

Conclusions: Initial results showed higher drug permeability through both RWM, and the oval window, which may impact SSHL therapies. The ex-vivo porcine model aids in understanding drug distribution and will inform targeted treatments.

Outer Hair Cells Stir Cochlear Fluids

Choongheon Lee*[1], Mohammad Shokrian[1], Kenneth Henry[1], Laurel Carney[1], Joseph Holt[1], Jong-Hoon Nam[1]

[1]*University of Rochester*

Background: Unlike other fluids in the body, the lymphatic fluids in the inner ear are nearly isolated and stationary. Substances in these fluids are mainly transported through diffusion, which occurs because of concentration differences. However, due to resorption before reaching the cochlea apex, effective transport over long distances remains challenging. A previous investigation indicated that outer hair cell motility could generate oscillatory fluid motion in the tunnel of Corti (ToC) within the organ of Corti (OoC). Recent measurements of OoC vibrations suggest that outer hair cells act as motors that enhance vibrations across traveling sound waves. Theoretically, peristaltic deformation of the OoC may induce non-oscillatory streaming along the ToC, although this phenomenon has yet to be explored experimentally. We hypothesized that active outer hair cells drive cochlear fluid circulation.
Methods: To test the hypothesis, we delivered the neurotoxin kainic acid to the round window of young gerbil cochleae. In some cases, sodium salicylate was also given, either intraperitoneally or to the round window, followed by round window kainic acid application. Multichannel electrodes were placed in the anteroventral cochlear nucleus (AVCN) to assess drug effects along the tonotopic cochlear length. As kainic acid diffused into the cochlea fluid spaces through the round window, we recorded neural activity in the AVCN during three acoustic conditions: broadband noise, pure-tone sound, and silence. Sounds presented at a moderate level of 75-80 dB SPL. Additionally, we employed computer models to simulate inner ear drug delivery, providing theoretical support for our experimental observations.
Results: Exposure to broadband sound significantly accelerated kainic acid delivery to the cochlear regions with characteristic frequencies above 4.5 kHz, while no significant effect was observed in the low-frequency regions below 4.5 kHz. When outer-hair-cell motility was blocked using salicylic acid, this sound-induced acceleration disappeared, making drug delivery times similar to those during the silence condition. This finding supports the hypothesis that outer-hair-cell motility is essential for faster drug delivery, particularly in mid-frequency regions. Additionally, a 0.5 kHz pure tone exposure facilitated drug delivery across both low-frequency (LESS THAN 4.5 kHz) and mid-frequency (GREATER THAN 4.5 kHz) regions. Finally, computer model stimulations demonstrated that low-frequency sound creates strong advective flow along the ToC, enhancing drug transport toward the cochlea apex.
Conclusions: These results suggest new strategies for improving drug delivery throughout the cochlear spiral. They also reveal a new function of outer hair cells beyond amplifying sounds.

Active outer hair cells deformed the OoC like a peristaltic tube to generate apically streaming flows along the ToC and basally streaming flows along the scala tympani. Both experimental measurements and simulations coherently indicate that broadband outer-hair-cell action plays a crucial role in driving cochlear fluid circulation.

Chemosensory Ciliated Cells of the Round Window Niche: Dual Functions in Sensation and Drug Transport to the Inner Ear

Adele Moatti*[1], Shannon Connard[2], Anna Vavakou[3], Ross Lampe[2], Mani Rai[2], Farimah Moazzam[2], Jorge Piedrahita[2], Lauren Schanbel[2], Doug Fitzpatrick[4], Kendall Hutson[5], Carlton Zdanski[5], Frances Ligler[6], Marcel Van Der Heijden[7], Kenneth Adler[2], Alon Greenbaum[2]

[1]*University of Pittsburgh,* [2]*North Carolina State University,* [3]*University Medical Center Göttingen,* [4]*University of North Carolina,* [5]*University of North Carolina at Chapel Hill,* [6]*Texas A and M University,* [7]*Erasmus Medical Center*

Background: Drugs injected into the middle ear must pass through the round window membrane (RWM) to reach the inner ear. However, the residence time of a drug in the depression or "niche" leading to the round window membrane is limited. In this niche, a directional flow of cilia-driven mucus affects the half-life of drugs in that space and subsequent drug transport across the round window membrane.

Methods: Here we assess the presence of cilia in the RW niche, complementing existing ciliation maps of the middle ear by delineating the gradient of ciliation and secretory cells via electron microscopy, H and E, PAS, and immuno-staining. Additionally, we investigate mucus release and transport dynamics when applying dexamethasone fluorescein (DexF) in the RW niche, along with examining transport in vivo via OCT. These findings illuminate the mechanism of drug removal from the RW niche via mucociliary transport into the ET. Additionally, our RNA sequencing and immunostaining data demonstrate the presence of taste receptors in the RW niche. We tested the effect of ciliary movement on drug passage across RWM via (1) inhibiting the motor activity of cilia (2) using bitter taste receptor blockage, and (3) inhibiting NO production, both in-vitro and in-vivo in gerbils and pigs.

Results: We discovered G-protein–coupled taste receptors in the niche cells; such receptors are known to modulate ciliary beating. We tested three paths to reversibly reduce ciliary beating and prolong the drug residence time in the round window niche: inhibiting dynein, inhibiting a bitter taste receptor, and inhibiting nitric oxide release. Of these approaches, inhibiting nitric oxide release was the most effective in vitro; the results were confirmed in a large animal model.

Conclusions: Collectively, the data reveal a previously unrecognized mechano- and chemosensory system that recognizes endogenous and exogenous agonists. These receptors and their ligands play roles in normal homeostatic and immune functions and suggest new drug targets for inner ear therapeutics and most importantly enhanced delivery.

Contrast Enhancement of Cochlea After Direct Microneedle Intracochlear Injection of Gadodiamide Through the Round Window Membrane With Minimal Dosage

Chaoqun Zhou*[1], Sharon Feng[1], Stephen Leong[1], Eugénie Breil[1], François Voruz[1], Chris Valentini[1], Daniella Hammer[1], Aykut Aksit[1], Elizabeth S. Olson[1], Jia Guo[1], Jeffrey Kysar[1], Anil Lalwani[1]

[1]*Columbia University*

Background: Contrast-enhanced magnetic resonance imaging (MRI) has been proposed as a method for diagnosing endolymphatic hydrops. However, its potential is largely limited by the long wait time required for contrast to reach cochlear turns following intravenous (IV) or intratympanic (IT) delivery, the high contrast dosage, and the inconsistent perilymph signal intensity enhancements among patients. In this study, we aim to address these limitations by investigating the utility of microneedle-mediated intracochlear (IC) injection of gadodiamide to achieve consistent and efficient contrast delivery with minimal contrast dosage.

Methods: A 100 μm diameter microneedle with 35 μm lumen was advanced to perforate the right side round window membrane (RWM) of guinea pig (n=7) and 1 μL of gadodiamide, diluted in artificial perilymph to a concentration of 17.4 mM, was injected into the cochlea. Serial MRI imaging was performed in a post-mortem animal using a 9.4T small-animal MRI. The fluid chambers within the injected cochlea, including scala tympani (ST), scala vestibuli (SV) and scala media (SM), were segmented. Each turn of contrast enhanced ST and SV chambers was separated into two halves, resulting in a total of 15 regions of interest (ROI). To evaluate contrast delivery efficiency, mean intensities in these 15 defined ROIs were compared to the mean intensities in the fluid region of the non-injected contralateral side using two-tailed paired t-tests. In addition, intensity enhancements were calculated to demonstrate the amount of contrast enhancement at different turns.

Results: The contrast was delivered locally across the RWM into the basal turn of ST, resulting in contrast being observed in both the basal turn of ST and SV in the first MRI scan for all subjects which was acquired as early as 35 minutes after injection. Two-tailed paired t-tests confirmed that contrast reached the first two turns of ST and the first two and half turns of SV within 60 minutes, and the second half of third turn and apical turns of ST and SV within 90 minutes (p LESS THAN 0.05). Intensity enhancements calculated at 90 minutes post-injection demonstrated substantial increases, exceeding 100% of that on the contralateral side in the first turn of both ST and SV and in the first half of the second turn of ST. The intensity enhancements ranged from 12% to 38% in the third and apical turns.

Conclusions: IC gadodiamide injection enables controllable and efficient contrast delivery utilizing a total contrast dosage three orders of magnitude smaller than those employed in IV and two orders of magnitude smaller than in IT administration. Additionally, our previous studies have shown no hearing loss and complete round window membrane healing following in vivo microneedle IC injection, thus, making IC injection a viable alternative to commonly used IV and IT injections for contrast-enhanced cochlear MRI.

New Insights Into Drug Distribution in the Cochlea: Translational Studies to Understand and Enhance the Cochlear Response to Implantation

Nathan Creber*[1], Hayden Eastwood[2], Justin Tan[2], Kate Brody[2], Dong Zhang[2], Stephen O'Leary[2]

[1]*The University of Sydney,* [2]*University of Melbourne*

Background: The ability to successfully provide hearing preservation cochlear implantation has widely expanded the cohort that may benefit from surgical hearing rehabilitation. The success of this is hindered by a tissue foreign body reaction and fibrosis of the cochlea. Steroids have been effective in mitigating this response; however, clinical studies have varied results. Translational studies are required to understand and enhance the pharmacokinetics and pharmacodynamics of steroid activity in the implanted cochlea.
Methods: We present a series of five translational animal studies assessing steroids' distribution and site of action in both naïve and implanted cochlea. In study 1, we assess local and systemic routes of administration to reveal drug distribution in both perilymph (mass spectroscopy) and tissues (immunohistochemistry), as well as the "end-point" of receptor activation. In study 2, we assess the potential of adjuvant agents to enhance round window permeability. In study 3, we assess implantation's impact on the cochlea's permeability and drug distribution. In study 4, we alternatively evaluate the response of mineralocorticoid activation. Finally, in study 5, we apply novel thin-sheet laser microscopy techniques to explore new regions of cochlear permeability following implantation.
Results: Following local administration typical basal to apical gradients in the perilymph and cochlear tissues are demonstrated. This gradient is absent following systemic administration. Cochlear concentrations are significantly lower following systemic administration. Despite this glucocorticoid receptor activation appears equivalent. Adjuvant agents are demonstrated to significantly enhance RWM permeability and steroid distribution, but were detrimental to hearing. The process of implantation increases blood labyrinthine barrier permeability to steroids. . Mineralocorticoid activation mitigates post implantation endolymphatic hydrops. Thin sheet laser microscopy images in fine detail a neovascularisation process that occupies the peri-implant tissue response, with substantial vessel permeability characteristic of immature vessels.
Conclusions: Here we demonstrate that radical perilymph drug concentration differences observed between local and systemic administration may not be relevant when assessing the end goal of receptor activation. Many earlier studies assess drug distribution and activity in "naive" cochlea. Our results indicate that the process of implantation has both an immediate and delayed effect on the permeability of the cochlea. Specifically, a new region of drug permeability is introduced via neovascularisation in the foreign body response. This suggests a window for specific peri-implant drug distribution following systemic therapies in the early post-operative period.

Delivery of CRISPR/Cas9 Using Mesenchymal Stem Cell Derived Extracellular Vesicles

Xiaoshu Pan[1], Peixin Huang[2], Athanasia Warnecke[3], Mei He[1], Hinrich Staecker*[2]

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Background: Gene therapy for clinical translation has been challenging, due to limitations in current delivery vehicles such as traditional viral vectors with constrained packaging capacity and long-lived transgene production. To overcome some of the limitations of current delivery methods we used CRISPR/Cas9 ribonucleoprotein (RNP)-sgRNA complexes engineered extracellular vesicles (EVs) for in vivo gene therapy. Using the Shaker-1 mouse model of dominant progressive

hearing loss, we evaluated the delivery of RNP-EVs into inner ear hair cells using EVs derived from two different cell sources.

Methods: : EVs were derived from HEI-OC1 cells and umbilical cord derived mesenchymal stem cells. Loading of EVs was carried out using a custom built microfluidic electroporation system. EVs were loaded with green fluorescent protein and injected into the posterior semicircular canal of 1 month old C57Bl/6 mice. Distribution of fluorescence was evaluated. Next EVs were loaded with CRISPR/Cas9 ribonucleoprotein (RNP)-sgRNA complexes targeting a point mutation in myosin 7a present in the Shaker 1 mouse. Both postnatal day 3 and 1 month old Shaker 1+/- mice were injected with the loaded EVs and hearing followed over a 6 month period. Controls consisted of untreated mice, mice treated with non loaded EVs and CRISPR/Cas9 ribonucleoprotein (RNP)-sgRNA complexes loaded into lipid nanoparticles.

Results: GFP delivered by the EVs could be detected throughout the inner ear. The CRISPR/Cas9 ribonucleoprotein (RNP)-sgRNA loaded EVs displayed much higher editing efficiency with a clear reduction of Myo7ash1 mRNA expression compared to RNP-loaded lipid-like nanoparticles (RNP-LNPs), leading to significant preservation of hearing measured by auditory brainstem responses (ABR).

Conclusions: Extracellular vesicles derived from either MSCs or HEI-OC1 cells can be loaded with CRISPR/Cas9 ribonucleoprotein (RNP)-sgRNA complexes and effectively used to treat a dominant progressive hearing loss.

Development of Optogenetic Microneedle for Minimally Invasive Neuro-Stimulation of Inner Ear

Subin Kim*[1], So-Young Chang[2], Keum Jin Yang[3], Seong su Won[4], Jiae Jeon[4], Jae Yun Jung[6], Dong-Kee Kim[4], Min Young Lee[5]

[1]*Soonchunhyang University College of Medicine, Cheonan, Republic of Korea,* [2]*Beckman Laser Institute Korea, Dankook University,* [3]*Daejeon Mary's Hospital,* [4]*College of Medicine, The Catholic University of Korea,* [5]*Dankook University Hospital*

Background: The auditory sensory deprivation can cause degeneration of related nervous structures and further lead to decrease of other brain functions such as cognition and emotion. The electrical stimulation device such as cochlear implant is used with great performance of hearing rehabilitation, but it requires invasive surgical intervention and discomfort of maintaining both inner and external devices.

The specific parameter of light energy can be delivered to inner ear with minimal trauma to trigger stimulation using optogenetic engineering technique which requires viral transfection of gene that can be activated by light. The hurdle is the delivery of this viral vector into the target cells since inner ear has complex anatomy and physiology. Compared to conventional viral injection, microneedle is expected to have slower release time and lesser leakage with better-performance.

Methods: The biodegradable hyaluronic acid-based microneedle which releases AAV viral vector is designed for trans round window delivery to cochlear scala tympani. This microneedle was evaluated in assembloid which are combination of cochlear progenitor cell organoid and spiral ganglion cells.In vivo experiments were performed for the safety and efficiency. Western blots of cochlea were performed to quantify the AAV delivery. As for experiments using assembloid,

epifluorescence analysis to confirm the viral transfection and multielectrode assay (triggered by light stimulus) to assess the neural electric responses were used. As for in vivo experiments, transfection efficiency using AAV-GFP to compare conventional viral delivery and microneedle delivery was analysed. Light (continuous, 532 nm, 0.38 mW) induced ABR response were measured in optogentically engineered rat using microneedle.

Results: Conventional AAV viral vector delivery using microcatheter and microneedle AAV viral vector were compared (same virus copy no.). The microneedle AAV-GFP delivery showed significantly increased GFP amount measured by western blot analysis compared to conventional method. For comparison of two methods AAV-GFP was used initially. In epifluorescence analysis, both methods showed good transfection in inner hair cells. But in microneedle AAV-GFP group, vivid transfection of spiral ganglion neuron was observed, which was observed rarely in conventional AAV delivery.

We incubated assembloid which contains well-structured inner ear hair cells and spiral ganglion with microneedle including AAV-mCherry-Halorhodopsin. In these assembloids mCherry expressions were observed in both hair cells and neuronal cells suggesting that virus are targeting both sensory and neural cells. With the light stimulus these structures showed robust phase-locked-electrical activities. These microneedle with AAV-mCherry-Halorhodopsin were transplanted into round window of rat and ABR response without sound was measured with the laser irradiation. With the laser stimulation, waveforms of irregular but increased amplitudes were observed.

Conclusions: This study observed the auditory-neural-signals by the light-stimuli in microneedle-groups. These outcomes will facilitate the further investigation and development of bioengineered materials for the use of auditory stimulation without using any attached mechanical devices.

Podium 2: Auditory Nerve: Mechanisms, Damage, and Protective Strategies

Moderators: Cathy Sung and Mark Rutherford

2:30 p.m. - 4:30 p.m.

Ocean Ballroom 9 - 12

Estrogen-Related Receptor Gamma (Esrrg) is Required for Auditory Innervation and is Essential for Hearing

Shri Vidhya Seshadri[1], Neil J. Ingham[1], Rhianna R. Mackenzie[1], Anwen Bullen[2], Darya Alcock[1], Katie E. Smith[2], Adam J. Carlton[3], Stuart L. Johnson[3], Walter Marcotti[3], Karen P. Steel[1], Lisa S. Nolan*[1]

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Background: Estrogen-related receptor gamma (Esrrg) encodes an orphan nuclear receptor with structural homology to the classical estrogen receptors. Previously, we showed that genetic variation in ESRRG is associated with susceptibility to age-related hearing loss in women of post-menopausal age. Here, we describe the phenotypic characterisation of mice carrying a conditional knockout for Esrrg in the inner ear.

Methods: Mice carrying a conditional allele for Esrrg with LoxP sites flanking exon 2 were crossed with mice carrying the Sox10-Cre transgene [Tg(Sox10-Cre)1Wdr/J; MGI ref: 3586900] to generate Esrrg-mutant mice carrying the recombined allele, Esrrgtm1d/tm1d. To characterise the role of Esrrg in hearing, an in-depth phenotypic analysis was performed encompassing in-vivo auditory physiological recordings, immunofluorescence analysis, single hair-cell electrophysiology and comparative transcriptome analysis by RNA-Seq.
Results: Auditory brainstem response (ABR) recordings revealed substantial hearing loss in Esrrg-mutant mice from 2 weeks of age that by 2 months, although showing some improvement, remained significantly elevated. In comparison, distortion product otoacoustic emissions and endocochlear potential recordings were not disrupted in Esrrg-mutant mice and immunolabelling experiments showed no obvious loss of inner nor outer hair cells. Conversely, the ABR waveform shape was abnormal in Esrrg-mutant mice and wave I amplitude was reduced. Subsequently, immunolabeling experiments with anti-CtBP2 and anti-GluA2 showed the number of colocalised synapses was significantly reduced in Esrrg-mutant mice and many pre- and post-synaptic puncta were orphans. Single hair-cell electrophysiological recordings revealed inner hair cells from Esrrg-mutant mice fail to develop into fully functional sensory receptors. Further, immunolabeling with anti-NF-H to examine the auditory innervation in conjunction with the plasma membrane dye CellMask™ orange, revealed the auditory nerve fibres are sparsely populated in Esrrg-mutant mice with many myelinated fibres appearing truncated. Additional immunolabelling with anti-Tuj1 revealed a reduced number of spiral ganglion neurons in Esrrg-mutant mice. These results will be discussed further in the context of comparative transcriptome analysis
Conclusions: Targeted disruption of Esrrg in mouse inner ear leads to an early onset hearing loss and a phenotype reminiscent of an auditory neuropathy.

ATP-Gated P2x7 Receptor Predominantly Expresses in the Type II Auditory Nerves and is Required for the Auditory Efferent System Function and Noise Protection

Chun Liang*[1], Tian-Ying Zhai[1], Li-Man Liu[1], Jin Chen[1], Ning Yu[2], Hong-Bo Zhao[1]

[1]*Yale University Medical School,* [2]*University of Kentucky Medical Center*

Background: Type II auditory nerves and the cochlear efferent system constitute a negative feedback loop to control outer hair cell electromotility and hearing sensitivity, which plays a critical role in the protection from noise trauma. However, little is known about the underlying channel mechanisms of this negative feedback loop, particularly the channel function in type II spiral ganglion neurons. Here, we report that ATP-gated P2x7 receptor has predominant expression in the cochlear efferent system and type II spiral ganglion neurons and is required for the cochlear efferent system function; deficiency of P2x7 increases susceptibility to noise.
Methods: P2x7 knockout mice (Stock Number, #005576, The Jackson Lab) were used. The cellular expression of P2x7 in the cochlea was examined by immunofluorescent staining with confocal microscopy. The susceptibility to noise was assessed by exposure to 98-100 dB SPL white noise for 2 hours, one time. Hearing function tests were also examined by ABR and DPOAE recordings. Acoustic startle response (ASR) was also recorded to assess animal behavioral changes. Outer hair cell (OHC) function was assessed by patch clamp recording.

Results: Immunofluorescent staining demonstrated that ATP-gated P2x7 receptor had a predominant expression at type II auditory nerves and lateral olivocochlear (LOC) and medial olivocochlear (MOC) efferent nerves. The intense labeling was detected in the type II spiral ganglion neuron's soma and nerve fibers. The intensive labeling also was visible at the MOC and LOC synaptic areas under OHCs and inner hair cells, respectively. Knockout (KO) of P2x7 increased sensitivity to sound stimulation measured by ASR. ABR and cochlear microphonics (CM) were increased. OHC electromotility associated nonlinear capacitance (NLC) was increased and Vpk of NLC was shifted to left hyperpolarization side. P2x7 KO mice are also susceptible to noise. After exposure to 98-100 dB SPL white noise for 2 hours, ABR thresholds of wild-type mice completely recovered at post-exposure day 7 (P7), whereas P2x7 KO mice retained ~40 dB SPL threshold shift at P7 and had 20-40 dB SPL of permanent threshold shift (PTS) at P28.
Conclusions: These data demonstrated that ATP-gated P2x7 receptors have a critical role in the type II spiral ganglion neurons and cochlear efferent system; deficiency of P2x7 receptors leads to reducing negative control of the cochlear efferent system, therefore increasing hearing sensitivity and susceptibility to noise.

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Background Noise Impairs Precise Timing of Consonant Cues by Single Auditory Nerve Fibers

Amarins Heeringa*[1], Christine Koeppl[1]

[1]*Carl Von Ossietzky University*

Background: Speech perception degrades in background noise, which is primarily caused by an inability to identify the consonants. In this study, we aim to determine how different consonants are represented in the spiking patterns of auditory nerve fibers and how these representations are affected when presented in speech-shaped background noise.
Methods: Single-unit auditory nerve fiber recordings were collected from fourteen normal-hearing, young-adult gerbils, using glass electrodes. Three different naturally spoken vowel-consonant-vowel constructs, including the consonants /b/, /m/, and /t/, were presented in quiet and in background noise at 5 dB SNR, imitating a realistic communication situation. Previous behavioral studies in gerbils using the same stimuli revealed that discrimination between these consonants was significantly compromised in these conditions.
Results: The voiced segment of consonant /m/ had a rate and temporal profile across the tonotopic axis that differed significantly from the rate and temporal profiles of responses to /b/ and /t/. Background noise eliminated these rate differences, while the temporal code during the voicing of /m/ remained significantly different compared to /b/ and /t/. The consonants /b/ and /t/ are further characterized by their closure times and release burst, which are encoded in quiet by both the low- and high-spontaneous rate fibers of the auditory nerve. Background noise however disrupted the representation of these timing cues for both consonants. Whereas coding in high-spontaneous rate fibers was impaired by rate saturation, coding in low-spontaneous rate fibers lacked accuracy in background noise.
Conclusions: In summary, the consonants were encoded differently by the spiking patterns of the auditory nerve, and background noise thus also degraded these codes differentially. While

temporal coding of voiced segments persisted in background noise, the timed representation of release bursts and closure times in the spiking activity of auditory nerve fibers was severely impaired. Our results suggest that the behavioral difficulties to discriminate the consonants in background noise originate peripherally.

Effects of Cochlear Synaptopathy on Single-Unit Auditory-Nerve Responses in the Budgerigar

Leslie Gonzales*[1], Margaret R. Youngman[1], Kenneth S. Henry[1]

[1]*University of Rochester Medical Center*

Background: As humans age it is common for auditory-nerve fibers (ANFs) to degenerate. The loss of synapses between hair cells and ANFs is referred to as cochlear synaptopathy (CS). Some studies suggest that CS may impair perception of sounds in noise. Across different animal models of CS, there are contradictory results on how ANF responses change in CS. The current animal model, the budgerigar (a parakeet species), has been used extensively in behavioral auditory research and demonstrates performance comparable to humans for various complex auditory discrimination tasks. Therefore, budgerigars can potentially provide insight to the neural mechanisms for perception of complex sounds. Based on emerging data suggesting that CS can amplify auditory-nerve onset responses and alter temporal dynamics, it is essential to conduct a follow-up study quantifying changes in peripheral encoding.

Methods: The current experiment compared basic ANF response properties, including frequency tuning curves, post-stimulus time histograms (PSTH), and rate-level functions in control and CS-induced budgerigars. Tuning curves were presented using an automated algorithm. To create PSTHs, a characteristic-frequency (CF) tone was presented at a fixed level of 30dB above threshold. Rate-level functions were created by randomly presenting CF tones of varying sound levels (0 to 80 dB SPL). In CS animals, substantial ANF loss was induced in both ears via intracochlear infusions of 1-2 mM glutamate analog, kainic acid. During this CS-inducing procedure, to ensure hair cells remain intact, distortion otoacoustic emissions were conducted. For ANF recordings, a craniotomy was made above the anterior, vertical semicircular canal. Then a glass electrode with 40 to 80 MΩ impedance was placed in the flocculus of the cerebellum and driven down to the auditory nerve to record single-fiber responses.

Results: Tuning curves of CS animals appeared similar to control animals. CS tuning curves were V-shaped, with characteristic frequencies within the range of the budgerigar's behavioral sensitivity (i.e., up to 5-6 kHz) and sharpness of tuning (Q10) was within the range of control animals. Strength of synchrony was calculated from PSTHs, which suggested CS ANFs were within a similar range as control ANFs. In response to tones, CS ANFs showed a typical strong onset response followed by adaptation towards a steady state rate and some synchronization to fine structure. From rate-level functions, we found CS ANFs have a monotonically increasing firing rate as sound levels increase as in control animals.

Conclusions: Preliminary results suggest that ANFs in the CS-induced budgerigars have similar characteristics as control ANFs. Further analyses will test population differences and characterize the temporal coding of complex sounds in CS animals. Research in this species can provide important insight into the neural bases of normal and hidden hearing loss.

This research was supported by R01-DC017519, R01-DC017519-S1, and 1F31DC021889-01A1.

Evaluation of Auditory Evoked Potential Biomarkers of Cochlear Synaptopathy in Listeners with Self-Reported Hearing Difficulties

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Background: There is no standard diagnostic test for cochlear synaptopathy (CS), even though this pathology affects the auditory nerve synapses and is associated with ageing, noise exposure and ototoxicity. CS is expected to affect a large part of the population, which motivates the need for more studies on potential CS biomarkers and their relation to hearing difficulties. This study contributes to this effort by evaluating the overall usability and potential of several EFR markers in comparison to ABR wave-I characteristics. We also investigate the power of these EFR markers in predicting overt or self-perceived hearing difficulties, age-related hearing loss and speech recognition in noise.

Methods: We recruited subjects across three groups: a control group (N=60, ages 18-77) with normal audiograms and without self-reported hearing difficulties, a test group with self-reported hearing difficulties who had a HHIE-s questionnaire score above 4 and normal (N=60, ages 18-77) or impaired audiograms (N=70, ages 18 - 87). We selected 10 subjects per age-decade to ensure a normal distribution of age across the cohort. To date, we present collected data from 30 control subjects and 49 test subjects, who underwent ABR and EFR testing using a medical device under clinical investigation (NCT06114680 – clinicaltrials.gov) along with standard and extended-high-frequency audiometry and a Matrix sentence test in stationary noise. The EFR conditions included a 110-Hz harmonic tone complex and rectangularly-amplitude-modulated (RAM) pure tones at 2, 4 and 6 kHz. Their modulation frequency was 110 Hz, and stimulus levels were 70 dB SPL for EFR, ABR as well as speech stimuli. Statistical analysis included pair-wise comparison t-tests as well as correlation statistics.

Results: EFRs recorded at 70 dB SPL were significantly smaller for participants with self-reported hearing difficulties and correlated with the HHIE-s score. EFRs showed a significant negative correlation with age (strongest for the 6, 4 and 0.11 kHz conditions), indicative of temporal envelope coding declines as we grow older irrespective of specific hearing complaints. In the control group, there was no relation between the EFR strength and the average audiometric thresholds nor SRTs, suggesting that individual differences in their overall stronger EFRs did not relate to hearing ability. A significant negative correlation between the EFR and audibility and the SRT was present, indicating that temporal coding strength predicted both these measures for listeners with self-reported hearing deficits. Overall, we saw a better sensitivity and of the EFR markers compared to the ABR wave-I amplitude.

Conclusions: Supra-threshold EFRs to different carrier frequencies provided a robust marker of self-reported hearing difficulties and objective speech-in-noise performance in those listeners with a HHIE-s score above 4. Furthermore, it appears that a certain degree of temporal coding deficits are necessary before hearing difficulties become apparent.

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Live Imaging of Macrophage Response to Excitotoxic Injury in Zebrafish Lateral Line

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Background: Macrophages respond to cochlear injury and may play a critical role in synaptic repair. Recent studies in murine models demonstrate macrophage recruitment to the cochlea following moderate-intensity noise exposure and impaired synaptic recovery with macrophage elimination. However, real-time visualization of macrophage-synapse interactions in the intact mammalian inner ear is not feasible. Zebrafish offer a useful model due to the ease of using fluorophores and time-lapse imaging. Herein, we employed live imaging methods to evaluate macrophage activity following excitotoxic trauma in the zebrafish lateral line.

Methods: 6-7 day old transgenic zebrafish expressing a red fluorophore in macrophages (tg(mpeg1:tdtom)) and either GFP in afferent neurons (tg(neurod:tGFP)) or a plasma membrane-targeted Ca2+ indicator (tg(hsp70:GCaMP6s:CAAX-Sill1)) were imaged. Fish were paralyzed with α-bungarotoxin and mounted in an imaging chamber before visualizing the neuromast hair cells and neurons with a spinning disc confocal microscope. After baseline images, fish were exposed to 100 μM AMPA for 15-30 minutes to simulate excitotoxic injury, then rinsed with embryo media. Time-lapse imaging followed for 2-3 hours, and results were compared to control fish without AMPA exposure.

Results: At baseline, we observed an average of ~2 macrophages within a 25 μm region around each neuromast. Following AMPA exposure, a majority of GCaMP6s-expressing afferent nerves (GREATER THAN 50%) showed a robust Ca2+ surge ~10-25 minutes after AMPA exposure. Macrophage activation and movement toward the neuromast occurred in a subset of neuromasts from both transgenic afferent nerve lines, peaking at ~50 minutes. In most of these cases, ~1-3 macrophages were localized adjacent to the neuromast, with pseudopodal extensions observed in nearly all the cases (~90%). There was no evidence of a Ca2+ surge or macrophage recruitment to the neuromast in the control condition. These experiments are ongoing.

Conclusions: Live imaging of the lateral line following excitotoxic injury revealed novel interactions between macrophages and lateral line afferent neurons, including evidence of a Ca2 + surge in the nerve terminals that was correlated with macrophage recruitment. Further work will involve imaging fish with fluorophores to visualize synaptic debris uptake (Ribeye-GFP) and optogenetics to simulate excitotoxicity at a specifically targeted neuromast.

Translational and Multidisciplinary Investigation of Age-Related Myelin Degeneration in the Auditory Nerve

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Background: Limited understanding of the age-related pathologies underlying auditory nerve (AN) dysfunction, and lack of appropriate biomarkers of this pathology, has impeded the

development of clinical assessments and effective interventions to treat age-related hearing loss. Though myelin degeneration is known to occur with increasing age, the contribution of myelin degeneration to AN dysfunction in older adults is largely unknown. We employed a translational and multidisciplinary approach that included molecular, pathology, electrocochleography, and imaging studies, with comparative studies in postmortem human temporal bones, and incorporates older adults and mouse models. Myelin degeneration is hypothesized to disrupt the microstructure of the AN, which can be measured using diffusion tensor imaging (DTI) metrics. To determine the specificity of these DTI metrics to myelin, we present preliminary evidence from ex vivo imaging of human temporal bones, where we can compare MRI metrics and the underlying myelination status (via histology examination) within the same tissue. We can then translate these same DTI metrics to examine myelin status in vivo. In addition, to understand the role of myelin degeneration, and to identify possibly functional biomarkers of myelin degeneration, we examine associations between myelin degeneration and AN function using electrocochleography in younger and older adults and a mouse model of age-related hearing loss.
Methods: We performed ex vivo DTI on human temporal bones and in vivo AN DTI in younger and older adults. DTI metrics of fractional anisotropy (FA), mean diffusivity (MD), and radial diffusivity (RD) were examined. Myelin structure was confirmed using quantitative analysis of FluoroMyelinTM stained AN sections of the temporal bones. AN function using electrocochleography in humans and in CBA/CaJ mice. Several AN measures were assessed, including response amplitude and neural synchrony (phase-locking value, PLV).
Results: Age-related axonal loss and decreased myelin structure was seen in temporal bones from older donors compared to younger donors and was associated with DTI metrics of myelin degeneration. DTI metrics consistent with myelin degeneration were associated with PLV (AN synchrony) in older adults. Moreover, our preliminary results demonstrate that in young mice, and young adults, AN response amplitude is strongly associated with PLV, with larger responses seen with better neural synchrony. In contrast, no association between AN response amplitude and PLV was observed in older mice and humans. Analyses will examine the extent to which the breakdown in the link between AN response amplitude and PLV observed in older adults relates to AN myelin pathology.
Conclusions: Our results suggest a role for myelin degeneration in age-related AN dysfunction and provide preliminary evidence for the feasibility of developing non-invasive in vivo imaging protocols for detection of human AN dysfunction, and novel electrophysiological protocols to characterize AN myelin degeneration in aging humans.

TRC051384 Prevents Noise Induced Hearing Loss Through Activation of the Heat Shock Response in Spiral Ganglion Neurons

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Background: Noise-induced hearing loss (NIHL) is a significant public health concern that can profoundly affect an individual's communication abilities and overall quality of life. Excessive noise exposure primarily damages cochlear sensory hair cells and the synaptic connections

between inner hair cells and spiral ganglion neurons (SGNs). Although many molecular mechanisms involved in the pathogenesis of NIHL have been clarified, there are currently no effective drugs to reverse or prevent its occurrence. Developing effective targeted therapies requires a detailed and in-depth understanding of the molecular mechanisms underlying NIHL, including the relevant molecules, pathways, and biological processes. Recently, using proteomics methods, we have shown that noise insults generate proteotoxicity and activate the proteostasis network in the cochlea. The goal of this study is to test whether targeted protein quality control systems can protect against NIHL by utilizing small molecule regulators of the proteostasis network.

Methods: Subjects were 8- or 16-week-old male CBA/CaJ mice. The acoustic overexposure stimulus was an octave band of noise (8–16 kHz) at 100 dB SPL for 2 hours. GeoMx spatial transcriptomics was performed after noise exposure to determine spatial gene expression. Auditory brainstem responses (ABRs) and Distortion Product Otoacoustic Emissions (DPOAEs) were recorded before noise exposure and at 1 day, 3 days, and 14 days post-noise overstimulation. To test the drug effect, TRC051384 was administered intraperitoneally (60 mg/kg) 2 hours before noise exposure. ABR thresholds and wave-I amplitude were measured and compared among the control group, drug-treated group, vehicle-treated group, and the group exposed only to noise. Hair cells and cochlear ribbon synapses were examined by immunofluorescence microscopy. To assess changes in the protein quality control system, a ubiquitin-proteasome system (UPS) reporter mouse (line Ub-G76V-GFP) was used in the study.

Results: In 16-week-old male CBA/CaJ mice, moderate noise exposure results in no permanent ABR threshold shift but causes significant synaptopathy and a reduction in ABR wave-I amplitude. However, permanent hearing loss is observed in 8-week-old male CBA/CaJ mice. A wave of protein chaperone gene expression is observed 4 hours following noise exposure, which causes cochlear synaptopathy. Additionally, intraperitoneal pre-treatment with TRC051384 activates heat shock factor 1 (Hsf1) gene expression in the cochlea. GeoMx spatial transcriptomics reveals that proteasome genes are upregulated in both SGNs and supporting cells, but the UPS is dysfunctional only in SGNs. Using Ub-G76V-GFP mice, we find that TRC051384 alleviates noise-induced impairment of the UPS in SGNs. Consequently, our results show that TRC051384 pre-treatment completely prevents the decrease in ABR wave-I amplitude and synaptopathy after noise exposure, and also accelerates the recovery of hearing thresholds to baseline.

Conclusions: Noise-induced hearing dysfunction and synaptopathy can be prevented by pre-administration of TRC051384, which activates the heat shock response in spiral ganglion neurons.

Poster Blitz Session I

4:45 p.m. - 5:45 p.m.

Ocean Ballroom 5 - 8

Cochlear Amplification Modulates Synaptic Transmission at the Endbulb of Held Synapse in the Cochlear Nucleus

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Background: In the cochlea, outer hair cells push and pull the basilar membrane, dramatically amplifying its vibration, and therefore greatly expand the dynamic range of hearing. But how neurons and synapses in the central nervous system cope with this cochlear amplification and expanded dynamic range is poorly understood.
Methods: We took advantage of a mouse line (Prestin-/-) where prestin, the motor protein in outer hair cells, was genetically knocked out, therefore removing cochlear amplification completely without changing the cellular structure of the cochlea significantly. Firstly, we recorded auditory brainstem responses (ABRs) of both WT and Prestin-/- mice and evaluated their hearing performance. Secondly, we conducted patch-clamp recording in whole-mounted cochleae and investigated functions of inner and outer hair cells. Lastly, we performed patch-clamp recording in bushy cells in brainstem slices and examined the excitability of bushy cells in the cochlear nucleus and synaptic transmission in the endbulb of Held synapse.
Results: As expected, we found greatly elevated ABR thresholds in Prestin-/- mice (40 ~ 60 dB), with reduced amplitudes and increased latencies in Wave I of ABRs. In the cochlea, both outer and inner hair cells became smaller based on their whole-cell capacitance, non-linear capacitance in outer hair cells was completely removed, but exocytosis from inner hair cells remained unchanged. In the cochlear nucleus, bushy cells exhibited a slightly more depolarized resting membrane potential (3.29 mV), and an increased input resistance (67.4%), along with a smaller and shorter after hyperpolarization following spikes, all of which indicate increased excitability in Prestin-/- bushy cells. With auditory nerve stimulation, we found that Prestin-/- auditory fibers were more excitable in that the stimulation voltage required to evoke EPSCs successfully in Prestin-/- bushy cells was only 55.9 % of that for their WT counterparts. Furthermore, we found that the amplitude of evoked EPSCs was reduced by 22.2%, and their decay time constant became shorter (0.878 vs 0.770 ms), while neither the amplitude nor the frequency of spontaneous EPSCs was significantly changed. For paired stimulation, we found that the EPSC ratio went from depression in WT bushy cells (0.769) to facilitation in their Prestin-/- counterparts (1.11). With 50 stimulations at 100 Hz, we found a smaller readily releasable pool (RRP) of synaptic vesicles (391 vs 248 vs), a quicker replenishment of RRP (54.0% vs 76.2% at 500 ms), and a reduced sustained release rate of synaptic vesicles (1881 vs 971 vs/s) in Prestin-/- bushy cells.
Conclusions: Taken together, we found that cochlear amplification causes significant and multifaceted changes in excitability of bushy cells and synaptic transmission in the endbulb of Held synapses, and these changes are likely part of mechanisms allowing central neural circuit to better serve expanded dynamic range of hearing.

Computational Loudness Model of an Electrically Stimulated Cochlea

Franklin Alvarez Cardinale*[1], Waldo Nogueira[2]

[1]*Hannover Medical School,* [2]*Medical University Hannover and Cluster of Excellence "Hearing4all"*

Background: Cochlear implants (CIs) are devices that restore the sense of hearing in people with severe sensorineural hearing loss. An electrode array inserted in the cochlea bypasses the natural transducer mechanism that transforms mechanical sound waves into neural activity, by artificially stimulating the auditory nerve fibers (ANFs) with electrical pulses. The perception of sounds is

possible because the brain extracts features from this neural activity, and loudness is arguably the perceptual feature closest to the neural activity at the periphery of the auditory pathway.
Methods: A computational framework that uses a three-dimensional model and a simplified model of the electrically stimulated cochlea are used to reproduce loudness summation experiments performed by real CI users. These experiments studied the effect of rate of stimulation, electrode separation and amplitude modulation when using sequential stimulation (only one electrode active at a time). To obtain a loudness index, a spatio-temporal integration of the loudness contribution was performed. An exponential transform function was used to convert the instantaneous neural excitation density into loudness contribution in time steps of 200 µs.
The simulated threshold of hearing (Th) and most comfortable loudness (MCL) levels for each electrode were determined using a proposed method where the loudness growth function (LGF) obtained at different stimulation rates were compared. The criteria was based on various features observed in published data of real CI users' LGFs.

Results: The dynamic range of all electrodes, which is defined as the difference in dB between Th and MCL level, monotonically increased with the rate of stimulation when using pulse trains stimuli. However, this increment was highly dependent on the shape and selectivity of the excitation profile. Using two-electrode interleaved stimuli, the computational model predicted almost no difference (bellow 1 dB) in loudness summation when separating the stimulating electrodes. Finally, the loudness index of amplitude modulated stimuli was closer to the loudness index obtained by non-modulated stimuli at peak level when the carrier stimulation rate was low and the stimulation current was high. Using the simplified model ended in similar results as with the three-dimensional model, however, the dynamic range and loudness summation was generally higher.
Conclusions: Results showed that the proposed computational model, using both the three-dimensional and simplified peripheral model, is able to reproduce a wide range of experiments with real CI users predicting loudness summation in sequential stimulation. The LGF at low stimulation currents was highly related to the increase of neural activity in the peripheral ANFs, however, without the exponential transform function, it was not possible to obtain the rapid increase of the LGF slope at higher stimulation levels. This observation suggests that a more central mechanism in the auditory pathway influences the loudness perception.

Functional Characterization of Non-Calyceal Inputs in the Medial Nucleus of the Trapezoid Body

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Background: Auditory processing requires temporal precise integration along with reliable supra-threshold output formation. Neurons of the medial nucleus of the trapezoid body (MNTB) possess these features to ensure rapid and precise feed-forward inhibition to various auditory integration centers. This temporal precise neuronal transmission is partially based on the large, somatic synapse, the calyx of Held. Under resting conditions, this highly specialized synapse engenders faithful and temporal precise one-to-one action potential (AP) transfer. However, during ongoing high-frequency activity, the temporal precision and fidelity deteriorate. Next to the large calyceal

inputs, small excitatory non-calyceal (NC) inputs innervate the soma and dendrites of the MNTB neurons. Besides the excitatory nature of these NC inputs, their synaptic physiology and functionality remain largely unknown. In this study, we characterize NC inputs and elucidate how they support and modulate AP initiation generated by the calyx of Held and thereby promote fidelity and precision in auditory processing.
Methods: Whole-cell recordings from MNTB neurons in actuate brain slices were conducted. We stimulated afferent fibers at different frequencies and pulse numbers to characterize synaptic transmission and short-term plasticity of NC and calyceal inputs. External calcium concentration was elevated, to gain insights into vesicle dynamics of NC inputs. From these data, we derived the EPSC kinetics, short-term plasticity, recovery from depletion, and amount of asynchronous release. To probe the functional relevance of NC inputs to AP precision and success, we paired conductance templates of scaled calyceal and NC inputs under dynamic clamp conditions.
Results: Compared to calyceal EPSCs, the EPSCs of NC inputs are ~30x smaller and show more variability in decay time (up to 8x slower). In elevated extracellular [Ca2+] (2.5 mM) the vesicle pool could be partially depleted and recovered bi-exponentially with time constants comparable to the calyx of Held. Under physiological extracellular [Ca2+] (1.2 mM) NC inputs showed robust frequency-dependent facilitation. During train stimulations, asynchronous release increased. After the stimulation, the decay time of the occurrence of asynchronous release depended on the stimulation frequency. AP generation, triggered by simulated calyx conductance templates, showed a frequency-dependent reduction in success rate and a deterioration of temporal precision. Simultaneous application of NC conductance templates, increased calyx evoked AP success rates and improved the temporal precision up to 50 µs. This improvement depended on the conductance size of the applied NC template.
Conclusions: Our findings demonstrate that NC inputs can promote the fidelity and temporal precision of calyx-generated APs under ongoing activity. Moreover, it indicates that MNTB neurons integrate information from different sources to modulate the timing and fidelity of their output. Thus, the NC inputs play a critical role in auditory information processing.

3D Reconstruction of the Inner Ear Membranous Labyrinth Using 7 Tesla Magnetic Resonance Imaging and Advanced Post-Processing Techniques

Syed Ahmad*[1], Joon Soo Kim[1], Diane Jung[2], Zahra Sayyid[1], Adrian Paez[1], John P. Carey[1], Jun Hua[1], Bryan K. Ward[1]

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Background: Recent advances in Magnetic resonance imaging (MRI) at 7 Tesla (T) have allowed for greater characterization and resolution of the inner ear's membranous labyrinth. Since inner ear disorders like Meniere's Disease may be associated with swelling of the membranous labyrinth called endolymphatic hydrops, visualizing this space in greater detail may help understand its etiology. We aimed to develop a protocol for 3D segmentation of the inner ear to conduct volumetric analysis and characterize healthy vs. pathologic ears.
Methods: Adult participants without inner ear or neurological disorders were recruited. Axial T2-weighted and 3D-Fluid Attenuated Inversion Recovery (FLAIR) sequences were obtained at 7T MRI before and four hours after intravenous gadolinium-based contrast agent administration. Following image co-registration and subtraction with Statistical Parametric Mapping (SPM12) and

MATLAB, images were uploaded into 3D Slicer. Voxels measuring 0.3 x 0.3 x 0.5 mm corresponding to areas of endolymph were manually identified and highlighted to create 3D reconstruction, delineation, and volume quantification of the a) utricle and semicircular canals (SCC), b) saccule, and c) the cochlea.
Results: 3D segmentation was completed in five participants, yielding a volumetric analysis of 10 ears. The mean age of participants was 25.8 years (SD 1.64 years), and the cohort included three (60%) females. Across the ten ears, the mean utricle and SCC volume was 60.73 mm3 (SD 10.78 mm3), the mean saccule volume was 3.62 mm3 (SD 1.45 mm3), and the mean cochlea volume was 31.93 mm3 (SD 9.90 mm3). Of the five participants, the mean total endolymph volume (all three compartments bilaterally) was 192.62 mm3 (SD 36.83 mm3).
Conclusions: Herein, we describe one of the first protocols for 3D reconstruction and characterization of 10 non-pathologic ears of living human subjects. Volumetric analysis of this space can serve as a proxy for endolymphatic hydrops—and, therefore, as a bridge to better understanding the etiology, diagnosis, and treatment of several inner ear disorders. Future directions include comparing the inner ear's membranous labyrinth among patients with Meniere's Disease, Vestibular Schwannoma, and healthy controls to characterize differences in pathologic vs. non-pathologic ears better.

Healthy Aging Increases the Neural Reliance on Higher-Level Processing in Competing Speech Comprehension

Vivien Barchet*[1], Andrea Bruera[1], Jasmin Wend[1], Johanna Rimmele[2], Jonas Obleser[3], Gesa Hartwigsen[1]

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Background: In everyday life, spoken speech streams are often masked by noise or competing speech streams in the surrounding, creating challenging listening situations, particularly for older adults. To navigate these situations, listeners reconstruct the continuous speech signal from incomplete sensory input using higher-level processing. In an EEG study, we investigated how acoustic and higher-level linguistic processing contribute to competing speech comprehension across the adult life span. Previous results on the link between the behavioral and the neural reliance on acoustic and higher-level information in language comprehension across the adult life span have been inconsistent. Our pre-registered hypothesis was that older adults compensate for declines in lower-level sensory processing by relying more on higher-level information on the behavioral and the neural levels to maintain speech comprehension.
Methods: 63 normally hearing participants (age range = 18 – 70 years) heard 240 trials of two sentences presented simultaneously and were instructed to follow one speaker while ignoring the other one. They subsequently repeated the target sentence. The individual task difficulty was adjusted using an adaptive staircase procedure to account for peripheral hearing differences. Additionally, they listened to 20 trials, in which target sentences were replaced by word lists composed of three words with low semantic similarity. The hypothesis was assessed using a generalized linear mixed effects model predicting word comprehension performance from the interactions of age with acoustic and linguistic word-level variables. Additionally, we assessed the

extent of neural representation of acoustic and linguistic features of the target and distractor sentences using the trial-wise, cross-validated fits of multivariate temporal response functions.
Results: Consistent with our hypothesis, comprehension differences between word lists and target sentences were positively predicted by age, indicating that older adults benefited more from the context provided in the target sentences (p LESS THAN .001). However, the influence of word surprisal on sentence comprehension was not influenced by age (p = .31). Neural results revealed an increased neural representation of linguistic word-level features over centro-parietal sensors with increasing age (p = .003), as well as an increased influence of the neural representation of these features on comprehension performance in older adults (p = .014). This indicates that older adults' comprehension performance was more strongly influenced by the neural representation of higher-level target information.
Conclusions: The results provide a link between a stronger behavioral and neural reliance on higher-level processing supporting speech comprehension with increasing age, which may provide an important compensatory strategy in well-functioning older adults.

Cochlear Anatomy Impacts Neural Health and Current Spread at the Electrode-Nerve Interface in Children with Bilateral Cochlear Implants

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Background: The objective of this study was to evaluate the impact of cochlear malformations on the ability of cochlear implants (CI) to deliver current to the auditory nerve. The effectiveness of this process depends on the ability of each electrode to target sites along the auditory nerve to best portray sound cues. However, abnormal current pathways due to malformation of the cochlea may hinder the current's ability to reach neural cells or cause electrodes to stimulate overlapping populations of auditory nerve cells, obscuring sound cues. Despite frequent and successful cochlear implantation in children with abnormal cochleae, the impact of malformations on current spread remains poorly understood and programming stimulation parameters is clinically challenging. This study aimed to test the hypotheses that abnormal cochlear shape exacerbates current spread, correlates with reduced neural responsivity, and can predict the programmed electrical stimulation parameters.
Methods: CI stimulation parameters, electrophysiological recordings, transimpedance measurements and other relevant clinical information were assessed for a large cohort of children with bilateral CIs with either typically developed cochleae (n=184) and cochlear malformations (n = 27). A mixed effects modelling analysis was conducted. Child-specific models of voltage spread in the cochlea were developed by optimizing the tissue properties and dimensions of 3-D models of the implanted cochlea to accurately reproduce the spread of current in the child's cochlea as measured by the transimpedance measurements.
Results: Wider current spread was associated with increased auditory nerve electrophysiological thresholds (mean(SE) = 0.83(0.59), p LESS THAN 0.05) in the malformed cochlea, but not in the typically developed cochlea (p = 0.21). However, higher CI electrical stimulation levels were required for electrodes with wider current spread in the typical and malformed cochleae groups

(mean(SE) = 5.50(0.77), p LESS THAN 0.001 for C-levels in the malformed cochlea; mean(SE) = 3.31(0.82), p LESS THAN 0.001 for C-levels in the typical cochlea; mean(SE) = 5.55(0.49), p LESS THAN 0.001 for T-levels in the malformed cochlea; mean(SE) = 4.73(0.38), p LESS THAN 0.001 for T-levels in the normal cochlea). Further, there was greater spread of CI current in the malformed cochlea group than the typical cochlea group in the mid (mean(SE) = 1.43(0.43) p LESS THAN 0.05) and apical portions of the array (mean(SE) = 1.18(0.53, p LESS THAN 0.05). Child-specific models of the voltage distribution in the cochlea indicated that the spread of current to the auditory nerve depends on child-specific anatomy.
Conclusions: The spread of current delivered by the CI in the cochlea is impacted by the of cochlear malformations, including electrode-nerve distance and extracochlear tissue properties. These differences can be captured by a child-specific model. The ability of CI electrodes to stimulate the auditory nerve is dependent on the anatomy of an individual CI user. CI programming protocols should account for these differences.

Sex Differences in the Auditory Processing of Musical Sounds as Revealed With the Frequency Following Response

Joseph Luetkehans*[1], Trent Nicol[1], Jennifer Krizman[1], Nina Kraus[1]

[1]*Northwestern University*

Background: Male and female young adults differ in the timing and amplitude of their Frequency Following Response (FFR) to complex sounds. These sex differences in auditory processing arise in adolescence, suggesting a hormonal mechanism that preserves the timing and amplitude of brain responses in females. Previous experiments investigating sex differences in speech-evoked FFRs from young adults have demonstrated shorter onset latencies in female responses, as well as stronger encoding of high-frequency harmonic information in the female temporal fine structure response. The current study investigates whether similar sex differences can be found in FFRs to a wide range of musical notes.
Methods: Frequency Following Responses from 45 participants (28 female) between the ages of 18-25 were collected using 36 200-ms musical note stimuli sampled from octaves 2-5 of a Rhodes Electric Piano. Each note is a complex synthesized tone with a fundamental frequency (F0) ranging from 65.41 to 493.88 Hz. All participants had normal hearing.
Results: Female participants demonstrated faster response onset timing than male participants. Females also had greater phase consistency, a measure of the consistency of frequency-specific timing in the response, than males at first harmonic frequencies. We did not observe a significant main effect of sex in response amplitudes at either fundamental frequencies or first harmonic frequencies across all notes. There was no sex difference in cross-trial consistency of response morphology or broadband signal-to-noise ratio.
Conclusions: The sex differences that we observed in musical note-evoked FFRs are closely aligned with those observed in previous investigations of sex differences in click ABRs and speech-evoked FFRs and indicate faster, more stable processing of sound in the brainstem and midbrain in females than males. These results support the idea that sex differences in auditory processing reflect more than physical sex differences (e.g. head size), and may be a product of sex hormones acting differently on the auditory system of females and males starting in adolescence. In addition, the timing consistency effect observed here further separates biological effects on

auditory processing from experiential effects on auditory processing and should be considered in future research on sex, musicianship, language experience, or other experience-induced effects that prompt changes in sound encoding as measured by the FFR.

Two-Dimensional Organ of Corti Motion in the Mouse Apex

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Background: Despite tremendous progress in uncovering cochlear micromechanics with the introduction of optical coherence tomography (OCT), two- and three-dimensional organ of Corti motions are still not well understood. In addition, recent work in gerbil (Cho and Puria, 2022) showed that the reticular lamina (RL) moved more than the basilar membrane (BM) and that the motion varied between rows of outer hair cells. Passive mechanics measurements in gerbil (Zhou et al., 2022) showed similar transverse motion between the BM and RL and increased radial motion in the RL. Previous work in the mouse apex in vivo (Lee et al., 2016) suggested that radial tuning was present in both the BM and RL and was of a similar magnitude in the two directions. However, their equipment did not provide the resolution to resolve motion along the different rows of hair cells. We aimed to explore radial and transverse motions of the BM and RL in the mouse apex at the three rows of outer hair cells in vivo.

Methods: We used our 905 nm center wavelength, single beam Thorlabs OCT system mounted on a six-axis robot arm to capture two-dimensional organ of Corti motion measurements at a higher resolution than previous experiments. We achieved planar rotations using a tool reference frame aligned to that of our OCT B-scan for measurements at the same longitudinal place. To quantify our measurement angles, we implemented the orientation program described in Frost et al., 2022 and Frost et al., 2023. Vibration measurements in mice were obtained using methods similar to those described in Cho and Puria (2022, Scientific Reports). Tone sweeps were presented through the ear canal, and vibrations of the basilar membrane and organ of Corti structures were measured using VibOCT and SyncAv—LabView-based programs built in-house.

Results: Our preliminary results suggested that the RL of the first row of hair cells moved similarly in the radial and transverse directions and that this motion was greater than that of the BM. Furthermore, high-level measurements showed a decrease in motion from the first to third row of hair cells. The BM did also show radial tuning, and this motion was less than in the transverse direction.

Conclusions: Combining our single-beam, high-resolution OCT system and six-axis robot arm allowed us to capture motion along different rows of outer hair cells. This is an important step in untangling the intricate motions within the organ of Corti and support future development towards three-dimensional motion measurements. [Supported by the Amelia Peabody Charitable Fund and NIDCD R01DC07910, F31DC021079, and T32 DC000038.]

Small Arms Fire-Like Noise Induced Hearing Loss (NIHL) May Possess Distinct Diagnostic Profile From Previously Studied Models of NIHL

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[1]*Purdue University*

Background: Noise exposure is the second most common cause of hearing loss, behind aging. Small arms fire-like (SAF) noise is an acute form of noise exposure found in military and law enforcement occupations and recreation. Clinically, SAF noise induced hearing loss (SAF-NIHL) is often diagnosed and treated similarly to other forms of hearing loss by addressing loss of hearing sensitivity through hearing amplification strategies. However, SAF exposure may differ from other forms of NIHL. To develop SAF specific diagnostics and therapeutics, it is imperative to investigate the pathophysiology of SAF-NIHL. In 2019, Altschuler et al. found increased thresholds, reduced wave 1 auditory brainstem amplitudes, and a reduction in cochlear synapses 12-15 weeks after noise exposure. While these measures characterize peripheral damage, they do not provide information about more central auditory changes or responses to complex sounds. Our study aims to identify SAF-NIHL biomarkers responsible for auditory processing throughout the peripheral and central auditory systems to inform a progression map of damage post-SAF exposure. We hypothesize SAF exposure disrupts temporal processing through damage to hair cells and ribbon synapses leading to downstream neurotransmitter imbalance and neuroinflammation throughout the auditory brainstem.
Methods: Rat subjects (3-6 months) were exposed to SAF noise (50 rounds of 12 biphasic 0.3 ms pulses, 1 round every 3 s) at either 120 dBpSPL (SAF group; n=8, F=4) or 60 dBpSPL (sham group; n=4, F=2). We analyzed distortion product otoacoustic emissions (DPOAEs)(4, 8, 10 kHz), auditory brainstem responses (ABRs)(click, 8 kHz), and auditory evoked potentials (AEPs) of complex stimuli at baseline and post exposure days (7, 14, 28, 56). Thresholds were found using click and 8 kHz ABRs. Complex AEP stimuli included dynamically amplitude modulated sweeps (dAMs)(8 kHz or noise carrier amplitude modulated exponentially) and speech tokens of a male voiced "Purdue" (8 kHz or noise carrier modulated by speech envelope).
Results: Thresholds were persistently elevated by 10-15 dB. DPOAE signal-to-noise ratio decreased in all post-exposure days at 8 and 10 kHz. ABR waveform analysis demonstrated an overall decrease in amplitude with a greater decrease in wave 5. dAM response energy decreased at all days for frequencies between 8-120 Hz. Speech token neural responses demonstrate an overall decrease in response to all frequencies, except for a heightened response seen at the onset of the stimulus for frequencies between 500-2500 Hz. All measures demonstrated a general trend of gradual damage (days 7-14) and minor recovery (days 28-56).
Conclusions: Our findings suggest the diagnostic profile of SAF-NIHL may differ from the previously studied models of NIHL. Future work will identify mechanisms of damage at different time points post-exposure through anatomical imaging of biomarkers including neurotransmitter, neuron-glia interaction, hair cell integrity, and synaptic ribbon puncta.

Transcriptomic and Epigenomic Characterization of Adult Mouse Vestibular Hair Cells

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[1]*University of Washington,* [2]*Creighton University,* [3]*Keck School of Medicine, University of Southern California,* [4]*The University of Washington, Virginia Merrill Bloedel Hearing Resource Center*

Background: The sensory organs in the mammalian vestibular system house specialized mechanosensory hair cells that detect head movements. These hair cells are currently classified into two types, I and II, that differ in shape, molecular markers, physiology, and innervation. There is limited knowledge regarding the actual genetic diversity of mature vestibular hair cells and the regulatory mechanisms that control this diversity. Defining gene expression patterns in each distinct hair cell type in vestibular organs will inform on their unique features and functions and on strategies to drive functional hair cell regeneration.
Methods: We are analyzing the transcriptomes and epigenomes of vestibular hair cells in mature mice at 6, 7, 10, 14, and 22 weeks of age. Single nucleus multiome sequencing (RNAseq and ATACseq) was performed on utricles using 10x Genomics. mRNA sequences were analyzed using Seurat Library, creating cell clusters based on transcriptional similarities, and were verified using known marker genes. Chromatin accessibility data were first analyzed using Signac and ArchR to infer enhancer-promoter interactions. Then, pseudo-bulk ATACseq data were derived based on transcriptome clusters to analyze cell type/subtype specific regulatory elements. Genes of interest (GREATER THAN 2x enriched in each subtype) were explored using data from other RNAseq datasets via the gEAR portal, along with gene ontology analysis using DAVID and ShinyGO. Some genes of interest were validated using fluorescent in situ hybridization.
Results: Our data revealed five distinct groups of hair cells. Two groups of type II hair cells (Calb2+, Sox2+) were differentiated by region (Ocm+/-). Three groups of type I hair cells (Spp1+) were differentiated by region (Ocm+/-) and gene expression level. We confirmed the spatial and cell type-specific expression of over 10 newly defined genes. For example, Dlk2 was validated as a pan type II hair cell gene, Bmp2 as a pan type I hair cell gene, and Paqr9 as a striolar type I hair cell gene. Cntnap5b shows specific gene expression in striolar type II hair cells. Mgat4c gene expression is limited to a subpopulation of type I hair cells. We also identified cell type/subtype-specific regulatory elements for those genes, including Dlk2 enhancer, Bmp2 promoter and enhancer, Paqr9 enhancer, and Mgat4c promoter and enhancer.
Conclusions: We identified transcriptionally and epigenetically distinct clusters of mature utricular hair cell that varied based on region and type. We will further study gene regulation in each cluster of mature hair cells to learn how hair cell diversity is maintained.

Pathology of Fresh Human Cochleae Imaged With OCT and Validated With Histological Assessment

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[1]*Harvard Medical School, Massachusetts Eye and Ear Infirmary,* [2]*Harvard Medical School,* [3]*Massachusetts Eye and Ear*

Background: The human cochlea, a small fluid-filled organ, is encapsulated by the hardest bone in our body and is further surrounded by the temporal bone. As a result, currently available clinical assessments of cochlear health are mostly limited to standard audiometric measurements, including otoacoustic emissions. While these methods can diagnose hearing loss and the dysfunction of outer hair cells (which help amplify small sounds), the etiology is generally unknown, and the anatomical state of the cochlea is never directly assessed in clinic. However, recent advances in imaging technologies such as optical coherence tomography (OCT) enable

visualization of cochlear structures in situ without invading the cochlea. Here, we carried out a coordinated study of human cochlear anatomy based on OCT imaging of fresh (4-24 hours postmortem), unfixed human cochleae combined with light microscopy imaging of histology prepared from the same donor to better interpret OCT images and assess OCT's ability to detect cochlear pathologies.

Methods: Fresh human temporal bones (4-24 hours postmortem) were obtained from donors with permission at Massachusetts General Hospital. From each case, one ear was prepared for OCT imaging whereas the contralateral ear was immediately fixed in formalin and prepared for histology. Imaging was performed in intact, unfixed cochleae through the round window membrane using a 900-nm center wavelength OCT system (Gan6201C1, Thorlabs, Germany) with an axial resolution of 2.23 μm (in water) and lateral resolution of ~8 μm as previously described (Cho et al., 2022 JARO 23(2):195-211). Commercially available software (ThorImageOCT 5.4.8) was used to collect cross-sectional images and 3D volumetric images of the cochlear partition. Following imaging, cochleae were fixed and histologically processed along with the contralateral ears using a novel and rapid embedding technique based on a plastic (methyl methacrylate) resin.

Results: OCT was able to image cochlear structures after surgically accessing the middle ear cavity via a mastoidectomy with an expanded facial recess approach. Direct comparisons of OCT images and histology from the same donor and cochlear region facilitated the interpretation of OCT images and identified structural pathologies such sensory hair cell loss. We also investigated the effects of histological processing artifacts due to chemical fixation and dehydration.

Conclusions: The ability to image and identify cochlear structures with OCT may enable us to visualize and diagnose pathologies in patients. Intracochlear imaging would be invaluable for the testing of new pharmaceutical therapies, as the success of these drugs may require that baseline conditions of cochlear health be met, such as the survival of supporting cells or a near-normal appearance of the organ of Corti. Our results aid the accurate interpretation of OCT images and aim to facilitate the use of OCT as a future non-invasive real-time method to diagnose cochlear pathology in the clinic.

Surgical Planning for Implantable Middle Ear Microphone in Sheep Using Temporal Bone Micro-CT

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Background: Totally-implantable cochlear implant development, which would address drawbacks from external microphones, is limited by a well-functioning implantable microphone. The umbo Microphone (UMic), a dual-layer PVDF sensor under development by our team, detects umbo motion and converts it into electrical charge. To support the UMic's development, we are preparing for a live sheep study by refining the surgical approach for placement and fixation of the UMic using cadaveric sheep temporal bones. In this study, we aim to use micro-CT imaging to evaluate sheep temporal bone anatomical variations (e.g. facial nerve course) that influence development of a universal implantable device, to guide the surgical process, and to confirm correct placement of the UMic.

Methods: Four cadaveric Hampshire sheep temporal bones were evaluated by pre- and post-surgical micro-CT scans. Bone structures were segmented from pre-surgery micro-CT scans and down-sampled to ensure smooth simulation in an open-source image processing software (3D Slicer). Critical anatomical landmarks were segmented, and drilling was simulated with the eraser tool. The simulated drilled bone was then exported to a CAD virtual design program (SolidWorks). To secure the UMic sensor in place, a 3D printed metal fixation device was designed with a ball-and-socket mechanism. The socket arm was available in different lengths to accommodate variations in the distance between the umbo and the mastoid cortex; the length was chosen preoperatively using micro-CT. Fixation devices of various lengths were virtually tested to ensure fit. We then surgically prepared the cadaveric specimen for facial recess access to the middle ear cavity. Subsequently, one specimen was implanted with the UMic. To secure the UMic, the fixation device was screwed into the mastoid cortex. Placement of the UMic was checked by microscopic visualization and post-operative micro-CT.
Results: All four specimens were surgically prepared. Micro-CT imaging and actual surgical drilling revealed a small or absent antrum, abundant middle ear mucosa, and poorly pneumatized mastoid in all four specimens. The round window was fully visualized in both the simulated and all the actual post-surgical specimens. The socket arm length for each temporal bone was determined by virtually fitting the fixation device into the simulated drilled bone. Implantation of the fixation device and UMic in one specimen confirmed that the pre-selected version provided the best fit. Post-surgical micro-CT confirmed that the sensor tip contacted the umbo.
Conclusions: Definition of the sheep middle ear anatomy via micro-CT analysis will aid in future translational studies. In addition, this study demonstrates a feasible approach for simulating surgery using open-source software without the need for specialized equipment. This enabled surgical planning and device selection for the UMic and could have wider applications for other implantable prostheses.

Paralemmin-3 – an Essential Constituent of the Submembrane Cytoskeleton of Auditory Hair Cells

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Background: In the mammalian inner ear, cochlear inner hair cells (IHCs) enable accurate and faithful synaptic sound encoding, while outer hair cells (OHCs) perform frequency-specific sound amplification and fine-tuning through their intrinsic voltage-dependent somatic electromotility. This latter process is facilitated by the unique trilaminate structure of the OHC lateral wall, which consists of the transmembrane motor protein Prestin found within the plasma membrane, the

subcortical actin- and spectrin-based cytoskeleton, and the cytoplasmic subsurface cisternae. This complex system is essential for both, mechanical rigidity and stability as well as cell expansion and contraction during electromotility. Whereas the ultrastructure of the lateral wall is well described, its molecular composition is incompletely understood. Here, we identified paralemmin-3 (Palm3) as a novel protein specifically localized to the lateral walls of auditory hair cells that may play a crucial role in connecting the plasma membrane to the underlying cytoskeleton.
Methods: A comprehensive characterization of the functional and morphological consequences of Palm3 deficiency was conducted in Palm3-KO mice. First, auditory brainstem response (ABR) and distortion product otoacoustic emissions (DPOAE) recordings were conducted on 3- and 10-weeks-old WT littermate and Palm3-KO mice. Second, tonotopic hair cell survival and the overall involvement of Palm3 in the structural integrity and maintenance of auditory hair cells were investigated using immunohistochemistry, light sheet fluorescence-, and confocal microscopy. Third, by employing electron tomography on high-pressure frozen and freeze-substituted organs of Corti, the structural integrity of the lateral wall of WT and Palm3-KO OHCs was examined on ultrastructural level. Finally, intracochlear delivery of adeno-associated viruses (AAV) encoding eYFP-tagged wild-type Palm3 was carried out to try to revert the pathophysiological phenotype of Palm3-KO.
Results: Palm3-KO mice exhibit early-onset and progressive hearing impairment resulting from diminished cochlear amplification. Subsequent multiscale morphological analyses of acutely dissected cochleae revealed structural collapse of OHCs, leading to progressive and extensive OHC loss along the tonotopic axis. Furthermore, Palm3-KO OHCs exhibit disrupted distribution and attenuated expression of several membrane-associated proteins – including Prestin and α2-Spectrin – suggesting a role of Palm3 in plasma membrane scaffolding. In line with this hypothesis, electron tomography of OHC lateral walls revealed significantly fewer and structurally perturbed cisternae structures in Palm3-KO compared to WT littermates. Finally, AAV-mediated rescue of Palm3 partly restored hearing function, enhanced OHC survival and restored OHC cell shape as well as Prestin and α2-Spectrin expression.
Conclusions: Palm3 is a novel protein of the lateral plasma membrane of auditory hair cells that is essential for adequate membrane scaffolding and OHC survival.

Does Stereocilia Separation-To-Height Ratio Accurately Define the Geometric Gain?

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Background: Geometric gain is a critical parameter in cochlear hair bundle (HB) models that influences the bundle's mechanical and electrical response to sound. It is often approximated as the ratio of the horizontal spacing between stereocilia pivots to the average stereociliary height. This approximation is used to estimate how mechanical deflections of the HB affect gating spring tension, which modulates ion channel activity. However, relying on this simplified ratio does not accurately capture the true mechanics of the system, leading to potential inaccuracies in predictions of HB sensitivity, stiffness, and related quantities from the models.
Methods: We analyzed geometric gain at five cochlear locations in adult mice (30 kHz, 12 kHz, 8 kHz, 2 kHz, and 750 Hz), comparing two definitions: the approximated "stick geometric gain,"

derived from two infinitesimally thin stereocilia and the "true geometric gain," based on the complete morphology of stereocilia using our fully nonlinear two-row isolated HB model without adaptation. We use morphological and mechanical data from published experiments. To assess the effects of these geometric gains on bundle sensitivity, operating range, and stiffness, we implemented the model developed by Tinevez et al. (Biophys. J., 93(11), 4053-4067 (2007)), referred to as the TJM model, without adaptation, as it employs the stick approximation. We then compared its predictions with our two-row model that inherently utilizes the true geometric gain.
Results: The stick geometric gains were calculated as 0.25, 0.17, 0.15, 0.11, and 0.09 across decreasing characteristic frequencies, while the corresponding true geometric gains were consistently lower at 0.09, 0.08, 0.06, 0.05, and 0.04. This reveals a substantial two- to three-fold overestimation of geometric gain by the stick model that neglects bundle morphology. As a result, the TJM model predicts larger changes in gating spring tension for the same set of model parameters defined in the two-row model, leading to overestimated bundle sensitivity and narrower activation curves. Stiffness predictions at ~0.4 open probability were similarly larger, with errors ranging from 75% to 125%.
Conclusions: The overestimation of bundle sensitivity and stiffness, coupled with an underestimated operating range, compromises the accuracy of HB dynamic models. The commonly used geometric gain approximation, based on a simplified stick model, fails to capture the complexity of HB morphology, particularly the length and inclination of the tip link. These structural factors significantly influence gating spring tension and sensitivity, highlighting the need for a precise definition of the geometric gain and models that accurately represent the true geometry of HBs. As the next step, we plan to explore how changes in the geometric gain affect response prediction when adaptation effects are included in the system.

Therapeutic Potential of ssAAV vs. scAAV in Inner Ear Gene Therapy

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Background: Gene therapy using adeno-associated virus (AAV) is a powerful approach for treating inner ear diseases, with more than 40 preclinical studies and ongoing clinical trials demonstrating its potential. To fully achieve the potential of AAV-based therapies for hearing loss and balance disorders, several factors need optimization. One limiting factor in transgene expression is the conversion of single-stranded (ss) DNA to double-stranded (ds) DNA. Self-complementary (sc) AAV vectors can bypass this step and may improve expression efficiency in hair cells, but it remains unclear if this leads to superior therapeutic efficacy compared to ssAAV vectors. This study aims to compare and evaluate the viral expression rates and therapeutic effects of ssAAV and scAAV in a mouse model of DFNB103 caused by CLIC5 mutations.
Methods: Synthetic AAV9-PHP.B vectors, single-stranded (ssAAV) and self-complementary (scAAV), both carrying the coding sequences of TurboGFP or Clic5, were generated and delivered

into the inner ears of Clic5c.680T GREATER THAN C mice via utricle injection at P0. Immunostaining was used to quantify and compare the transduction rate between the two AAVs. Measurements of hearing function were performed using auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs), followed by behavioral assays such as Rotarod and open field tests to evaluate vestibular function. Sensory cell morphology in both the vestibular and auditory systems was assessed using immunofluorescence and scanning electron microscopy.
Results: Both ssAAV and scAAV demonstrated high expression efficiency in inner ear sensory cells, with scAAV.GFP demonstrating superior enhanced efficiency compared to ssAAV.GFP. Injection of either ssAAV or scAAV vectors encoding Clic5 rescued hearing in deaf Clic5c.680T GREATER THAN C mice by restoring Clic5 expression in hair cells. Compared to the untreated group, the treated mice had a higher survival rate of hair cells and reduced degeneration of hair bundle morphology. In the vestibular system, both ssAAV.Clic5 or scAAV.Clic5 rescued vestibular function, evidenced by decreased circling behavior and improved motor performance in treated mice. While the scAAV demonstrated enhanced transduction efficiency, the ssAAV showed a slightly more favorable trend for comprehensive therapeutic effects. However, this improvement was not statistically significant when compared to the scAAV.
Conclusions: Our findings demonstrate the feasibility of restoring Clic5 expression, reducing cell death and morphological degeneration, and rescuing auditory and vestibular function in Clic5-deficient mice. This was achieved using both ssAAV and scAAV. While scAAV demonstrated enhanced expression efficacy, this did not translate to significantly improved therapeutical outcomes in this model.

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An Auditory Cortex Network Represents Both Vocal Categories and Family Dialects

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Background: In some species, variability in the acoustic features of specific vocalizations can convey information about an individual's identity or family, sometimes referred to as vocal dialects. Thus, a fundamental question is how the central nervous system represents a general vocal category, while also discriminating between the subtle acoustic differences that may communicate social information.
Although there is a rich understanding of vocalization representations throughout the auditory neuraxis, the general approach has been to probe auditory neurons with relatively few vocalization exemplars, and often from vocalizers with an unknown relationship to the receiver. Here, we address the issue of call categorization by analyzing the response of adult gerbil auditory cortex (AC) neurons to a large array of variants recorded from the animal's own family and those of two other families.

Methods: We investigated the ability of AC neural populations to categorize 4 vocalization types in awake, freely-moving adult Mongolian gerbils (Meriones unguiculatus) (n=5; 3M, 2F). Gerbils are a highly social rodent species that live as multi-generational families and produce a rich vocal repertoire. We used chronically-implanted high-density silicon probes to record wirelessly from single AC neurons while presenting a large set of variants (n=300) for each of 4 vocalization categories (n=1200 stimuli, 5 trials each). The vocalizations were obtained from overnight audio recordings of individual gerbil families, one of which was always the implanted animal's own family. The response of each neuron to pure tones and amplitude modulated (AM) white noise was also used to characterize spectral and envelope responses. Initial analyses focused on AC neuron rate coding using a population decoder (support vector machine).
Results: Single unit responses generally displayed a highly variable response to the 300 variants within a call category. Despite this within-group variance at the single-neuron level, AC populations were able to decode categories significantly above chance (compared to shuffled trials; paired t-test, p LESS THAN 0.001). The sensitivity for decoding each call type was measured using the area under the curve (AUC) of the ROC curve, comparing trials of a given category versus all other categories. The average AUC across individual animals was 0.89 (range: 0.78-0.94). AC populations were also able to decode the family identity of each of the 4 call types, with an average AUC across individual animals of 0.63 (range: 0.57-0.69). Current research is investigating the extent to which AC neuron spectrotemporal tuning contributes to call and family categorization.
Conclusions: Our results suggest that vocal categories and family differences are represented by the same auditory cortex network, even for single syllables. Future work will investigate whether family information is more potent when vocalizations are presented in their natural bout structure.

Optical Imaging of Auditory Cortex Responses in the Awake Common Marmoset (Callithrix Jacchus) With Unilateral Cochlear Implants

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Background: Our laboratory has recently developed a through-skull wide-field optical imaging method in awake common marmosets (Callithrix jacchus) (Song et al, 2022). This non-invasive imaging method has shown to be an efficient tool for identifying tonotopic gradients in the auditory cortex. It also serves as a localizer for directing the parcellation of the auditory cortex, separating the primary auditory cortex (area A1) and belt subregions. Although this approach has been extensively investigated under acoustic stimuli, it has never been examined in the context of electrical stimulation from cochlear implant (CI) devices.
Methods: In this study, we implemented this optical imaging method in the awake marmoset models with unilateral CI, a highly vocal non-human primate with a similar hearing range as humans, to investigate cortical response patterns under CI stimulation. Imaging sessions were recorded in three awake marmosets from both hemispheres while varying current levels across all CI electrodes using three different CI configurations (monopolar/MP, partial tripolar/pTP, and tripolar/TP) in random order.
Results: Results showed the feasibility of this imaging technique to demonstrate tonotopic gradients across the hemispheres under CI stimuli. In general, for all paradigms tested, increasing

current level corresponded with increased activation amplitudes and areas. However, we also observed non-monotonic responses in the left hemisphere under TP and pTP stimulation. In comparison between left and right hemispheres, we found that CI stimulation was more effective at activating neuronal responses in the left hemisphere (contralateral to the CI ear). Between MP and TP stimulation, MP induced greater activation areas, whereas TP activation areas were more focused.

Conclusions: This through-skull mapping approach provides an alternate perspective for understanding how the auditory cortex processes the electrical stimulation from the CI device and may help address some deficits observed in the performance of CI users in perceptual tasks.

AI-Driven Automatic Speech Perception Scoring

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Background: Speech perception testing is a cornerstone for evaluating outcomes in cochlear implant (CI) users, guiding clinical interventions and long-term care (Holden et al., 2013; Gifford et al., 2008). This process typically requires participants to listen to words or sentences and articulate their responses, which are manually scored against the original speech materials. However, manual scoring is labor-intensive, introduces variability, and can lead to inconsistent results (Kuk and Lad, 2010; Wolfe and Gurgel, 2019). We investigated the potential of using Whisper, a high-accuracy, open-source speech-to-text tool (Radford et al., 2023; Sinha and Azadpour, 2024), to automate CNC word and phoneme scoring in CI users.

Methods: Two experienced CI users completed word recognition tests with six CNC30 word list pairs (Holden et al., 2013). Audio recordings were transcribed using Whisper, supported by voice activity detection (VAD, Bain et al. 2023) and grapheme-to-phoneme (g2p) (Park and Kim, 2024) mapping. The g2p function allowed for phoneme-level comparisons for accurate speech perception scoring by addressing variations in spelling and pronunciation. Orthographic transcriptions provided by CI users served as the benchmark for evaluating scoring accuracy of AI-driven transcriptions and manual scoring by expert human scorers.

Results: Across CNC list pairs, Whisper-produced transcription errors averaged at -1.38% ± 3.35% for words and -1.00% ± 1.87% for phonemes. Expert human scorers had mean transcription errors of 2.38% ± 2.71% for words and 2.11% ± 1.43% for phonemes. Speech perception scores based on orthographic transcriptions averaged 73.67% ± 4.3% and 40.08% ± 4.72% for the two CI subjects. We found no measured difference in the absolute error rate between whisper and human scorers (paired t-test, $p=0.38$). We also found no measured difference in variance in the error rates, between Whisper and human scorers (Levene's test for equality of variances, $p = 0.451$; and F-test for variance, $p = 0.755$).

Conclusions: Whisper demonstrates strong potential as an AI-based tool for automating speech perception scoring in CI users, closely matching the benchmark orthographic transcriptions provided by CI users themselves. Notably, our findings reveal that expert human scorers consistently overshot the orthographic benchmark scores while Whisper undershot it. However, absolute error rates between Whisper and Human scoring were statistically indistinguishable. By automating speech scoring, Whisper could significantly streamline clinical workflows and enable

more consistent CI outcome tracking, particularly in large-scale or remote settings. While minimal manual adjustments were required for audio file formatting in this study, the process holds the potential to be fully automated. Future work will focus on expanding the subject pool to ensure broader representation of CNC word and phoneme score performances, integrating AI confidence measures to trigger manual review when needed, and testing additional speech-to-text models like NeMo Canary (Kuchaiev et al., 2019) to further improve accuracy and robustness.

Central Processing of Optical Hearing in the Anteroventral Cochlear Nucleus

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Background: Future cochlear implants may use light instead of electricity to restore hearing to people with profound hearing loss. Unlike electricity, light can be spatially confined in the cochlear fluid and could therefore restore hearing with a greater frequency selectivity. The anteroventral cochlear nucleus (AVCN) is the first processing unit of the auditory pathway, where excitatory inputs from the spiral ganglion neurons are integrated. Acoustically, the representation of the sound into AVCN spike trains varies greatly, reflecting a large diversity of cell types, morphology, connectivity and molecular makeup.
Methods: In this study, we aim at characterizing the AVCN neural processing of cochlear optogenetic stimulation and interrogating the synaptic integration mechanism underlying the observed responses. Activation of the auditory pathway was achieved by optogenetic stimulation of spiral ganglion neurons (SGNs) expressing f-Chrimson in Mongolian gerbil. We employed a semi-stochastic stimulus to examine over 200 combinations of light pulses and inter-pulse-intervals; and sequentially performed juxtacellular recordings of SGNs or AVCN neurons. Additionally, optically evoked compound action potentials were simultaneously recorded as a control of the cochlear activation.
Results: Our data show the diversity of neuronal responses to optogenetic stimulation at early stages of auditory processing. We also observe increase of fidelity of responses in AVCN neurons compared to SGNs.
Conclusions: Our findings advance our understanding of input processing in the central auditory system and inform the development of optical hearing restoration strategies.

Poster Blitz Session II

4:45 p.m. - 5:45 p.m.

Ocean Ballroom 9 - 12

Cochlear Nitrative Stress and Associated Signaling in Noise-Induced Hearing Loss

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Background: Noise-induced hearing loss (NIHL) is a significant public health issue worldwide. Nitrative stress is emerging as an important factor in NIHL, but the underlying mechanisms are not fully understood. This study investigates cochlear nitrative stress, identifies nitrated cochlear proteins, and elucidates associated signaling mechanisms in noise-induced auditory dysfunction in mice.
Methods: Young adult CBA/J mice were exposed to 90 dB broadband noise 2 h/day for two weeks. Hearing threshold shift was evaluated by recording Auditory Brainstem Responses (ABR). Outer hair cell (OHC) activity was measured by recording Distortion Product Otoacoustic Emissions (DPOAE), and hair cell loss was assessed by immunohistochemistry. Cochlear synaptic dysfunction was evaluated by measuring ABR wave-I amplitude and latency and the functional synapses in the cochlea were estimated by immunostaining with anti-GluR2 and anti-CtBP2. The levels of the nitrosative stress marker 3-nitrotyrosine in the mice cochleae were measured by immunostaining with anti-nitrotyrosine. The nitrated cochlear proteins were immunoprecipitated with anti-nitrotyrosine and analyzed by mass spectrometry, and associated signaling pathways were identified using bioinformatics analysis.
Results: Noise exposure increased the hearing thresholds by 10-20 dB (p LESS THAN 0.05; n=6), affected the activity of OHCs (16, 24, and 32 kHz; p LESS THAN 0.05), and induced hair cell loss in the basal turn of the cochlea (p LESS THAN 0.05). Noise exposure decreased wave-I amplitude (Click and 32 kHz; p LESS THAN 0.05) and increased wave-I latency (24, 32 kHz; p LESS THAN 0.05), suggesting noise-induced cochlear synaptopathy. In agreement, an examination of the pairing of pre- and post-synaptic markers indicated that noise exposure decreased the number of paired synapses (p LESS THAN 0.05; n=3) in the basal turn of the cochlea. Moreover, noise exposure significantly increased the nitrotyrosine levels in the OHCs and spiral ganglion cells (p LESS THAN 0.05), suggesting noise-induced cochlear nitrative stress. Proteomics analysis revealed that noise exposure induced the nitration of 744 cochlear proteins (n=4) while bioinformatics analysis of associated KEGG pathways indicated that the citrate cycle, oxidative phosphorylation, ribosome, and synaptic vesicle cycle were among the most highly enriched cellular processes.
Conclusions: This study indicates that noise exposure induces cochlear nitrative stress, resulting in the nitration of several cochlear proteins. More importantly, many of the nitrated cochlear proteins are associated with critical signaling pathways that regulate auditory function. Together, these findings provide new insights into the role of nitrative stress in NIHL.

Modeling Normal and Impaired Hearing With Deep Neural Networks Optimized for Ecological Tasks

Mark Saddler*[1], Torsten Dau[1], Josh McDermott[2]

[1]*Technical University of Denmark,* [2]*MIT*

Background: Computational models that perform real-world hearing tasks using cochlear input could help link the peripheral effects of hearing loss to real-world perceptual consequences. Deep artificial neural networks, optimized separately for sound localization and recognition tasks, have been shown to account for many aspects of normal hearing behavior. Here, we extend this

approach to model the behavioral consequences of hearing loss using a network jointly optimized for multiple tasks.
Methods: We trained a single deep neural network model to localize and recognize speech, voices, and environmental sounds using simulated auditory nerve representations of naturalistic scenes. Once trained, we compared the model's spatial hearing and speech recognition performance to that of humans. We also measured the model's psychoacoustic thresholds (tone detection in quiet/noise, temporal gap detection, and spectral/temporal modulation detection) by training linear classifiers to make binary judgments using the model's learned features. These classifiers are intended to represent the decision rules that humans use to perform simple hearing tests, relying on relatively fixed internal representations that were plausibly optimized for ecological tasks over longer timescales. To investigate the perceptual consequences of hearing loss, we altered the model's peripheral input and measured the resulting effects on behavior. Different types of hearing loss were simulated by manipulating the number and functionality of inner hair cells (IHCs), outer hair cells (OHCs), and auditory nerve fibers (ANFs).
Results: When equipped with healthy cochleae, the model accounted for several aspects of binaural speech perception in humans with normal hearing, reproducing the effects of noise, reverberation, and spatial separation between speech and noise. When healthy cochleae were replaced with damaged cochleae, the model's performance resembled that of humans with hearing loss: speech recognition deteriorated (especially at low SNRs) and spatial release from masking was reduced. Psychoacoustic thresholds measured from the model similarly reproduced patterns of normal and impaired human hearing. Despite never being fit to human data, the model replicated the effects of noise carrier bandwidth, modulation rate, and masker modulations on human amplitude modulation detection thresholds. Simulations of plausible and idealized hearing loss phenotypes (i.e., combined OHC and ANF loss vs. isolated OHC or ANF loss) suggest that both OHC and ANF loss contribute to real-world hearing difficulties, but they produce distinct behavioral outcomes in psychoacoustic listening tests.
Conclusions: The results provide a normative account for fundamental aspects of human hearing, suggesting phenomena like spatial release from masking and modulation frequency selectivity can be understood as consequences of optimization for ecological tasks. Machine-learning-based models that generate behavior from simulated auditory nerve input can predict aspects of hearing-impaired behavior and may help disentangle the perceptual consequences of different types of hearing loss.

Accuracy and Efficiency of a Swept Modulation Depth Stimulus for Cross-Species Neurometric Physiological Analyses

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Background: Despite advancements in hearing-aid technology, understanding speech in noise remains a significant challenge for hearing-aid users. Understanding the neural mechanisms underlying speech perception in noise, and how these mechanisms are degraded with hearing loss, is crucial for improving hearing-aid efficacy. Amplitude modulation (AM) plays an essential role in speech, and previous studies have demonstrated a relationship between modulation detection and speech intelligibility. Physiological studies have also shown that sensorineural hearing loss impacts modulation detection, particularly in noisy environments. Neurometric analyses can be

used to estimate modulation detection in physiological studies by quantifying the discriminability of modulation depth based on neural coding statistics. However, estimating modulation detection through physiological recordings involves repeating measurements for each modulation depth and comparing to a reference (unmodulated tone). This process is time-consuming and poses challenges, especially in neurophysiological recordings from a single auditory-nerve (AN) fiber. While this measurement results in a unique and valuable dataset for studying the neural mechanisms underlying modulation coding, the measurement is constrained by time as the fiber may be lost before the modulation depth threshold can be determined. Similarly in human studies, long behavioral experiment durations introduce subject fatigue and reduced attention, while lower signal-to-noise ratios (SNR) in human electrophysiological measurements such as envelope following response (EFRs) and EEG require more repetitions, further extending the duration of these experiments.

Methods: We propose using a swept modulation depth stimulus, where the modulation depth changes continuously over time while keeping the root mean square of the signal constant. This stimulus offers an alternative method for studying modulation coding and determining modulation depth thresholds. Spiking data from computational models (Zilany et al., 2023 [without efferent] and Farhadi et al., 2023 [with MOC efferent]) are used to analyze phase-locking to the temporal envelope of AM stimuli. The effects of modulation frequency, direction of the sweep (upward or downward), and sound level are examined. This stimulus will be evaluated for application in physiological recordings, including AN-fiber recordings (animal models), EEG, and EFR in both human and animal studies.

Results: Preliminary results from EFRs recording in chinchilla and computational modeling confirm that a swept modulation depth stimulus can be both efficient and accurate for physiological recordings, particularly in AN-fiber single-unit recordings as time for measuring data from each fiber is limited and variable across fibers and specifically degraded in animals with hearing loss.

Conclusions: The swept modulation depth stimulus has the potential to improve amplitude-modulation detection methods in a range of physiological studies. Further research is needed to evaluate the practical application of this stimulus in cross-species experimental setups. Additionally, the effects of efferent activity should be explored through computational modeling and physiological methods.

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Technical Details on Single-Molecule Microscopy of MYO7A Trafficking in Live Hair Cell Stereocilia

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and Other Communication Disorders, [6]Harada Ear Institute, [7]NIDCD / NIH, [8]Southern Illinois University School of Medicine

Background: Stereocilia are a bundle of cylindrical F-actin protrusions that develop on the apical surface of hair cells and function as biological mechanosensors for sound and acceleration. Although wild-type motor activities of unconventional myosins are essential for developing functional stereocilia, it is not fully understood how each myosin molecule localizes itself and its cargo using the energy from ATP hydrolysis. Here, we introduce the technical details of our workflow for single-molecule microscopy in live hair cell stereocilia to approach this open question with recent updates demonstrating our current analyses of MYO7A's trafficking in stereocilia.

Methods: Explant cultures of utricles or saccules (hereafter "vestibules") were prepared from mouse neonates at postnatal day 2–5 and transfected using a Helios® gene gun to express HaloTag-fused proteins. EGFP or EGFP-fused protein was co-expressed as a transfection marker. HaloTag-fused protein molecules were fluorescently labeled using JFX554-ligands and illuminated by a 561-nm light sheet at 0.2 kW/cm2 using a dual-inverted selective plane illumination microscope (diSPIM). HaloTag-fused human β-actin (HaloTag-actin) was used to characterize fluorescent puncta. Trafficking of MYO7A was analyzed using HaloTag-fused full-length MYO7A, MYO7A-HMM (a head + neck fragment) and MYO7A-R/K (a mutant disabling the tail-mediated motor autoinhibition). The p.F36V mutant of FK506-binding protein 12 (FKBP) was fused to the C-terminus of MYO7A-HMM for conditional homodimerization using an FK506 derivative, AP20187. Conditional heterodimerization between FKBP and the FKBP-rapamycin binding domain (FRB) by a Rapalog, AP21987, was used to tether MYO7A-HMM to the plasma membrane or the F-actin core.

Results: In vestibular hair cells expressing HaloTag-fused proteins, the density of fluorescent puncta decreases as the concentration of JFX554 ligands decreases. HaloTag-actin molecules are distinguished from each other with 0.01–0.03 nM of JFX554 ligands. Fluorescent intensities of HaloTag-actin puncta show a quantum distribution consistent with single-molecule detection. Single molecules of HaloTag-fused MYO7A and its fragments are visible with a slightly higher concentration of JFX554 ligands, 0.3–0.6 nM, suggesting their low expression level. Imaging of MYO7A in stereocilia is established using HaloTag-MYO7A-HMM-FKBP conditionally dimerized by 200 nM AP20187. Kymograms generated from time-lapse acquisition every 1 sec are useful for visualizing processive movements of MYO7A-HMM, which occur at 101 ± 53 nm/s (n = 42; mean ± SD) in stereocilia. Processive movements are also detected for a constitutively active mutant, MYO7A-R/K, but not for full-length MYO7A suggesting that MYO7A can dimerize (or oligomerize) in stereocilia when the motor domain is exposed. MYO7A-HMM does not show processive movements when tethered to the plasma membrane or F-actin, both of which are possible with MYO7A's interacting partners.

Conclusions: An experimental workflow to visualize single protein molecules in live hair cells was established and utilized to analyze MYO7A's trafficking in stereocilia. We are improving this workflow to analyze various protein-protein interactions necessary for developing functional stereocilia.

Hyperosmotic Sisomicin Infusion: A Mouse Model for Hearing Loss

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Background: Losing either type of cochlear sensory hair cells leads to hearing impairment. Inner hair cells act as primary mechanoelectrical transducers, while outer hair cells enhance sound-induced vibrations within the organ of Corti. Established inner ear damage models, such as systemic administration of ototoxic aminoglycosides, yield inconsistent and variable hair cell death in mice.
Methods: To overcome this limitation, we developed a method involving surgical delivery of a hyperosmotic sisomicin solution into the posterior semicircular canal of adult mice.
Results: This procedure induced rapid and synchronous apoptotic demise of outer hair cells within 14 h, leading to irreversible hearing loss. The combination of sisomicin and hyperosmotic stress caused consistent and synergistic ototoxic damage. Inner hair cells remained until three days post-treatment, after which deterioration in structure and number was observed, culminating in a complete hair cell loss by day seven.
Conclusions: This robust animal model provides a valuable tool for otoregenerative research, facilitating single-cell and omics-based studies toward exploring preclinical therapeutic strategies.

Neural Synchrony is a Sensitive Measure of Early Age-Related Auditory Deficits in Mice

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Background: Aging is associated with deficits in auditory structure and function. Recognizing age-related changes that emerge in middle-age may be crucial for characterizing the initial functional impairments that occur with age, and the temporal and spatial progression of age-related pathophysiology in the auditory pathway. There is increasing evidence that suprathreshold measures of auditory nerve (AN) function may be able to detect age-related auditory deficits earlier than reduced pure-tone thresholds. These age-related AN deficits may be exacerbated in the presence of background noise. We hypothesize that early age-related auditory deficits will be more pronounced when analyzing auditory evoked responses recorded in noise compared to quiet. Specifically, we predict that in noisy conditions, middle-aged mice will exhibit decreased response amplitude and weaker neural synchrony (measured by phase-locking value; PLV) than younger mice.
Methods: To test our hypothesis, we collected auditory brainstem responses (ABRs) from young (2-4 months, n=19), middle-aged (12-15 months, n=20), and aged (+24 months, n=23) mice. ABRs were collected in silence and in broad-band background noise (-5 to +5 dB SNR) in response to 11.3 kHz tones. ABR wave amplitude, to measure aggregated neural activity, and PLV, to measure neural synchrony, were collected from wave I and V for the quiet and noise conditions. The number of synapses per inner hair cells for each age group were quantified to examine if an early age-related synapse loss predicted age-related functional changes.
Results: We compared the PLV, amplitude and latency of waves I and V to examine age-related functional changes in noise and quiet conditions. We found significant interactions between age, noise, and wave (I and V). We found that in quiet, AN PLV from middle-aged mice was significantly weaker than younger mice. In contrast, AN amplitudes were similar in middle-aged and younger mice. In the presence of background noise, AN responses from middle-aged mice

become more similar to aged mice, with significant decreases in amplitude and weaker synchrony in middle-aged relative to younger mice. We found that PLV at the midbrain response (wave V) in middle-aged mice was similar to young mice in quiet and similar to old mice in noise. There were no significant differences in wave V amplitude between any age group. AN and midbrain response latencies in middle-aged mice were not significantly different from young mice in either condition.

Conclusions: These data show the importance of measures such as PLV as a sensitive tool for the early detection of age-related auditory functional decline and suggest that AN pathological alterations that affect neural synchrony may exhibit the earliest age-related deficits. Effects of noise demonstrate that using noise as a challenge to auditory functioning can reveal early age-related deficits. The possible contribution of synaptopathy to age-related synchrony deficits will be further analyzed and discussed.

Refining Convolutional Neural Networks for Temporal Bone Imaging Segmentation Using 3-Dimensional Distance Maps

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Background: Three-dimensional (3D) visualization of relevant structures within the temporal bone can be useful for pre-operative planning and image navigation but often requires manual segmentation of patient imaging, which can be tedious and time-consuming. Automated methods using convolutional neural networks (CNNs) for segmenting multiple geometrically complex structures have recently been described, but accuracy particularly for small neurovascular structures, has room for improvement. In this study, we present a novel loss function for neural network training that can provide more anatomically accurate segmentations for smaller structures in the temporal bone.

Methods: Fifteen deidentified, high-resolution cone-beam temporal bone computed tomography (CT) datasets were included in this study. Sixteen anatomical structures, including ossicles, inner ear, facial nerve, chorda tympani, and branches of the vestibular cochlear nerve were manually segmented. Five-fold cross-validation (75-25 train-validation split) was conducted using nnUNet, an open-source 3D semantic segmentation CNN, using the standard combination loss function of cross-entropy and Dice loss. Cross-validation was then repeated on nnUNet using a novel loss function of cross-entropy and distance-weighted Dice loss for training. Predicted segmentations from both models were compared against ground truth manual segmentations using modified Hausdorff distances (mHDs) and Dice scores.

Results: Training for 300 epochs took 4.2 hours per fold on a dedicated 24 GB VRAM GPU workstation. Modified Hausdorff distances and Dice scores between ground truth labels and predictions from standard nnUNet were as follows for select structures: malleus [mHD: 0.044±0.024 mm, Dice: 0.914±0.035], incus [mHD: 0.051±0.027 mm, Dice: 0.916±0.034], stapes [mHD: 0.147±0.113 mm, Dice: 0.560±0.106], inner ear [mHD: 0.038±0.031 mm, Dice: 0.952±0.017], facial nerve [mHD: 0.139±0.072 mm, Dice: 0.862±0.039]. Metrics for distance-weighted nnUNet were similar for these structures: malleus [mHD: 0.046±0.026 mm, Dice: 0.910±0.037], incus [mHD: 0.053±0.028 mm, Dice: 0.911±0.045], stapes [mHD: 0.139±0.096 mm, Dice: 0.565±0.115], inner ear [mHD: 0.039±0.028 mm, Dice: 0.951±0.015], facial nerve

[mHD: 0.221±0.280 mm, Dice: 0.858±0.037]. Distance-weighted nnUNet [mHD: 0.474±0.478 mm, Dice: 0.504±0.232] trended toward greater accuracy for labelling the inferior vestibular nerve compared to standard nnUNet [mHD: 1.222±2.458 mm, Dice: 0.471±0.200], though this difference was not significant (p=0.290).
Conclusions: This study sets a foundation for refining an open-source deep learning pipeline for semantic CT segmentation of temporal bone anatomy. By using a distance-weighted loss function, we have demonstrated that nnUNet labels temporal bone CTs with submillimeter accuracy compared to hand-segmented labels for all structures included in this study. This pipeline has the potential to streamline pre-operative planning workflows for a variety of temporal bone procedures and integrate with developing image-guidance and robot-assisted systems for surgical innovation.

Evoked Calcium Signals in Intact Vestibular Epithelium and Their Relationship to Electrical Changes in Hair Cells and Afferent Neurons

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Background: During head motions, each vestibular hair cell generates receptor potentials that reflect the direction, size and frequency content of the motion. Current knowledge of how mammalian vestibular epithelia respond to motion is largely based on data collected serially from individual hair cells or primary afferents, as well as recordings of summated potentials that reflect output of the vestibular inner ear. But such recordings don't provide us with information on how hair cell subpopulations of vestibular epithelia and their afferents are representing head motion moment-by-moment.
Methods: Progress with genetically encoded calcium indicators allowed us to produce mouse lines with GCaMP8m expression in specific cell types: hair cells and afferent, allowing us to record simultaneously the responses of multiple cells to stimuli on both hair cell and afferent stages in the intact epithelium of the mouse utricle (Luong et al., this meeting).
Results: We are exposing mechanosensitive hair bundles by removing otoconia and stimulating them with a fluid jet. Hair bundle deflection leads to entry of cations, including Ca2+, through transduction channels and the resulting receptor potential activates voltage-gated CaV1.3 channels in the basolateral membrane. Stimulus-evoked transmission to afferent synaptic terminals gives rise to postsynaptic Ca2+ signals likely due to voltage activation of CaV1.2 channels near the spike initiation zone. But we lack information on calcium dynamics in vestibular hair cells and afferents. Here we are investigating the relationship between current/voltage changes and Ca2+ responses in individual hair cells and afferent neurons.
Conclusions: Direct comparisons of single-cell Ca2+ and electrical signals will guide our interpretation of population Ca2+ signals and modeling of how different populations within the vestibular epithelium and nerve represent head motions (Lutz et al., this meeting).

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Android-Based Mobile Application to Estimate the User's Audiometric Hearing Thresholds and Auditory Temporal Resolution

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Background: This study presents an Android mobile application developed to perform two key auditory tests: 1) standard pure tone audiometry, which measures the user's audiometric hearing thresholds, and 2) temporal masking (TM) test that assesses the temporal resolution of the user's auditory system. While several mobile applications have been created to automate audiometry for at-home testing, these solutions often lack explicit calibration with the hardware characteristics of connected headphones, leading to uncertainties in the true sound intensities delivered. To address this limitation, the developed mobile app adapts to the specific frequency response of the connected headphones and conducts pure tone audiometry using von Bekesy's method. Additionally, the app performs TM which is a psychoacoustic test for estimating the user's ability to distinguish time-varying (temporal) cues in sound signals. This is an important ability for perceiving sounds properly, especially in the presence of non-stationary noise.
Methods: A total of 48 participants were recruited: 24 with normal hearing (mean age=49.2 years, SD=15.0) and 24 hearing-impaired individuals (mean age=59.0 years, SD=9.0). Clinical tone audiometry was manually conducted on both ears by an experienced audiologist at frequencies between 250 and 8000Hz in a sound booth. Participants then took the app-based audiometry test using SONY WH-1000XM3 headphones in a quiet room. Results from both tests were statistically analyzed using paired t-tests and Pearson correlation (r) in IBM SPSS, with a two-tailed significance level of 0.05. Our TM paradigm consisted of a fixed-intensity narrow-band noise masker at 80dB, followed by a silent gap and a 50ms-long tone (target). During this test, the gap duration varied, and hearing thresholds of the target were measured as a function of gap duration. The TM test was conducted on all test participants at gap durations of 5, 10, 20, and 80ms and tone frequencies of 0.5, 2, and 4kHz.
Results: The app-based audiometry results closely matched clinical outcomes. The mean absolute error (MAE) between the two methods was 5.1dB for normal-hearing participants and 7.3dB for hearing-impaired participants, averaged across all frequencies. Paired t-tests revealed no significant differences between the two methods at any of the eight tested frequencies. The correlation between the two methods was highly significant (p LESS THAN 0.05) and consistently strong across all frequencies (r=0.90 on average). For the TM test, One-Way ANOVA showed that the slope of the TM function was significantly (p LESS THAN 0.05) steeper in the normal-hearing group (-0.22 at 2kHz, -0.22 at 4kHz) than in the hearing-impaired group (-0.14 at 2kHz, -0.10 at 4kHz).
Conclusions: These findings indicate that our mobile app can accurately estimate the clinical audiogram. Furthermore, the TM results showed that hearing-impaired individuals gain only minimal benefit from the temporal cues, represented by the gap duration, in the signal.

Relationship Between Natural Head Orientation and Unaided and Aided Spatial Hearing Outcomes

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Background: People naturally move their heads to listen better in group conversations. Recently, there has been growing interest in using head movement data to improve hearing aid algorithms. Previous studies have shown that orienting the head 30 degrees away from the target speaker can improve speech reception thresholds (SRT) by 1-3 dB in the presence of spatially separated interferers (Grange et al., 2018). However, the effects of natural head orientation during competing speech, particularly in aided listening, remain underexplored. This study aims to identify natural head orientation/movement tendencies across four listening configurations (target at the front, target at the side, co-located, and separated) both with and without two different directional microphones (Omni and Directional). It also seeks to examine the impact of unaided versus aided hearing on 'functional spatial boundaries (FSB)'—the spatial separation required to segregate competing speech effectively.
Methods: This study recruited adult listeners with symmetrical moderate to moderately severe hearing loss. We measured SRTs under both head-fixed and head-free conditions, with the target and masker co-located at the front and side. Additionally, we determined the FSB required to achieve 9 dB of Spatial Release from Masking (SRM) by adaptively adjusting the angular separation between the target and masker in both head-fixed and head-free conditions. These tests were performed under unaided, aided-omni, and aided-directional microphone conditions. During testing, head orientation and movement (in pitch, yaw, and roll), were tracked with an optical motion capture system.
Results: There was no effect of head orientation/movement when the target and masker were co-located. However, when the masker was separated from the target at the front, both head orientation and variance significantly increased (p LESS THAN .05), with a weak tendency to orient their head between the target and masker. Conversely, when the target was presented from the side and the masker was separated from the target, no significant difference in head orientation was observed compared to the co-located condition. For the FSB with the target located at the front, a negative correlation was found in the unaided condition (p LESS THAN .05), a positive correlation in the omnidirectional mode (p LESS THAN .05), and no correlation in the directional microphone mode. Moreover, head orientation and movement in roll were significantly greater in the directional microphone condition compared to the omnidirectional mode (p LESS THAN .05).
Conclusions: These findings suggest that natural head orientation and movement can differentially impact hearing outcomes depending on whether a hearing aid is used, the target location, and the type of directional microphone employed. Additionally, there appears to be an interactive influence, as the type of hearing aid directionality also affects head orientation.

Osteoprotegerin Deficiency in the Human Otic Capsule as a Potential Driver of Otosclerosis

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Background: Otosclerosis features pathologically accelerated bone remodeling in the otic capsule. Previous research in mice suggests osteoprotegerin (OPG), an inhibitor of bone remodeling, governs the naturally low level of bone remodeling in the otic capsule. Absence of OPG thus may drive otosclerosis. This study explores OPG distribution pattern in the human

normal and otosclerotic otic capsule, its possible anatomical distribution routs, and its potential role in otosclerosis.
Methods: (i) OPG immunolabeling on human temporal bone sections from individuals with and without otosclerosis, focusing on cochlea and otic capsule bone. Confocal microscopy was used to examine OPG distribution. (ii) Fresh human temporal bone samples were perfused with a fluorescent tracer for different incubation times and confocal microscopy on non-decalcified, plastic-embedded tissue sections evaluated tracer distribution. (iii) Lacuno-canalicular network pores surface area from the cochlear wall were evaluated using scanning electron microscopy.
Results: OPG labeling was strongest in cochlear supporting cells and showed a radial gradient in the surrounding otic capsule. In the otic capsule, OPG labeling was localized in osteocyte lacunae and extracellular clefts within globuli interossei. The fluorescent tracer outlined osteocyte lacunae and clefts within globuli interossei similarly to OPG-immunolabeling, indicating diffusion into the otic capsule via a continuous lacuno-canalicular network.
Conclusions: Our findings support OPG's role in inhibiting bone remodeling in the human otic capsule. Cochlear supporting cells are a probable major OPG source, likely secreting it into inner ear fluids, where it diffuses through the otic capsule via a continuous lacuno-canalicular network. Disruption of this network may lead to localized OPG-deficiency, potentially driving otosclerotic bone remodeling.

Characterization of a Progressive Early Onset Hearing Loss in SIRT3 Knock-Out Mice

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Background: Sirtuins are a highly conserved family of NAD+ dependent histone deacetylases, consisting of seven members (SIRT1-7) in mammals. SIRT3 is found in the mitochondria where it regulates oxidative phosphorylation. The major proteins which interact with SIRT3 are SOD2, FOXO3A, and IDH2. Together, they contribute to antioxidant and redox signaling and suppress reactive oxygen species. Despite its critical roles in various cellular process, complete knockout of SIRT3 (SIRT3-/-) only leads to mild phenotypes in the animals. SIRT3 has been associated with age-related, noise-induced, and drug-induced hearing loss using animal models. However, the molecular mechanism of SIRT3 in hearing protection requires more research.
Methods: A constitutive SIRT3 knock-out mouse model (129-Sirt3tm1.1Fwa/J) was obtained from Jackson Laboratory. The hearing of the three genotypes (+/+, +/-, and -/-) was assessed by auditory brainstem response measured 6-, 8-, and 12-week-postnatal. At the endpoint, the mice were euthanized, and their cochleae were collected. Cochlear whole mount segments in frequency range of 9-36 kHz were dissected after decalcification and they were stained with antibodies targeting SIRT3, SIRT5, and pre- and post-synaptic components. In addition, fresh cochlear whole mounts were collected from four animals each for SIRT3+/+ and SIRT3-/- genotypes for RT-qPCR. Sixteen gene targets were selected from existing literatures which had investigated SIRT3 previously.
Results: At week 6, SIRT3+/+ (n = 33), SIRT3+/- (n=39), and SIRT3-/- (n = 29) mice did not show significant difference in ABR thresholds at all frequencies measured. However, male SIRT3-/- mice showed significantly higher (51.16 dB ±5.05, SD) median ABR thresholds than females

(35.06 dB ±1.65, SD) at 36 kHz (p LESS THAN 0.01). Similar patterns were observed at week 8 and 12, and a progressive ABR threshold elevation was observed in male SIRT3-/- mice. The progressive early onset hearing loss was not observed in SIRT3+/+ and SIRT3+/- groups. Immunostaining showed that SIRT3 was expressed in the hair cells of all other animals except SIRT3-/- mice. RT-qPCR showed that SIRT5 was upregulated ~ 1.74 fold (2^-(ΔΔCt)) in male SIRT3-/- mice compared to male SIRT3+/+ mice. In females, SIRT3-/- mice showed downregulation of GPX4 with ~ 0.38 fold difference compared to SIRT3+/+.
Conclusions: We report an early onset hearing loss in male SIRT3-/- mice, which initiated by as early as 6 weeks and progressed until up to 12 weeks, but not in female SIRT3-/- mice. The hearing impairment was not observed in SIRT3+/+ and SIRT3+/- mice. These results indicate that SIRT3 deficiency is more punishing in the hearing of the males than that of the females. SIRT3 deficiency may drive a compensatory antioxidant mechanism through overexpression of SIRT5, although insufficient for rescuing the hearing of the males. In females, SIRT3 deficiency may have caused downregulation of GPX4, a key antioxidant enzyme, but their hearing was unaffected.

In-Silico Framework for Benchmarking Optogenetic Hearing Restoration

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Background: Optogenetic cochlear implants (oCIs) represent a promising means to better restore hearing in individuals impacted by severe sensorineural hearing loss than possible with electrical cochlear implants (eCIs). The wide spread of current and channel interactions in eCIs limit comprehension of speech in noisy environments and the enjoyment of music. By reducing the spread of neural activation, oCIs promise a greater number of independent stimulation channels.
Methods: A computational framework for the evaluation of oCIs in the human cochlea was developed using four main modules. First, a generic n-of-m sound coding strategy was implemented, which could be easily adjusted to evaluate various parameters. Second, a three-dimensional ray-tracing model of a reconstructed human cochlea was used to investigate light propagation. Third, a biophysical model of spiral ganglion neurons (SGNs) was built to simulate optogenetically evoked firing. Fourth, a similarity measure was developed to compare the input sound spectrograph to the output spikes pattern. Finally, these stages were integrated to generate a comprehensive model capable of processing an audio files dataset and computing a similarity score.
Results: The major findings indicate that the spatial spread of light using µLED- and waveguide-based oCIs is narrower than the electrical current spread. Moreover, the impact of variables such as emitter-to-SGN distance, emitter rotation, and scar tissue formation on the irradiance at SGNs was evaluated. The improved spectral resolution of oCIs compensates for the currently lower temporal fidelity of optogenetically driven firing.
Conclusions: The computational framework provides a valuable resource for researchers to explore the complex interplays of sound processing, light delivery, and optogenetic stimulation. This study supports the notion that optogenetic stimulation of the cochlea could improve the speech understanding of CI users.

Loss of tmc1/2 Function Induces Expansion of tmc1/2b+ Cells in the Zebrafish Inner Ear

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Background: Detection of sound and head movement requires mechanoelectrical transduction (MET) channels at tips of hair-cell stereocilia. In vertebrates, the transmembrane channel-like (TMC) proteins TMC1 and TMC2 fulfill critical roles in MET. During normal development in zebrafish, hair-cell progenitors within the supporting cell layer first express tmc1 and tmc2b and then initially express all three tmc1/2a/2b genes (tmc2 is duplicated in zebrafish) before migrating to the upper layer of the neuroepithelium.
Methods: We assessed the development of the inner ear endoorgans in a tmc1/2a/2b triple mutant larvae and discovered a potential developmental regulatory loop involving the tmc genes.
Results: We find that ectopic expression of tmc1/2b transcripts in peripheral cells occurs in tmc1/2b double and tmc1/2a/2b triple mutants in a gene dosage-dependent manner. Results from tmc single and double mutants reveal that expression of wild-type tmc1 or tmc2b is sufficient for suppressing ectopic expression of tmc1/2b. In addition, our experiments indicate that ectopic expression of tmc1/2b is not universal to all endorgans. Instead, it is specific to otolithic organs and progressively increases during development in tmc triple mutants. To determine whether tmc1/2b ectopic expression is caused by the absence of mechanotransduction and/or mislocalization of Tmc proteins, we examined expression in mechanotransduction mutants carrying strong alleles of tmie and tomt and did not observe ectopic expression of tmc1/2b. We also examined loss of function alleles for the known interacting partners of Tmc1/2 proteins such as Pcdh15a, Lhfpl5a and Cib2/3 auxiliary subunits and found no effect on tmc1/2b expression.
Conclusions: Our preliminary data suggest that loss of Tmc1/2b proteins specifically result in upregulation of tmc1/2b expression in peripheral cells. The mechanism is unclear and may involve a secondary role for Tmc1 and Tmc2b in signaling to peripheral cells to differentiate into hair cells. Whether ectopic expression involves canonical developmental or transdifferentiation pathways remains to be determined.

Parvalbumin and Somatostatin in the Songbird Auditory Cortex Suggest Conserved Mechanisms for Inhibition

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Background: Vocal learning is a rare ability that requires the memorization and sensorimotor development of vocalizations. The songbird is an exemplary model for evaluating the role of auditory processing in vocal learning. In the mammalian cortex, nearly all inhibitory neurons express the neuropeptides parvalbumin (PV), somatostatin (SST), or the serotonin receptor 5HT3aR. The expression of these neuronal subtypes differs across cortical layers and contributes to auditory plasticity, selectivity, and attention. In general, PV neurons provide fast spiking, peri-

somatic inhibition. SST neurons provide inhibition to dendrites and are part of a disinhibition circuit with 5HT3aR neurons expressing vasoactive intestinal peptide (VIP). Despite extensive study in mammals, the role of interneuron subtypes in auditory-vocal communication is not well known. Recent in-situ hybridization studies have matched mammalian PV, SST and VIP marker genes to avian GABA3/4, GABA2, and GABA5 genes respectively. We hypothesize that the subtypes of inhibitory neurons in auditory cortex serve distinct roles in vocal learning. Here, we examined PV and SST expression across the superficial, intermediate, deep, and secondary regions of the songbird auditory cortex.

Methods: We performed immunohistochemistry in the zebra finch (Taeniopygia gutatta) and long-tailed finch (Poephila acuticauda). Commercially available antibodies for GABA, parvalbumin and somatostatin were used in conjugation with fluorescent antibodies to label and map auditory interneurons by subtype. The percentage of neurons that expressed PV or SST was correlated with known physiology of cortical subregions. These percentages, along with spontaneous firing rates, were used to establish and differentiate putative PV-Like and SST-Like neurons. Species differences in vocal acoustics were also considered when evaluating interneuron subtype firing latencies.

Results: We found that the expression and localization of PV and SST differed across cortical regions. We also observed differences in PV expression along the mediolateral axis. Physiologically, PV-Like neurons exhibited significantly shorter firing latencies to tone bursts in both deep and secondary regions. Putative interneuron subtypes also differed in their frequency tuning curves and response to song stimuli. While both finch species expressed GABAergic neurons with PV or SST, we surprisingly observed PV+ neurons without GABA expression. These neurons may represent PV+ fast spiking excitatory neurons, previously only seen in the songbird robust arcopallial nucleus (RA).

Conclusions: The expression and localization of interneuron subtypes likely have critical and differential contributions to vocal learning and auditory selectivity towards birdsong. Future studies using adeno-associated virus (AAV) and brain clearing could evaluate interneuron microcircuits and synapse location. Mammalian studies that utilize PV or SST specific silencing should be replicated in the songbird. These studies could support the idea that vocal learning in mammals and birds has conserved mechanisms for complex, differentiated inhibition.

Unraveling the Role of Mitochondrial Protein ACO2 in Hearing Loss

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Background: Hearing relies on the proper function of cochlear hair cells that detect sound vibrations, and auditory nerves that transmit this information to the brainstem. Mitochondrial dysfunction disproportionately affects cells requiring large amounts of energy such as neurons. Mutations in mitochondrial DNA or nuclear genes encoding mitochondrial proteins can affect mitochondrial biogenesis or energy metabolism, impacting auditory function. Mutations in the nuclear-encoded mitochondrial enzyme aconitase hydratase 2 (ACO2) are associated with severe neurological disorders and deafness in children. ACO2 is a tricarboxylic acid cycle (TCA) enzyme that catalyzes the interconversion of citrate into isocitrate. The cellular mechanisms underlying ACO2-associated disease remain poorly understood due to the lack of useful animal models.

Methods: Using whole genome sequencing, we identified an arginine to leucine substitution in Aco2 in a mouse stock showing circling behavior at The Jackson Laboratory. Interestingly, Aco2R56L animals are viable and fertile as homozygotes whereas an Aco2 knock-out strain is lethal before birth. We measure ABR and DPOAEs to test auditory function and use immunofluorescence and confocal microscopy on whole and cryosectioned samples to assess cochlear structure. Mitochondria are genetically labeled with a cell-specific, sparse Cre driver using the Mito-Dendra2 reporter. We use organotypic culture of the cochlea to test metabolic supplementation approaches that can be next used in vivo to rescue or delay ACO2 dysfunction.
Results: Homozygous Aco2R56L mutants have elevated ABR thresholds compared to wild-type and heterozygote littermates with shifts worsening between postnatal (P) day 28 and P47. DPOAEs are normal and comparable between all genotypes at P56, when harvested inner ears show normal OHC and IHC apical morphology (F-actin labeling). SGN quantifications suggest a normal spiral ganglion neuron (SGN) count at 3 weeks, a 17-23% reduction at the apical and mid-cochlear turns at 4 weeks, and a 33-60% reduction affecting all positions at 8 weeks. Ongoing work aims to visualize and quantitatively assess the mitochondrial network in SGNs before and during degeneration. We hypothesize that mitochondrial dysfunction leads to SGN-inner hair cell synapse loss and/or peripheral dendrite defects, which in turn cause SGN loss. We are currently culturing the cochlea including SGNs to apply TCA metabolites and antioxidants with the goal of preventing SGN loss and earlier defects in Aco2 mutants.
Conclusions: The Aco2R56L strain is a unique new model to investigate the role of ACO2 in auditory function and disease. Normal DPOAEs, largely intact hair cells and SGN degeneration suggest that Aco2R56L mutants and ACO2 patients suffer from early onset, rapidly progressing auditory neuropathy. Our objective is to use compounds first validated in cochlear explants for supplementation in vivo to rescue or slow down auditory dysfunction. The Aco2R56L strain and metabolic supplementation also have the potential to model and mitigate other ACO2 neurological disorders including cerebellar-retinal degeneration.

Quantifying Binaural Speech Fusion Using a Dichotic Formant, Vowel Identification Task in Children and Adults With Cochlear Implants

Emily Burg*[1], Caroline Paroby[2], Matthew Fitzgerald[3], Duane Watson[1], Rene Gifford[1]

[1]*Vanderbilt University,* [2]*University of North Carolina at Chapel Hill,* [3]*Stanford University*

Background: The auditory system's ability to compare and integrate information across ears, known as binaural hearing, is critical for navigating complex acoustic environments. Binaural hearing allows a listener to distinguish a friend's voice from background noise in a crowded coffee shop, and identify where a car is coming from on a busy street. To access these functional benefits, the normal hearing (NH) auditory system analyzes the correlation of incoming signals at each ear to fuse sounds originating from the same source into a single percept; this is known as binaural fusion. Previous work has shown that cochlear implant (CI) users generally cannot access binaural hearing benefits to the same degree as NH listeners. This may be due to impaired binaural fusion stemming from pathological, surgical, and device related asymmetries that reduce interaural correlation. The goal of this study was to establish an ecologically valid measure of binaural speech fusion for CI users that is not dependent on their subjective perception of fusion.

Methods: To quantify binaural fusion, we employed a dichotic formant vowel identification task. Vowels are primarily identified by the first two formants, and previous studies have shown that NH listeners can identify vowels with alternate formants presented to each ear. This task requires effective binaural integration of formant information to correctly identify the vowel. Stimuli consisted of six vowels in an h-Vowel-d word context. Words were processed to preserve or remove specific formants (F), and participants were tested in five conditions: 1) Formants 1-4 presented bilaterally (Diotic F1-F4) to quantify baseline vowel recognition, 2) Formants 1-2 presented bilaterally (Diotic F1F2) to ensure that two formants were sufficient for good vowel recognition, 3) Formants 1 and 2 presented to opposite ears (Dichotic F1/F2), and 4/5) F1 or F2 presented bilaterally (Diotic F1 and Diotic F2) to ensure that vowels were not identifiable by a single formant alone. We hypothesized that, if listeners could effectively fuse formants 1 and 2 across ears, performance in condition three would be similar to condition two. Four participant groups were tested: NH adults, NH children, bilateral CI (BiCI) adults, and BiCI children.
Results: Results indicated good baseline vowel recognition in the Diotic F1-F4 condition for all groups. Performance in the Diotic F1F2 condition was similar to baseline, indicating that two formants were sufficient for good vowel recognition. Single formant conditions revealed that some vowels were identifiable by one formant alone; these were generally consistent across groups. Finally, all groups demonstrated some degree of fusion, but NH groups were able fully fuse and reach Diotic F1F2 performance, whereas BiCI groups did not. Confusion matrices and binaural unmasking results will also be presented.
Conclusions: Results indicate that reduced binaural hearing benefits in CI listeners may stem from impaired binaural fusion.

A First Look at Human Inner Ear Pathology in POU4F3 Variants: Findings From Three Human Temporal Bone Donors

Diana Correa*[1], Jennifer T O'Malley *[1], Christopher Giardina[1], Alison Brown[2], Sami Amr[3], Alicia Quesnel[1]

[1]*Massachusetts Eye and Ear, Harvard Medical School,* [2]*Biobank Genomics Core, Mass General Brigham Personalized Medicine,* [3]*Brigham and Women's Hospital, Harvard Medical School, Biobank Genomics Core, Mass General Brigham Personalized Medicine*

Background: Genetic variants account for nearly half of sensorineural hearing loss (SNHL) cases worldwide. The POU4F3 gene, a class IV POU domain transcription factor, is essential for inner ear hair cell survival. Mutations in POU4F3 underlie DFNA15, an autosomal dominant non-syndromic hearing loss with variable clinical presentations. While animal models offer insights, human temporal bone histopathology for POU4F3 mutations has not been reported. This study describes the histopathological findings in three donors with POU4F3 variants, complementing existing clinical and animal data.
Methods: Sixty-nine temporal bone donors with histories suggestive of hereditary hearing loss were identified. Samples were obtained via buccal swabs (n=29) or frozen muscle specimens (n=40) and Whole Exome Sequencing was performed using the Illumina NextSeq 500. Temporal bones from candidate donors were embedded in celloidin, sectioned, and stained with hematoxylin and eosin (H and E). Clinical records and family histories were reviewed using data from the NIDCD National Temporal Bone Registry. Histopathological analysis was conducted to identify

inner ear abnormalities, and differential interference contrast (DIC) microscopy was used to assess hair cell and supporting cell survival. A machine learning algorithm in Dragonfly 3D was used to evaluate spiral ganglion neuron (SGN) counts, which were then compared to age-matched controls.

Results: Three donors with POU4F3 variants were identified: two with the c.602T GREATER THAN C p.Leu201Pro missense variant (likely pathogenic) and one with the c.709T GREATER THAN G p.Ser237Ala (variant of unknown significance). The cases included two females and one male, all in their 10th decade of life. Hearing loss onset ranged from childhood to middle adulthood, with a strong family history suggesting autosomal dominant inheritance. Audiological history showed progressive bilateral SNHL, varying from moderate to profound, with better preservation of lower frequencies.

Histopathology revealed consistent findings: a flat organ of Corti in the basal cochlea with no surviving hair or supporting cells. In the apical regions, inner hair cell survival increased to 65-80%, while outer hair cell survival remained below 30%. Supporting cells were absent in the basal turn; toward the apex, some regions showed a collapsed tunnel of Corti with partial differentiation into supporting cells but without normal architecture. Other areas had more preserved cytoarchitecture but with evident loss of outer pillar cells.

SGN counts ranged from 10,550 to 15,820 (raw counts, multiplied by ten, as only every 10th section was counted, no correction factor used). These values corresponded to 61.98% to 96.5% of the SGN counts found in age-matched controls.

Conclusions: Our analysis presents the first human histopathological insights into two POU4F3 variants, revealing severe hair cell loss and supporting cell disruption, especially in the basal regions. These findings enhance our understanding of the underlying pathology and raise important questions about potential therapeutic approaches for this patient group.

Memorial Symposium for Eric Young: From the Auditory Nerve to Cochlear Nucleus to cortex, and Back

4:45 p.m. - 6:45 p.m.

Ocean Ballroom 1 - 4

Chair: Lina Reiss*, Oregon Health and Science University*
Co-chair: George Spirou*, University of South Florida*
Co-chair: Tilak Ratnanather*, Johns Hopkins University*

Eric Young at Johns Hopkins

Xiaoqin Wang

Johns Hopkins University School of Medicine

Individual Abstract: Beginning with his doctorate with Murray Sachs in 1972, Eric spent his entire career at Johns Hopkins (save a postdoc with Jay Goldberg at U. Chicago), rising to Professor of Biomedical Engineering in 1987. From 1991-2015 Eric directed the Center for Hearing Sciences. Throughout that time, he served as the scientific polestar, inspiring, advising

and leading hearing research throughout the University. In addition to unbroken funding for his own lab, Eric was the initiator, organizer and director of multiple program and training grants from the NIH that benefited the entire Center. Most importantly, Eric's dedicated and generous leadership enabled the multi-departmental Center to thrive and to enrich research and training for all its faculty and students.

Eric Young's Contributions to Quantifying and Modeling Neural Representations of Sound
Laurel H. Carney

University of Rochester

Individual Abstract: Eric Young made fundamental contributions to our understanding of neural coding at the level of the auditory nerve (AN). He studied coding of both simple and complex sounds, in quiet and in noise backgrounds. Two major papers published in 1979 with Murray Sachs are still required reading for any student of neural coding of complex periodic sounds. In this work, they fleshed out the limitations of both rate-based and temporal-fine-structure-based coding of synthetic vowels for all three spontaneous-rate types. Eric detailed a strategy, referred to as the average localized synchronized rate (ALSR), for quantifying the temporal-fine-structure information in AN responses to harmonic complex sounds, in this case synthetic speech. Eric was a major inspiration, an enthusiastic cheerleader, and a primary source of the quantitative detail required for the development of computational models for AN fibers. His quantitative metrics for AN response properties in cats with both normal-hearing and with noise-induced sensorineural hearing loss were critical contributions to this work. He worked with Ian Bruce and Murray Sachs on an important version of the AN model that included simulations of AN responses in cats after acoustic trauma. AN models that he worked on directly, or inspired in other labs, incorporate details of both temporal fine structure and average discharge rates of AN responses. These models are now widely used throughout the world, from the level of auditory-nerve physiology to human psychophysics. Eric's work is distinct from that of many contemporaries in that he embraced the nonlinearities in the auditory periphery, rather than assuming that the central system would work around them and somehow linearize the problem. As such, his work continues to influence ongoing studies of how nonlinearities, and changes in those nonlinearities, influence neural representations of sound in the healthy and impaired ear.

Auditory Nerve Encoding With Acoustic Trauma
Ian Bruce

McMaster University

Individual Abstract: Following from seminal studies that Drs. Eric Young and Murray Sachs conducted into mean-rate and spike-timing coding of vowels in cat auditory-nerve fibers (ANFs) (Sachs and Young, JASA 1979; Young and Sachs, JASA 1979), their lab began two decades later to investigate how acoustic trauma affects these neural representations. A series of studies conducted by postdoctoral fellow Roger Miller and graduate students under Eric's supervision showed that: i) the tonotopic representation of vowel formants in ANF spike-timing responses is greatly degraded in cats with hair-cell damage, and ii) conventional hearing-aid amplification schemes are unlikely to restore neural representations to normal. They then investigated a novel formant-contrast-enhancement scheme that showed greater promise for normalizing tonotopic vowel representations. These studies are reviewed by Sachs et al. (ABME 2002).

To allow these investigations to be expanded to running speech and a greater variety of hearing-aid amplification approaches, Eric and Murray decided to develop a computational model of cat auditory periphery that could incorporate hair-cell impairment. Postdoctoral fellow Ian Bruce led this model development in the late 1990s and early 2000s under the supervision of Eric and Murray and in collaboration with Dr. Laurel Carney, then at Boston University. The first model version with hair-cell impairment (Bruce et al., JASA 2003) replicated many physiological phenomena from Roger Miller's recordings. Over the last 22 years, Dr. Bruce's own lab at McMaster University has continued to improve and refine this model, with major contributions by doctoral student Muhammad Zilany and Dr. Carney's lab.

In the early-to-mid 2000s, postdoctoral fellow Mike Heinz investigated with Eric how sound-level coding in cat ANFs is affected by acoustic trauma. They demonstrated that cats with hair-cell damage have abnormal rate-level functions, but that these abnormalities don't fit with common conceptual models of loudness recruitment, suggesting that neural correlates of loudness recruitment originate in the central auditory system (Heinz et al., JARO 2005). Dr. Heinz's own lab at Purdue University subsequently extended neurophysiological investigations of effects of various forms of SNHL on ANF coding using advanced computational analyses that followed in Eric's footsteps. Led by Ken Henry, nonlinear system-identification techniques (Wiener kernels) generalized the phenomenon of distorted tonotopy (DT) (Henry et al., J. Neurosci, 2016, 2019). Satya Parida developed and applied a unifying framework of spectrally specific spike-train temporal analyses to identify DT as the most relevant factor increasing neural-coding susceptibility for speech in background noise (Parida and Heinz, Hear. Res. 2022).

Dorsal Cochlear Nucleus: Functional Anatomy and Circuitry for Auditory Processing

Israel Nelken

Hebrew University

Individual Abstract: One of Eric Young's greatest successes was the elucidation of the functional circuitry of the Dorsal Cochlear Nucleus (DCN). The DCN was known to have two input systems, an auditory one through the auditory nerve and the Ventral Cochlear Nucleus (VCN), and a multimodal one through its external, cerebellar-like layer. Eric's work in the DCN began with the finding that the neurons in the decerebrate, unanesthetized cat show distinct response properties to pure tones, with widespread inhibition (Young and Brownell 1976). He then identified the type II and type IV units as the major response classes in the DCN. The type IV neurons turned out to be the principal neurons of the DCN, projecting to the inferior colliculus, while type II responses are associated with an inhibitory interneuron, the vertical neuron. By the mid 1990s the relationships between the type II and type IV neurons were largely worked out by Eric and his coworkers (with important contributions of the late Herb Voigt, George Spirou and Israel Nelken) and a second inhibitory input, nicknamed 'the wideband inhibitor' (WBI), was postulated. The WBI is believed to be a class of neurons in the VCN (the D-stellate neurons) that provide inhibitory input to the DCN. This circuit, which combines feedforward excitation from the auditory nerve fibers and VCN neurons with feedforward inhibition from the type II neurons and the WBI, largely accounts for the response properties of type IV neurons to pure tones, broadband noise, and spectral notches.

The resulting auditory processing in the DCN is remarkably rich, contributing to many important computational tasks of the auditory system with particular importance to spatial information processing.

Beyond the STRF: Random Spectrum Stimuli (RSS) Models

Lina Reiss

Oregon Health and Science University

Individual Abstract: Another of Eric Young's contributions was in non-linear approaches to characterizing auditory neurons at various stages in the auditory pathway. One domain was in the area of input-output (I-O) function estimation. His initial foray, with Peter Kim, was the application of reverse correlation and spectro-temporal receptive field (STRF) estimation to the auditory nerve, for which STRFs performed better than reverse correlation at estimating tuning curves, but were limited by their narrow dynamic range of applicability, i.e. linearity (Kim and Young, 1994). This led Eric, together with Barbara Calhoun and Jane Yu, to develop what he termed "random spectrum stimuli (RSS)": broadband stimuli with random fluctuations in spectral amplitude in 1/8 or 1/16 octave bins, with neural responses to a set of such stimuli used to determine the neural weighting of energy in each frequency bin, analogous to STRFs but limited to the frequency domain (Yu and Young, 2000; Young and Calhoun, 2005). This tradeoff allowed for computation of second-order (nonlinear) weights, in addition to first-order weights, and for the detection of curvature in the rate-level function as well as suppressive or facilitative interactions between frequency components. These models were evaluated based on accuracy of prediction of neural responses, and performed well for linear neurons such as auditory nerve fibers and chopper units in the ventral cochlear nucleus, but not for non-monotonic neurons in the dorsal cochlear nucleus or inferior colliculus. Further work revealed that the second order terms were necessary to predict non-linear sensitivity such as a preference for rising spectral edges (Young, Yu, and Reiss, 2005), and that smaller stimulus contrasts and level-dependent functions are necessary for accurate prediction (Reiss, Bandyopadhyay, and Young, 2007; Bandyopadhyay, Reiss and Young, 2007). Later, binaural interaction terms were incorporated to investigate excitatory-inhibitory interactions in the inferior colliculus (Yu and Young, 2013; Slee and Young, 2013). Beyond parametric models, Eric also applied information theoretic approaches to studying nonlinear neurons, and a series of papers with Steven Chase demonstrated that first-spike latency and firing rate provide independent information about sound localization cues in the inferior colliculus (Chase and Young, 2006, 2008).

This work was transformative for the field as it spurred other researchers toward greater rigor in ensuring that STRFs and other I-O functions predict neural responses in order to be considered sufficient characterizations, and to adopt the use of nonlinear estimation methods in their models.

Multimodal Processing in the (Dorsal) Cochlear Nucleus

Patrick Kanold

Johns Hopkins University

Individual Abstract: We sense the world by multiple sensory modalities and sensory systems. Sensory systems influence each other already at very early stages of processing presumably to support rapid multisensory behaviors. One of Eric Young's major contributions was to identify the

dorsal cochlear nucleus (DCN) as the first site of auditory-somatosensory integration in the auditory pathway. A trail-blazing publication in J. Neurophysiology in 1995 showed that the superficial DCN receives inputs from somatosensory brainstem nuclei such as the cuneate nucleus and the trigeminal nucleus (Young, Nelken, and Conley, 1995). Subsequent work with Kevin Davis and Roger Miller (Davis, Miller, and Young 1996; Davis and Young 1997) worked out how this cerebellum like circuitry in the superficial DCN processed this non-auditory input. Further work showed that these somatosensory nuclei relayed proprioceptive information from cervical nerves innervating the head, ear, and shoulder (Kanold and Young 2001). These findings finally illuminated the role of the superficial DCN, implicating a role in integration of proprioceptive information about pinna and head orientation in computation of auditory sound source location. Additional work by the Young lab and later by others revealed that activation of these inputs can have a remarkable and persistent influence on auditory processing (Kanold, Davis and Young 2011; Koehler, Pradhan, Manis, Shore 2011) and that thereby non-auditory inputs can alter sound processing.

These integrative multisensory properties also suggest that altered somatosensory input can give rise to auditory processing disorders such as tinnitus. Conversely, manipulation of multisensory input to the auditory system might be used to alter auditory processing to compensate for auditory dysfunctions. Indeed, this groundbreaking work was adapted by others as the basis for patents and in recent commercial applications to mitigate tinnitus. Thus, with his curiosity and drive to achieve a complete circuit level description of the cochlear nucleus, Dr. Young opened up a major creative and non-invasive path for treating auditory disorders.

Eric Young's Impact Internationally and Within the ARO

Alan Palmer

Hearing Sciences, University of Nottingham

Individual Abstract: Eric Young had a major impact on his colleagues around the world who held him in the highest esteem. This obviously reflected the quality and quantity (Google Scholar: 12557 citations H index 59) of his publications. However, that is only part of the story. When Eric embarked on his illustrious career the "digital age" had not dawned. There was no internet, no X, no Meta and no exchange of electronic manuscripts and papers. We waited for the printed papers to appear in our university libraries. Consequently, the lead time was huge and attendance at meetings was essential to keep up-to-date. From the outset, Eric was invited to attend meetings outside the USA. This hugely raised his profile internationally in three ways. First, his lucid presentation of his own research and that of his post docs and students. Second, Eric clearly understood papers presented by others and asked apposite and penetrating questions that grabbed everyone's attention, although never in a manner that belittled the presenter. Thirdly, Eric was generous with his time and wisdom and sometimes the informal chats were the most informative part of the meeting. Over the years I had the pleasure of his company at several "International Symposia on Hearing", two speech meetings in Utrecht, one meeting in Ascona and of course the cochlear nucleus meeting in Salamanca. On a personal level, Eric had a major impact on the way that I conducted my research. Much of his early work on single unit classification and signal processing in the dorsal cochlear nucleus was hugely influential in the scientific development of post-graduate and post-doctoral members of my laboratory. Any paper of Eric's was always subjected to detailed analysis and discussion.

Though hard to quantify, Eric Young certainly had a major impact on auditory research internationally.

Eric was also a great supporter of the ARO, attending and bringing his students and postdocs to the meeting every year, and serving on ARO committees. The huge significance of his corpus of work was recognized by the ARO with the Award of Merit in 2007. He later served as the chair of the Awards committee. However, his most lasting contribution (and impact) within the ARO was certainly becoming the founding editor of JARO. When the Association decided to launch a journal, it turned to Eric and under his guidance JARO quickly became established as the excellent journal that if remains today.

Sunday, February 23, 2025

Symposium 1: Inter-Areal Contributions to Auditory-Guided Behavior

Chair: Ross Williamson*, University of Pittsburgh*
Co-chair: Justin Yao*, Rutgers University*
8:00 a.m. - 10:00 a.m.

Ocean Ballroom 1 - 4

Symposium Description: Auditory-guided behavior is ubiquitous in everyday life, whenever auditory information is used to guide the decisions we make and the actions we take. Though we have made great strides in identifying the functional role of many individual brain regions, we still do not understand how interactions between distinct areas cooperate to orchestrate auditory-guided behavior. During active listening, distinct populations of neurons across brain-wide neural circuits work to encode relevant sensory information and transform it into an appropriate behavioral response. Modern systems neuroscience techniques (electrical/optical physiology, opto/chemogenetics, viral genetics) now allow us to monitor, manipulate, and model neural circuits with unprecedented precision. Our primary goal for this symposium is to highlight and synthesize recent cutting-edge studies of auditory cortical (ACtx) structure-function relationships that span multiple brain regions. Our selected abstracts focus on several distinct aspects of inter-areal connectivity during auditory-guided behavior to provide a holistic "whole-brain" view of ACtx processing. Our symposium presenters will showcase work spanning three critical areas in inter-areal communication: thalamo-cortical interactions (how and why the ACtx integrates ascending, bottom-up information), cortico-fugal/cortico-cortical processing (how and why the ACtx propagates information to its many downstream cortical and sub-cortical targets), and top-down modulation (how and why ACtx representations are modulated by upstream brain regions in association and frontal cortex). Combined, these abstracts will lead to a cohesive, cutting-edge, overview of how the exquisite structure of brain-wide ACtx circuits can guide and inform purposeful behavior. We have assembled a diverse array of speakers with the aim of engaging a broad ARO audience, including those interested in modern systems neuroscience, sensory-driven decision making and perception, neural encoding/decoding, neuroanatomy, and computational neuroscience.

Interactions Between the Ascending and Descending Flow of Information in the Auditory System

Andrew King*, University of Oxford*

Individual Abstract: This presentation will summarize recent findings illustrating how neural representations of sound are transformed by the network of ascending and descending connections in the brain. By recording from neurons at several levels of the auditory pathway, we found that much of the nonlinear encoding of sounds in auditory cortex can be explained by transformations that take place in the midbrain and thalamus. Modeling cortical neurons in terms of their inputs across these subcortical populations enables their responses to be predicted with unprecedented accuracy. In contrast, subcortical responses cannot be predicted from their extensive descending cortical inputs, indicating that the ascending feedforward transformations are irreversible, resulting in increasingly lossy, higher-order sound representations across the

auditory pathway. Rather, optogenetic manipulations show that auditory cortex selectively modulates the nonlinear aspects of thalamic auditory responses and the functional coupling between subcortical neurons, without affecting the linear encoding of sound. Descending corticofugal projections also influence subcortical auditory processing in other ways. Inputs from the primary somatosensory cortex to the auditory midbrain and thalamus are involved in integrating multisensory signals, which are then transmitted to the auditory cortex. These subcortical loops therefore provide a basis for communication between different sensory cortical areas. Furthermore, projections from the auditory cortex to the inferior colliculus are required for the perceptual learning that enables accurate sound localization to be restored in the presence of unilateral conductive hearing loss. The training-dependent recovery of localization behavior observed after plugging one ear is mirrored by adaptive changes in the representation of sound source location in the inferior colliculus, raising the possibility that this midbrain plasticity is driven by top-down cortical signals. While consistent with other evidence suggesting that descending pathways dynamically update the responses of auditory neurons depending on the behavioral and sensory context in which particular sounds are heard, we have also shown that an animal's behavioral choice can be accurately read out from the activity of corticorecipient neurons in the inferior colliculus even if the auditory cortical input is removed. Together, these findings highlight the fundamental role of subcortical transformations in shaping cortical responses and illustrate how auditory processing and perception rely on the dynamic interplay of bottom-up and top-down signalling.

Diverse Cortical Layer 1 Circuits for Context-Dependent Auditory Perception

Anne Takesian*, Harvard Medical School*

Individual Abstract: Auditory perception is powerfully influenced by behavioral state and learned associations. Recent work implicates layer 1 (L1) of auditory cortex as a hub for integrating sound information with neuromodulatory and top-down signals that convey contextual information such as vigilance, locomotion, and the acquisition of rewards. The interneurons that populate L1 are composed of multiple subtypes, characterized by the selective expression of distinct molecular markers. However, the mechanisms by which these diverse interneuron subtypes participate in unique circuits to control context-dependent processing are not fully understood. Combining retrograde monosynaptic tracing with in vitro electrophysiological approaches, we have discovered several unexpected features about the functional connectivity of L1 interneurons in mouse auditory cortex. We find that the vast majority of auditory thalamic inputs to these interneurons arise from the ventral subdivision of the medial geniculate body (MGBv), the tonotopically-organized primary auditory thalamus. Moreover, specific L1 interneuron subtypes are recruited by distinct neuromodulatory inputs. Finally, the L1 interneurons form complex inhibitory networks that powerfully control their activity. Together, these connectivity studies suggest that L1 interneurons are uniquely positioned to integrate thalamic inputs conveying precise sensory information with neuromodulatory inputs. Parallel experiments using two-photon imaging in behaving mice reveal that subsets of L1 interneurons are robustly activated by sound stimuli and narrowly tuned to spectrotemporal features of sounds. However, the sound-evoked responses of these interneurons show high trial-to-trial variability that can be attributed to changes in behavioral state and interactions between neighboring L1 interneurons. Ongoing two-photon holographic activation studies are determining the effects of activating specific, small ensembles of L1 interneurons on

cortical network activity and behavioral perception. Together, these results contribute to our understanding of the L1 circuits and provide new insight into the cortical mechanisms that mediate context-dependent auditory processing and learning.

Deep-Layer Projection Neurons Develop Representations of Perceptual Categories and Behavioral Choice

Ross Williamson, *University of Pittsburgh*

Individual Abstract: Auditory-guided behavior is a fundamental aspect of our daily lives, whenever auditory information guides our decisions and actions. Nestled amongst several populations, extratelencephalic (ET) neurons reside in the deep layers of auditory cortex (ACtx) and provide a primary means of routing auditory information to sub-cortical targets associated with decision-making and action. To investigate the behavioral role of L5 ET neurons, we trained head-fixed mice to categorize the rate of sinusoidal amplitude-modulated (sAM) noise bursts as either fast or slow to receive a water reward. We then used two-photon calcium imaging alongside selective GCaMP8s expression to monitor the activity of L5 ET, as well as layer (L)2/3 and L5 intratelencephalic (IT) populations.

L5 ET neurons significantly changed their stimulus tuning across learning. Longitudinal recordings revealed that these neurons dynamically shifted their responses to selectively encode the slow and fast categories of the trained stimuli. This categorical selectivity correlated with performance and was completely absent in untrained mice. In trained mice, these L5 ET neurons showed notably weaker selectivity during passive listening, suggestive of top-down modulatory input. Furthermore, decoding analyses revealed a robust representation of category identity in the L5 ET population which grew with learning. Categorical information was weaker and stayed relatively constant in both L2/3 and L5 IT populations, implicating L5 ET cells specifically in the acquisition of auditory categories.

Behavioral choice could also be robustly predicted from L5 ET activity. Choice activity grew with learning and preceded motion onset, emphasizing that these signals were separate from motor activity. Choice signals were only weakly present in L2/3 and L5 IT populations and did not change across learning. However, while L5 IT neurons did not show categorical selectivity at stimulus onset, they did display categorical selectivity following the animal's choice. This effect was only present in the earliest days of learning, hinting at a role for these neurons in early association learning of auditory stimuli. Together, these results suggest that ACtx projection neuron sub-types differentially encode behaviorally relevant stimuli throughout learning, emphasizing the divergent pathways from ACtx and their contributions to auditory-guided behavior.

Projection-Specific Cortical Processing of Vocalizations Driving Mouse Maternal Behavior

Amy LeMessurier, *New York University School of Medicine*

Individual Abstract: How does the brain derive meaning from social vocalizations and drive behavior in response? Vocalizations are complex stimuli that must require integration across multiple time-scales and appropriate contextualization. Thus, it is likely that top-down

modulation of feed-forward auditory processing is needed for these computations. To test this hypothesis, we took advantage of a robust mouse behavior, in which parental animals search for lost pups in response to infant ultrasonic vocalizations (USVs). Experienced moms are experts at this behavior, but nulliparous females can also learn to respond to infant distress calls after cohousing with lactating moms and litters, and emergence of pup retrieval behavior is correlated with oxytocin-dependent plasticity in the left auditory cortex (Marlin et al. 2015, Schiavo et al. 2020).

We first asked whether projections from auditory cortex to subcortical areas are important for pup retrieval behavior in experienced maternal mice by chemogenetically silencing activity in left auditory cortex layer 5 during retrieval. Pup retrieval was reduced after CNO treatment vs vehicle control sessions (N=6 mice, p LESS THAN 0.05). Silencing only neurons projecting to inferior colliculus led to a similar decrease (N=6, p LESS THAN 0.05), while silencing only neurons projecting to the tail of striatum had no effect (N=6, p GREATER THAN 0.05), indicating that corticocollicular (CC) projections are particularly critical for linking perception to behavior. We next used 2-photon Ca2+ imaging in awake head-fixed mice to compare USV responses of corticostriatal (CS) and CC neurons. CC neurons in expert retrievers (N=4 mice) had sustained increases in activity during USVs compared to pure tones, while activity was similar during USV and tone presentation in CS neurons (N=3 mice). To precisely quantify the time course of these USV responses, we performed in vivo whole-cell recordings from optotagged projection neurons (n=8 CC, n=5 CS neurons). We also performed simultaneous Neuropixels recordings from auditory cortex, midbrain, and thalamus (N=6 mice) and found a subpopulation of neurons that did not respond to single call syllables but instead integrated over longer USV sequences.

To examine whether sustained activity in corticofugal neurons develops with experience, we tracked activity in CC and CS neurons with 2-photon imaging over days of co-housing. This revealed robust responses to USVs on each day; however, responses were variable across individual neurons and days. In both groups, delayed and sustained responses to USVs were larger on days in which mice had reached expert performance, which may reflect upregulated activity in recurrent auditory circuits.

Orbitofrontal Cortex Modulates Auditory Cortical Sensitivity and Sound Perception

Melissa Caras, *University of Maryland*

Individual Abstract: Sound perception is highly malleable, subject to influence by both the internal state of the listener and the behavioral relevance of the acoustic signal. A wealth of evidence indicates that these dynamics are mediated by adaptive shifts in auditory cortical activity and tuning, but the neural circuits that orchestrate these fluctuations are only beginning to be understood. Recent data points to the orbitofrontal cortex (OFC) as a brain region of interest, due to its ability to encode contextual information and transmit task-relevant signals to the auditory cortex. However, the role of OFC in sound encoding and perception remains uncertain. We addressed this issue by monitoring and manipulating OFC activity and assessing the downstream impact on auditory cortical activity and behavior.

We first recorded extracellular single unit responses of OFC neurons in freely moving Mongolian gerbils under two behavioral contexts: during passive sound exposure and during the performance of an amplitude modulation (AM) detection task. The majority of neurons were sensitive to behavioral context, exhibiting significant increases or decreases in tonic firing during task performance. In a subset of neurons, these firing rate changes persisted into a passive sound exposure session immediately following task performance, mirroring dynamics previously observed in the auditory cortex. We then used muscimol infusions to reversibly silence the OFC while simultaneously recording sound-evoked responses in the auditory cortex. OFC inactivation prevented task-dependent enhancements of auditory cortical sensitivity, and, in turn, impaired the ability of animals to detect AM noise. Together, our results suggest that OFC neurons provide contextual information to the auditory cortex to facilitate the perception of behaviorally relevant sounds.

The Asynchronous Maturation of the Left and Right Auditory Cortex Could Underpin Specialized Sound Processing Development

Hysell Oviedo*, Washington University*

Individual Abstract: It is unknown how the human and rodent Auditory Cortices develop distinct communication processing capabilities. Decoding auditory signals is a challenging task due to the fleeting nature of the auditory scene. From humans to mice, one strategy believed to be important in decoding auditory signals is distributing the computational load between the left and right Auditory Cortices (i.e. lateralization). The circuitry underlying these sound processing tasks is most receptive to refinement during critical periods when the brain is shaped by neural activity reflecting sensory information in the current environment. Therefore, we hypothesized that differences in the timing of critical periods between hemispheres could serve as a potential mechanism underlying the development of functional specializations. To investigate, we compared measures of thalamocortical and intracortical maturation that have been previously associated with critical periods in sensory cortices. To compare the maturation of thalamocortical (TC) projections, we utilized voltage-sensitive dye imaging (VSDi) and a new slice preparation to capture auditory TC slices from both hemispheres in an animal. Our findings revealed that the transition from immature to mature patterning in TC activation of the left Auditory Cortex lags behind that of the right Auditory Cortex. Additionally, we examined indicators of GABA and AMPA synapse maturation. Through measuring miniature inhibitory postsynaptic currents (mIPSCs), we discovered that GABAergic synaptic function matures earlier in the right ACx. Similarly, the maturation of mEPSCs occurs earlier in the right ACx but occurs after the maturation of GABAergic synapses as observed in previous studies. To evaluate the functional relevance of this asynchronous maturation between the Auditory Cortices, we manipulated the acoustic environment of male and female juvenile mice and tested the impact on tonotopic map re-arrangement in adulthood. We exposed mice to a 7kHz tone during the early critical period (P12-P15, as indicated by our VSDi and mPSC data) and mapped tonotopy by recording frequency responsiveness in adult, anesthetized mice using multi-channel silicone probes. Notably, tone rearing male mice resulted in an overrepresentation of 7kHz in the right Auditory Cortex, while tone rearing within the same time window led to the opposite trend in females, with overrepresentation in the left Auditory Cortex. These findings suggest that differential

timing in hemisphere development could precipitate lateralized auditory functioning in a sex-specific manner. Using spatial transcriptomics, we aim to uncover the molecular pathways that could coordinate asynchronous maturation between the hemispheres.

Contribution of Top-Down and Bottom-Up Processing in Auditory Decision-Making
Yale Cohen*, University of Pennsylvania School of Medicine*

Individual Abstract: Auditory perception and decision-making are mediated by both bottom-up (feedforward) and top-down (feedback) processes. Bottom-up processes interpret incoming sensory information to form a categorical perceptual decision (e.g., did I hear "boomer" or "groomer"?). In contrast, top-down processes, such as cued or expectation-driven attention, improve auditory perception by enhancing the neural representations of behaviorally relevant stimuli, while simultaneously suppressing neural representations of irrelevant stimuli. As a consequence of this interaction between bottom-up and top-down processes, a listener can flexibly direct their perceptual and cognitive resources in the pursuit of goal-directed behavior. Despite the importance of these bottom-up and top-down interactions, little is known about the neural mechanisms that govern them. Here, we review our studies that have shown how these processes modulate activity in the ventral auditory pathway. The ventral pathway is often conceptualized as a bottom-up pathway with processing beginning in the anterolateral belt region of the auditory cortex, progressing through the parabelt, and concluding in the ventrolateral prefrontal cortex. However, this model of information processing is inadequate because there are considerable top-down connections in the ventral auditory pathway whose functional roles in auditory perception and decision-making are not understood. To test how top-down and bottom-up processes interact in the ventral auditory pathway, we record simultaneous in multiple brain regions (e.g., the auditory cortex and the ventrolateral prefrontal cortex) while rhesus monkeys perform a threshold-level psychophysical task (i.e., reporting whether a tone burst is "low" or "high" frequency when embedded in various amounts of background noise). During this task, we manipulate the monkeys' bottom-up and/or top-down expectations. As expected from analogous human studies, the monkeys' psychometric and chronometric behavior is differentially modulated by these processes. Our neurophysiological recordings show that bottom-up and/or top-down expectations elicit differential brain activity. In general, we found that neural coherence, which quantifies the degree to which two brain regions synchronize their activity, is modulated by these expectations. The degree of this modulation and the directionality of the brain synchronization is a function of the recording sites (both cortical field and cortical layer). We compare our findings with classical models of predictive coding, which specify the cortical lamina and neural frequencies that mediate top-down versus bottom-up expectations.

Young Investigator Symposium 1: Cochlear Health after Cochlear Implants, Biomarkers, Therapeutics, and Outcomes

Chair: Seba Ausili*, University of Miami*

8:00 a.m. - 10:00 a.m.

Ocean Ballroom 5 - 8

Drug-Eluting Cochlear Implants

Stephen O'Leary*, University of Melbourne*

Individual Abstract: Introduction: Drug-eluting cochlear implants fill a specific niche in cochlear pharmacotherapy, providing drugs direct access to the neurosensory epithelium. The first clinical implementation will be a dexamethasone-eluting implant. This is now in regulatory clinical trials.

"Why" drug-eluting cochlear implants: A major challenge of cochlear implantation is preserving cochlear structure and function following implant surgery. The implant procedure puts the acoustic hearing at risk and causes fibrosis and new bone formation. Both negatively impact cochlear health with adverse effects on implant function. Steroids (dexamethasone) mitigate these effects and reduce fibrosis. Drug-eluting implants are required because other approaches, such as topical or systemic administration, have been ineffective.

"How" to measure that dexamethasone-eluting implants improve cochlear health: This presentation will focus on an in-depth appraisal of the biomarkers developed to measure the impact of steroids on the local cochlear environment. Residual hearing is one index, but it is only available in individuals with recordable acoustic thresholds before surgery. For fibrosis, electrode impedances have emerged as a proxy. With the emergence of light-sheet imaging of the intact, implanted cochlea, it is now possible to reconstruct in 3D all the tissue response to implantation and relate this to the position of the electrode contacts. In the implanted guinea pig, we see highly significant (p LESS THAN 0.001), moderate correlations (r ~ 0.45-0.55) between monopolar or bipolar impedance and the volume of immunoreactive tissue labelled for the extracellular matrix (fibronectin) and fibroblasts (smooth-muscle actin) within the tissue response. There are significantly fewer fibroblasts in the tissue response of animals receiving steroid-eluting electrodes.

Clinical trials: The first human trial was with a steroid-eluting investigational perimodiolar electrode [PMID32143111]. This non-randomised placebo-controlled study over 12 months revealed significantly lower impedances in patients receiving a steroid-eluting array. Here, we present more recent data. First, we show that the low impedances in the first study have persisted unchanged for 8 years. Second, a recent placebo-controlled RCT (NCT04750642) with a new-generation perimodiolar electrode has reported a reduction of 4.7 kΩ (95% CI:3.7-5.6) in monopolar impedance for the steroid-eluting electrode at 6 months. The 12-month results will be presented at this symposium.

The clinical "why": The clinical results suggest that dexamethasone reduces cochlear fibrosis. This means, first, longer battery life, which is directly related to electrode impedance. Second, improved preservation of residual acoustic hearing is anticipated, and the reduced fibrosis should make reimplantation easier. Finally, better cochlear health to prepare the ear for receiving future regenerative therapies.

A Glimpse Into the Implanted Ear: Modeling and Modulating Electrode-Tissue Interface

Federico Di Lella, *Hospital Italiano de Buenos Aires*

Individual Abstract: Cochlear implants (CIs) are the most successful sensory prostheses, restoring hearing to thousands of people worldwide. As their design evolves, a critical focus is on protecting the delicate inner ear structures. This involves new surgical techniques, thinner electrodes, and recently, the hybrid use of self-eluting dexamethasone electrodes. Steroids, such as dexamethasone, are known to reduce inflammatory effects, thus minimizing the foreign body reaction process. However, quantifying their effects can be challenging.
To understand the relationship between the implanted electrode and the inner ear medium, impedances serve as a potential tool to capture important changes. Our approach models the electrode-tissue relationship and captures the impedance subcomponents. Each of these components relates to specific physiological processes: the access resistance corresponds to the bulky tissue surrounding the electrode, while the polarization impedance associates with the thin layer between the electrode and the cochlear medium.

To assess the effects following cochlear implantation, we measured daily impedances in patients from CI surgery up to two months, capturing the complex impedances and various physiological processes between the implanted biomaterial and the cochlear medium. We also examined the effects of glucocorticoids through two different approaches: topical application and self-eluting dexamethasone electrodes. This unique approach allows us to observe daily changes in the inner ear medium using complex impedance as a proxy for cochlear health and the foreign body reaction process.

Our current results with self-eluting dexamethasone electrodes show a significant reduction in tissue growth up to six months post-implantation. In this presentation, we will discuss the elements of complex impedance, offering a new way to understand the underlying mechanisms of the cochlear response to cochlear implantation.

Therapeutic Hypothermia for Inner Ear Functional Preservation: Translational Journey From Preclinical Research to Clinical Applications

Maria Fernanda Yepes Restrepo, *University of Miami*

Individual Abstract: Trauma to the inner ear, whether from cochlear electrode implantation surgeries, noise or blast overexposures, adversely impact residual hearing and may lead to balance dysfunctions. The cellular and molecular mechanisms can lead to irreversible damage to the sensory neuroepitheium including the hair cells, neuronal cell bodies, and synapses. Therapeutic options such as dexamethasone, N-acetyle-cysteine, and other anti-oxidants, photobiomodulation and improvements in surgical techniques and materials have been studied to mitigate post-injury functional decline. Dexamethosone-eluting cochlear implants under clinical studies show promise. Unfortunately, there are currently no FDA-approved medications for prevention or treatment. Mild therapeutic hypothermia (MTH) has shown potential in counteracting the pro-inflammatory pathways, reactive oxygen species accumulation, and apoptosis that contribute to these injuries. In preclinical studies, we have demonstrated that acute application of MTH has an otoprotective effect following device implantation. We have shown its translational potential through cadaver temporal bone models. This protective effect has been validated through electrophysiological assessments via auditory brainstem responses,

immunohistochemical analyses via inner ear hair cell counts, and mechanistic studies. We also developed a non-invasive localized cooling technique to avoid the systemic and invasive side effects associated with other methods. These techniques, which involve a delivery of MTH through the mastoid and/or a balloon catheter positioned in the ear canal, have been shown to be feasible, safe, and effective, protecting against auditory dysfunction, hair cell loss, synapse damage and spiral ganglion death, while also reducing detrimental gene expression. These methods were further validated using computational and physical models of full cadaver heads to account for the physical attributes of skin, muscle, and vasculature absent in temporal bone or animal models. Our extensive research is leading to NIH-funded randomized clinical studies aimed at testing the safety and efficacy of MTH in patients. The present report will focus on this significant step towards translating preclinical findings into clinical practice, while offering a novel and efficacious approach to protect inner ear functions.

Using the Panoramic ECAP Method to Characterize Current Spread and Neural Responsiveness in Cochlear Implant Users

Charlotte Garcia*, University of Cambridge*

Individual Abstract: It is generally understood that the health of the auditory nerve has implications for cochlear-implant (CI) outcomes. As it is not possible to assess this directly in human CI users, multiple behavioral and objective markers of neural health have been proposed and evaluated over the last few decades. One of these, the Panoramic ECAP Method (PECAP), uses Electrically Evoked Compound Action Potentials (ECAPs) and provides estimates of the relative contribution of spread of electrical current and responsiveness of neurons along the length of a cochlear implant electrode array. It has the advantage of being a primarily objective metric that characterizes the electrode-neuron interface in detail, describing the variation in both the current spread and the neural responsiveness within the cochlea.

PECAP's neural-responsiveness estimate can detect localized areas of reduced neural responsiveness, temporarily imposed by adapting neurons that respond to stimulation of individual electrodes (Garcia, et al., 2021). Its current-spread estimate can detect localized areas of increased current spread, artificially increased by simultaneously stimulating multiple adjacent electrodes (Garcia, et al., 2024). In a large-scale, multi-center assessment, PECAP has also been able to detect differences between different electrode array types in Cochlear © devices, suggesting a location-specific effect of narrower current spread at the apex in pre-curved arrays (Garcia and Carlyon, 2024). We have also taken steps to increase potential clinical translatability of PECAP, developing a procedure called 'SpeedCAP' to reduce the data-collection time required down from ≈ 45 to ≈ 8 minutes in Cochlear devices (Garcia, et al., 2023). Recent work suggests that SpeedCAP could be further reduced to LESS THAN 5 minutes, and has also involved development of a web application for PECAP analysis and some data-collection procedures, increasing accessibility.

This talk will describe the benefits but also the limitations of the PECAP Method in characterizing these patient-specific neural activation patterns, and compare it to some of the other methods for characterizing aspects of cochlear neural health, such as focused thresholds (Garcia, et al. 2021) and variation in monopolar vs bipolar thresholds along the array (Peng, et

al., 2023). It will also discuss the potential benefits of optimizing cochlear implant programming based on each patient's unique neural activation patterns, and some of the future work needed in order to achieve this using PECAP.

Contributions of Auditory Nerve Density and Synchrony to Speech Encoding in Aging Cochlear Implant Listeners

Kara Schvartz, *University of Maryland*

Individual Abstract: Previous studies have demonstrated that the number (or density) of surviving auditory nerve (AN) fibers in an implanted ear helps to predict speech understanding in cochlear implant (CI) users. However, the role of AN across-fiber synchrony is not well understood. Previous research in non-implanted older adults shows that AN pathophysiology, to include AN density and synchrony, independently contribute to senescent declines in speech understanding. These findings are important to consider in CI users given that the majority of adult recipients are over the age of 65. A multi-metric approach to describe AN pathophysiology has not yet been explored to help explain speech recognition outcomes in aging CI users. This presentation focuses on recent work exploring estimates of AN health in aging CI listeners and discusses implications for speech encoding.

This prospective study examined electrically evoked compound action potentials (ECAPs) in 47 adult CI recipients. We measured the ECAP interphase gap (IPG) effect to estimate AN density, as developed previously in our animal model. We used a novel ECAP N1-P2 interpeak latency measure to estimate AN synchrony and will discuss how this compares to estimates of AN synchrony in non-implanted listeners. Linear regression models were used to examine the relationship between estimates of AN pathophysiology, age, and speech recognition in noise outcomes across participants.

Estimates of AN density and synchrony worsened with increasing age in CI users but were not correlated with one another indicating that they represent unique features of AN health. Estimates of lower AN density and synchrony were independently associated with poorer speech in noise understanding among participants. Multiple regression models showed that 1) AN synchrony rather than density is the primary contributing factor to poorer speech understanding in older CI users, and 2) when controlling for listener age, AN synchrony remains a significant predictor of speech recognition outcomes in adult CI recipients.

These results suggest that AN dyssynchrony rather than density is the primary contributing factor to age-related declines in speech in noise understanding in CI users. The interpeak latency measure used here is novel but can be easily calculated from standard clinical ECAP measures. These results help to explain poorer outcomes often observed in older CI recipients. Despite electrical stimulation of the AN, dyssynchrony persists in older listeners. These findings have important implications for evaluating older listeners for consideration of a CI, and also for our counseling of these patients post-operatively

Measures for CI Outcomes Prediction: Deep Learning With Temporal Bone Histology, Advances in Electrophysiology, and Quantification of the Electrode-Neural Interface

Christopher Giardina*, Harvard Medical School*

Individual Abstract: A patient receiving a cochlear implant (CI) has little predictive knowledge of how well he or she will ultimately perform in speech comprehension. This wide range of outcomes is affected by pre-operative, intra-operative, and post-operative factors, all of which are estimated using surrogate markers for 'cochlear health'. The purpose of this talk is to survey and present new findings using several of these predictors of CI speech outcomes, including 1) spiral ganglion neuron (SGN) survival, 2) SGN function, and 3) a combined approach integrating SGN survival/function with CI positioning to quantify the overall integrity of the electrode-neural interface (ENI).

First, 'cochlear health' will be discussed in terms of SGN quantification and 3D orientation with unprecedented accuracy. SGNs from entire serial-sectioned human temporal bones (TBs), including infant donors as well as adults, are quantified and described as a background for normal and pathologic expectations with presbycusis. Next, SGN counts and cochlear morphology will be described in temporal bones from 6 CI users, which each have robust audiometric and clinical data available. In this way, the otopathology provides definitive metrics of 'cochlear health' in a manner which also allows for direct outcomes analysis. Intrinsic to this approach is the development of a deep-learning segmentation model, trained on human TBs to facilitate rapid analysis, which has obtained 96% accuracy relative to hand-counting methods.

Second, 'cochlear health' will be briefly discussed in terms of SGN function when estimated non-invasively by 1) acoustically-evoked auditory nerve neurophonics (ANNs) as well as 2) electrically-evoked CAPs (eCAPs). These objective measures, which interrogate the electric characteristics of residual neuronal populations, are described with particular relevance to CI speech outcome predictions in a cohort of 123 subjects with auditory neuropathy spectrum disorder (ANSD).

Third and finally, 'cochlear health' will be discussed in terms of CI positioning relative to dynamically surviving SGNs, estimated subjectively using an inverted electrostatic model of the scala tympani and Rosenthal's canal. Here, in 18 experienced CI users, both CI position (validated against CTs) and SGN survival were independently estimated from behavioral

thresholds to monopolar and focused CI simulation. These metrics ultimately predicted changes seen in speech performance after selectively deactivating electrodes over regions with varying degrees of SGN survival (r^2=71%).

Overall, in this talk, SGN counts, SGN function, and CI array positioning are all utilized to quantify cochlear health, characterize the ENI, and ultimately predict speech outcomes.

Podium 3: Hair Cell Anatomy and Physiology: Molecular Dynamics, Structural Components, and Pathways to Protection

Moderators: Leslie Gonzales and Sonja Pyott

8:00 a.m. - 10:00 a.m.

Ocean Ballroom 9 - 12

Sensory Transduction Plays an Essential Role in the Maturation of Inner Hair Cells, Afferent Ribbon Synapses and Auditory Nerve Fibers

Thibault Peineau*[1], John Lee[1], Brikha Shrestha[2], Wu Zhou[3], Hong Zhu[3], Jeffrey Holt[1], Gwenaelle Geleoc[1]

[1]*Boston Children's Hospital, Harvard Medical School,* [2]*Mass Eye and Ear/Harvard Medical School,* [3]*University of Mississippi Medical Center*

Background: Inner hair cells (IHCs) convert sound information into electric signals through activation of mechanosensory transduction channels (TMC1 and TMC2) localized at the tips of stereocilia (Pan et al., 2013; 2018). TMCs are associated with other proteins, notably TMIE and CIB2 (Xiong et al. 2012; Zhao et al. 2014). Hair cells acquire transduction, progressively, from the basal end of the cochlea to the apical end, during the first postnatal week in mice (Lelli et al., 2009). Alteration in sensory transduction has been shown to affect hair cell physiology (Marcotti et al. 2006, Corn et al. 2018) and maturation of IHC synapse morphology (Lee et al. 2021). Here we further investigate how sensory transduction affects IHC physiology, afferent ribbon synapses, as well as downstream type-I auditory nerve fibers (ANF) properties.
Methods: To tackle this question, we took advantage of several mouse models with altered sensory transduction: mice lacking TMC proteins and mice carrying a recessive mutation in TMIE (Spinner mice). We performed single cell electrophysiological recordings to assess voltage-dependent calcium currents, exocytosis and potassium current, and examined ribbon synapses with immunostaining and transmission electron microscopy before and after the onset of hearing, respectively 2 and 3 postnatal weeks. We assessed the spontaneous and evoked firing properties of ANF, in vivo, using single fiber recording in anesthetized mice (2-4 months). We assessed RNA expression of the ANF fibers by RNA single cell sequencing in P24-P28 mice. Finally, we also performed utricle injection of AAV vectors expressing a human version of TMC1 to determine if the viral expression could rescue the defects observed.
Results: Our work demonstrated preservation of synaptic properties in Tmc2 KO mice. However, in Tmie, Tmc1 KO and double Tmc1/Tmc2 KO mice, we observed alterations in voltage-dependent calcium currents and fast potassium currents, along with impairment in

sustained exocytosis at 2 and 3 weeks. These changes were also associated with alteration in the morphology of the synapse as we demonstrated previously (Lee et al., 2021). Specifically, we observed alterations in the localization of Ca2+ channel clusters near the ribbon synapse in the absence of Tmc1 or Tmc1/Tmc2 and concomitantly reduced ribbon size. We observed reductions in spontaneous firing rates of ANF in the absence of Tmc1, Tmc1/2 and Tmc2. And we observed changes in RNA expression in the absence of Tmc1, Tmc1/2. Finally, we observed complete rescue of all functions in mice lacking Tmc1 that were virally injected with TMC1 at P2.
Conclusions: Our work demonstrates that sensory transduction plays an important role in the development and maturation of hair cells, their afferent synaptic machinery, as well as maturation of the ANF.
Work supported by: NIH R01DC008853

Characterization of the Lipid Scramblase Activity of TMC1 and TMC2: New Perspectives on Mechanotransduction and Disease Mechanisms

Yein Christina Park*[1], Hubert Lee[2], Jayashree Balaraman[2], Angela Ballesteros[2]

[1]*National Institute of Health,* [2]*Section on Sensory Physiology and Biophysics, National Institute on Deafness and other Communication Disorders*

Background: The perception of mechanical stimuli, including sound, touch, gravity, and vibration, is crucial for vertebrate survival. In the inner ear, sensory hair cells are essential for sound and balance perception. These specialized cells rely on MechanoElectrical Transduction (MET) channel complexes to convert mechanical stimuli into electrical signals with exceptional precision, which are then transmitted to the central nervous system. Recent research has identified Transmembrane channel-like (TMC) proteins 1 and 2 (TMC1 and TMC2) as the pore-forming subunits of the MET channel. TMC proteins are structurally related to the TMEM16 lipid scramblases as well as the TMEM63/OSCA mechanosensitive channels, raising the possibility that functional properties may also be shared between three protein families. While TMC1 and TMC2 are known to function as mechanosensitive channels, their potential lipid scramblase function remains unexplored.
Methods: In this study, we investigated the lipid scramblase activity of TMC proteins using fluorescent lipid-based assays with purified proteins reconstituted into liposomes of varying compositions.
Results: Our results show that TMC1 and TMC2 can translocate lipids across a lipid bilayer in a concentration-dependent manner, indicating that TMC proteins can function as lipid scramblases. Importantly, control experiments confirmed that TMC scramblase activity is not an artifact of large pore formation. We further compared the activity of full-length TMC proteins with that of two deletion constructs, and examined the effects of calcium, established MET blockers, and specific lipid compositions on TMC scrambling. Notably, TMC1 mutations associated with deafness, which lead to constitutive phosphatidylserine externalization in hair cells, showed increased scramblase activity compared to the wild-type protein.
Conclusions: The characterization of TMC scramblase function enhances our understanding of its physiological role and the molecular mechanisms underlying TMC-related diseases, potentially guiding new strategies for the prevention and treatment of these disorders.

Analyses of MYO7A-Driven Active Cargo Transport in Stereocilia Using Single-Molecule Microscopy in Live Hair Cells

Takushi Miyoshi*[1], Mrudhula Sajeevadathan[2], Harshad Vishwasrao[3], Inna Belyantseva[4], Yasuko Ishibashi[5], Samuel Adadey[6], Narinobu Harada[7], Hari Shroff[8], Thomas Friedman[9]

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Background: Wild-type motor activities of unconventional myosins are necessary for developing and maintaining functional stereocilia. These myosins have tails different from one another and can localize specific proteins and phospholipids as their "cargo". MYO7A is one of the unconventional myosins whose variants are associated with human hereditary hearing loss. Although MYO7A is a key component of the tip-link complex mediating mechanosensing, it is not fully understood how this myosin localizes itself and its cargo using the energy from ATP hydrolysis. Here, we analyze the trafficking of MYO7A in stereocilia using our novel workflow for single-molecule microscopy in live hair cells.

Methods: Utricles or saccules (hereafter "vestibules") were harvested from mouse neonates at postnatal days 2–5 and transfected using a Helios® gene gun to express HaloTag-fused proteins of interest. HaloTag-fused protein molecules were fluorescently labeled using JFX554-ligands and imaged using a diSPIM light-sheet microscope. Full-length MYO7A, MYO7A-HMM (a head + neck fragment), MYO7A-R/K (a missense mutant disabling the tail-mediated motor autoinhibition) and MYO7A-M/F1 (a fragment truncated after the first MyTH4-FERM domain) were fused to HaloTag at the N-terminus. For conditional homodimerization, MYO7A-HMM was fused with the p.F36V mutant of FK506-binding protein 12 (FKBP) at the C-terminus and treated by an FK506 derivative, AP20187. MYO7A-HMM was also conditionally tethered to the plasma membrane or F-actin using the α subunit of human Interleukin receptor 2 (IL2Rα) or the PST domain of Harmonin b, respectively, mediated by a ternary complex of FKBP, the FKBP-rapamycin binding domain (FRB) and a Rapalog, AP21987.

Results: Under single-molecule microscopy, HaloTag-MYO7A-HMM-FKBP molecules move directionally toward stereocilia tips only when dimerized by the AP20187 treatment. Trajectories of moving molecules in kymograms are continuous and consistent with processive "walking" on F-actin. Similar processive movements are observed for MYO7A-R/K and MYO7A-M/F1 but not for full-length MYO7A. These results indicate that MYO7A can "walk" on F-actin, likely as a dimer (or an oligomer), when the motor domain is exposed. The first MyTH4-FERM domain may be involved in this "walking" because processive movements are observed for MYO7A-M/F1 but not for MYO7A-HMM monomers. MYO7A-HMM tethered to the plasma membrane or F-actin moves less frequently and over limited distances in stereocilia while the positive control, monomeric MYO10 head, does show processive movements in these conditions. Restricted movements of MYO7A-HMM tethered to the plasma membrane or F-actin suggest that MYO7A cannot move efficiently in stereocilia using PCDH15, CDH23 or Harmonin b as a scaffold to transmit force to F-actin.

Conclusions: Our study suggests a scenario in which some factors, likely MYO7A's cargo such as the components of the tip-link complex, unleash MYO7A from the autoinhibitory state and activate its motor activity. We are currently identifying physiological activators of MYO7A trafficking in stereocilia.

The Auditory Hair Cell Mechanotransduction Complex Regulates Stereocilia Membrane Mechanics

Shefin George*[1], Anthony Ricci[1]

[1]*Stanford University*

Background: Hair cells are the mechanoreceptors of the auditory and vestibular sensory systems. The sensory hair bundle is the organelle that senses movement and converts this movement to an electrical signal using mechanically gated (MET) ion channels. These channels can operate at high frequencies and with sensitivities at molecular dimensions. How is this achieved? A growing body of data support the hypothesis that the stereocilia membrane plays a role in modulating mechanotransduction. Biochemical modulations happen with PIP2, for example, regulating both permeation and conductance. Mechanical modulation is implicated pharmacologically and by fluorescent recovery after photobleaching which demonstrated that the MET open probability co-varied with membrane diffusivity; lower diffusivity correlated with more open channels.
Methods: Recent data has suggested that transmembrane channel like proteins (TMCs) are part of a large family of molecules that can serve as ion channels or membrane scramblases and are an essential component of the MET machinery. We investigated the role of TMCs in modulating membrane properties to determine if the MET machinery was directly controlling its local membrane environment. We used a newly developed viscosity sensor BODIPY 1c whose changes in fluorescence lifetime allowed precise spatial and dynamic monitoring of membrane properties within live hair cells. We also monitored membrane scramblase activity more conventionally, using PS-specific binding protein Annexin V (AnV).
Results: We find a strong correlation between MET channel activity and membrane viscosity both during development and in mutant mice that disrupt mechanotransduction (TMC1, TMC2, TMC1/TMC2 double and TMIE mutants). Biophysically dissecting current, voltage and calcium demonstrates that scramblase activity associated with the MET channel is responsible for the lower membrane viscosity. Conventional MET channel blockers block the scramblase activity resulting in an elevation in viscosity. The increased viscosity suggests either rapid diffusion or the presence of membrane flippase/floppases that counter the activity of the scramblase.
Conclusions: Together these data suggest that TMCs are mechanosensitive scramblases that directly modulate membrane mechanics. We further hypothesize a yet undefined system creating an asymmetric membrane that is then regulated by the MET channel's scramblase activity. The reduced viscosity will directly impact MET channel kinetics and sensitivity.

Identification of a Novel Principal Component of Outer Hair Cell Stereocilia – Tectorial Membrane Connectors

Dennis Derstroff[1], Antonia Löhnes[1], Vijay Vijay Renigunta[2], Boris A. Stuck[1], Nicola Strenzke[3], Dominik Oliver[2], Katrin Reimann*[1]

[1]*University Hospital Marburg, Philipps-University Marburg*[2]*, Institute of Physiology and Pathophysiology, Philipps-Universität Marburg*[3]*Institute for Auditory Neuroscience, University Medical Center Göttingen*

Background: OHC stereocilia (In contrast to inner hair cells (IHCs)), are linked to each other by horizontal top connectors (HTCs) and the tallest stereocilia are physically connected to the overlaying tectorial membrane (TM) by tectorial membrane attachment crowns (TM-ACs). Both protein complexes comprise the secreted proteins stereocilin, otogelin, otogelin-like and colocalize with the intracellular adaptor protein, tubby. The absence or mutations of either of the extracellular components results in the loss of both types of stereociliary links and results in hearing loss in humans (DFNB16; DFNB18B; DFNB84B, respectively) and mice. Strikingly, mutation or genetic ablation of tubby similarly disrupts both links. The complete composition and assembly of both protein complexes is unknown. Moreover, how the extracellular components can interact functionally or structurally with the cytoplasmic tubby protein is unclear. Here, we describe the identification of a novel transmembrane component of these complexes that is indispensable for the formation of HTCs and TM-ACs.
Methods: Expression and subcellular localization of hair cell proteins was determined using immunohistochemistry with fluorescence-labelled secondary antibodies in whole-mount preparations of the mouse organ of Corti. Three-dimensional distribution of proteins was imaged by confocal and by STED super-resolution microscopy. Scanning electron microscopy was used to examine the structural integrity of hair bundles.
Results: We identified a so far uncharacterized membrane protein, which we provisionally term TM_OHC that is selectively expressed in outer hair cells. TM_OHC immunoreactivity was restricted to the tips of OHC stereocilia, where it closely co-localized with both tubby and stereocilin in confocal imaging. Super-resolution STED microscopy revealed a ring-like distribution of both TM_OHC and tubby around the tips of the longest stereocilia, indicating their close spatial association with TM-ACs as outlined by previous electron microscopy studies. Additionally, TM_OHC labeling co-localized with tubby to the sites of HTCs. In TM_OHC knockout mice, both stereocilin and tubby were lost from developing and mature OHC hair bundles, and stereocilin immunoreactivity at the stereocilia contact sites of the tectorial membrane (imprints) was abolished. The structural integrity of OHC stereocilia bundles was compromised in the KO mice, as shown by SEM.
Conclusions: We propose that TM_OHC is a novel integral component of the OHC stereocilia TM-AC and HTC complexes. Future studies will reveal the precise role of TM_OHC in structure, assembly, and maintenance of the OHC stereociliary connectors, and help to understand the specific mechanical framework of the OHC hair bundle and its role for OHC transduction and cochlear mechanics.

Effects of the Abolition of Salt Bridges on Prestin Voltage-Sensor Charge Movements

Jie Yang*[1], Chenou Zhang[2], Jun-Ping Bai[1], Richard Mariadasse[1], Joseph Santos-Sacchi[1], Oliver Beckstein[2], Dhasakumar Navaratnam[1]

[1]*Yale University School of Medicine,* [2]*Arizona State University*

Background: Prestin is the membrane protein in outer hair cells (OHCs) responsible for electromotility. Voltage driven expansion and contraction of the protein in the lateral plasma membrane gives rise to electromotility. We have established the cryoEM structure of the protein at 3.6 Å that we believe to be in the contracted state. In the absence of clear structural data on its expanded state, we used Molecular Dynamic (MD) simulations to model its transition to the expanded state.
Methods: We used MD simulations to explore how the protein undergoes conformational change towards its expanded state. Based on these simulation data, we identified individual residues that formed salt bridges coincident with its transition from a contracted to an expanded state. We then measured the gating charge movement of individual residue mutations of the protein expressed in CHO cells to assess the effects of disrupting these salt bridges on its possible transition from a contracted to an expanded state.
Results: Several different algorithms identified a salt bridge between H365 and E404 in our static cryoEM structure of prestin in the contracted state. However, in our MD simulations at 0mV we noted the formation of a salt bridge between E404 and either K276 or K283 in one of the protomers (B), but not the other (A). These salt bridges formed coincident with the transition from the contracted to expanded state of protomer B (protomer A remained in the contracted state where E404 remained proximate to H365 (LESS THAN 4 Å)). We individually mutated these charged residues (E404, K276 or K283) to glutamine and measured its gating charge parameters. Specific charge density (Qsp) in E404Q mutant was significantly reduced. A significantly decreased z value was observed in all three mutants, indicating diminished charge movement of each individual motor. Moreover, both K276Q and K283Q mutants showed a similar right-shifted Vh, while E404Q mutant showed left-shifted Vh.
Conclusions: The formation of salt bridges between K276 or K283 with E404, coincident with the transition from a contracted to an expanded state, suggests that it could be related. Mutation of these residues consistently reduced measures of z indicating that these salt bridges may play a role in its transition from a contracted to an expanded state. Moreover, the abolition of either K276-E404 or K283-E404 could also affect the frequency-dependent kinetics of prestin's charge movement. The dynamic variation in distance between these residues indicates that there might be a more complex interaction between these two salt bridges.

Lipid Flippase ATP8B1 in the Function and Degeneration of Sensory Hair Cells

Henry De Hoyos*[1], Jun-Sub Im[1], Betsy Szeto[1], Emma Kim[1], Nikhil Amin[1], Jung-Bum Shin[1]

[1]*University of Virginia*

Background: Hair cell mechanotransduction occurs within the sensory hair bundle, and while the role of proteins in this process is well-characterized, the function of lipids within the hair bundle membrane remains poorly understood. One lipid of particular interest is phosphatidylserine (PS), which is typically confined to the inner leaflet of the membrane. Previous research has shown that damaging stimuli, such as noise and ototoxic drugs, can cause PS to translocate to the outer membrane. Recent findings indicate that this translocation requires a newly discovered scramblase activity of TMC1, a key component of the mechanotransduction

channel. Furthermore, genetic mutations affecting lipid flippases, which restore PS to the inner leaflet and maintain its asymmetric distribution, have been associated with hearing loss. My project explores the role of PS homeostasis, specifically the function of the flippase ATP8B1 and its co-factor TMEM30B, in hair cell health and degeneration. We hypothesize that ATP8B1 is essential for maintaining the lipid environment necessary for the maturation of hair cell mechanotransduction and the integrity of stereocilia. Supporting this, our preliminary data from ATP8B1 and TMEM30B knock-out (KO) mouse models demonstrate their critical role in hearing, while expression and localization studies using knock-in (KI) models offer clear evidence of their involvement in these processes.
Methods: We generated hemagglutinin (HA)-tagged Atp8b1 and Tmem30b mouse models to examine protein localization and expression levels via confocal and STED microscopy. We also generated Atp8b1 and Tmem30b KO mouse lines and tested auditory brainstem response (ABR) and abnormal externalization of PS using the fluorescent PS-binding probe Annexin V. Additionally, we crossed HA-Atp8b1 and HA-Tmem30b KI mice to a mouse line that lacks hair cell mechanotransduction (Cib2 KO), to test whether the correct expression and localization of ATP8B1 (and TMEM30B) is dependent on mechanotransduction (MET) activity.
Results: Using our HA-tagged mouse models, we found that ATP8B1 and TMEM30B are enriched in hair bundles of outer hair cells after the onset of MET and show a rapid increase in expression following the onset of hearing. Analysis of the HA-Atp8b1/Cib2 and HA-Tmem30b/Cib2 double KO mouse lines proved that the expression and localization of ATP8B1 and TMEM30B depend on MET activity. Atp8b1 and Tmem30b KO mice exhibited severe, early-onset hearing loss compared to WT controls. Atp8b1 and Tmem30b KO mice showed significant hair cell loss by P20 compared to WT mice. Additionally, Annexin V bound to hair cells in both Atp8b1 and Tmem30b KO mice, indicating externalized PS and loss of membrane asymmetry.
Conclusions: Our data suggest that ATP8B1 and its cofactor TMEM30B are essential for maintaining phospholipid asymmetry in the hair bundle. Their absence may compromise hair cell MET specifically and cause progressive loss of structural integrity of the plasma membrane, leading to elevated ABR thresholds and hair cell loss.

The Mechanotransduction Complex of Inner Hair Cells is the Primary Target of Noise-Induced Hearing Loss

Samuel Webb*[1], Stuart Johnson[1]

[1]*University of Sheffield*

Background: Exposure to excessive loud noise causes a wide range of anatomical and physiological changes within the cochlea that lead to hearing loss. A key cell type identified as a target of noise-induced hearing loss are the sensory inner hair cells (IHCs). Some of the changes to IHCs considered to be responsible for hearing loss include stereocilia damage, synapse dysfunction and cell death. Although it is known that noise can cause these problems in IHCs, it is not clear what the primary mechanism responsible for hearing loss is, and what are consequences following on from the initial damage.
Methods: To identify the primary target of noise exposure, experiments were performed on 1-month old 6N-repaired mice immediately after and 1-day after noise exposure (120dB for 2

hours at 1-16KHz) and controls. Auditory brainstem response (ABR) tests were used to confirm noise-induced hearing loss. Scanning electron microscopy was used to image IHC stereocilia. Whole-cell patch-clamp electrophysiology was combined with fluid jet displacement and fast camera imaging to measure the stiffness of IHC stereocilia and the mechanotransduction channel properties. Patch-clamp electrophysiology was used to measure the K+ channel profile and pre-synaptic functioning of IHCs. Immunolabelling and confocal microscopy were used to quantify pre- and post-synaptic connections.
Results: Noise exposure caused substantial hearing loss in ABR thresholds across all frequencies immediately and 1-day after noise. From the electron micrographs, it was not clear that there was damage to stereocilia. Calculating the steady-state stiffness by displacing the stereocilia with a fluid jet demonstrated that noise exposure does not affect the stiffness of the hair bundles, however, the mechanotransduction channels required more force to be opened. The inwardly-rectifying current carried through KCNQ4 channels was no different from controls immediately after noise, even when hearing is impaired, but became completely absent after 1-day. Also, there was no evidence of a loss of pre- and post-synaptic puncta 1-day after noise. When measuring the physiology of the synapses in IHCs, there was no loss of function observed immediately after noise, but after 1-day, the synapses ability to process sustained stimuli became significantly reduced. Unlike the mechanotransduction complex that became dysfunctional immediately after noise-induced hearing loss, the KCNQ4 K+ channels and synapses of IHCs remained functional until 1-day following noise exposure.
Conclusions: Overall, these results provide evidence that the primary target of noise-induced hearing loss is the IHC mechanotransduction complex, and mechanisms such as the KCNQ4 channels and synapses become dysfunctional as a secondary consequence.

Symposium 2: Vestibular Disorders: Breakthroughs in Diagnosis and Management

Chair: John Lee*, National Institute on Deafness and Other Communication Disorders*
10:30 a.m. - 12:30 p.m.

Ocean Ballroom 1 - 4

Symposium Description: Vestibular disorders significantly impact patients' quality of life, often leading to chronic dizziness, imbalance, and falls. In clinical practice, the available vestibular test battery (e.g., rotary chair testing, vestibular evoked myogenic potentials, caloric irrigations, vestibular head impulse test) suffers from several limitations including: the extensive time required for test administration, high costs and lack of insurance coverage for testing, and a lack of standardized guidelines for when tests should be included in patient evaluation. Additionally, the equipment required is expensive and frequently unavailable at most clinics. As a result, there is an overreliance on patient symptoms to indicate the presence of underlying vestibular dysfunction despite the fact that symptoms can be highly variable or even absent. Novel solutions are needed to achieve accurate and efficient diagnosis and clinical management of vestibular disorders. This symposium will highlight modern and innovative approaches as speakers share insights into the latest advancements in vestibular assessment techniques including pediatric vestibular testing, vestibular perception, kinematics, and machine learning.

Additionally, the symposium will highlight vestibular rehabilitation and how more accurate diagnosis of vestibular dysfunction can lead to more targeted and effective rehabilitation strategies. By bringing together leading researchers and clinicians, this symposium aims to bridge the gap between cutting-edge vestibular research and practical clinical application.

Quantifying Clinical Measures of Vestibular Function and Perception in Children With Vestibular Loss

Kristen Janky*, Boys Town National Research Hospital*

Individual Abstract: Children with hearing loss are at risk for having vestibular dysfunction. This risk increases as the degree of hearing loss increases. Despite this association, pediatric vestibular testing is not completed routinely in clinical practice. This may be in part because the spectrum of consequences associated with pediatric vestibular dysfunction have not been fully defined. Pediatric vestibular dysfunction has been associated with significant reductions in balance and dynamic visual acuity ability; however, the cascading effects of balance and dynamic visual acuity dysfunction are unknown. The purpose of this presentation is to outline novel vestibular assessments and the consequences of pediatric vestibular dysfunction. First, vestibular assessment in infants and young children will be reviewed. Pilot data will be presented demonstrating that with the use of remote-camera video head impulse testing (vHIT) and cervical vestibular evoked myogenic potential testing, degree of vestibular dysfunction can be characterized in infants and young children, allowing inquiries on the natural history of pediatric vestibular loss and the initiation and effectiveness of vestibular therapies at a young age (6 mos to 3 years). Second, the ability to assess vestibular perception in children will be reviewed. Pilot data will be presented on the feasibility of measuring vestibular perception in children and the ability to measure vestibular perception using a standard rotational chair in adults and children with varying degree of vestibular involvement. Lastly, the consequences of pediatric vestibular dysfunction will be outlined, which ultimately drive the need for pediatric vestibular assessment.

Are We Underestimating Fall Risk in Balance Disordered Patients?

Christopher Zalewski*, NIH - NIDCD*

Individual Abstract: Clinical breakthroughs in balance assessment and management have been slow to evolve over the past few decades. This is driven, in part, by the complexities associated with the vestibular system and its sophisticated integration with the central nervous system. Many vestibular diagnoses and functional outcome measures continue to be made from results measured from individual assessments. However, diagnoses made from siloed test-results derived from discrete responses often fail to wholistically describe vestibular function and may provide some explanation to the poor correlations often seen between objective and subjective outcome measures. This presentation will present data that will improve our understanding of how we evaluate our patient's functional outcome measures.

Balance function, or specifically postural stability, is a reflection of one's ability to maintain quiet stance over a base of support. However, in the presence of pathology, an individual's base of support as well as their postural sway can be significantly modified, potentially increasing a patient's risk for fall. An integral component to one's ability to maintain postural stability is determined by one's personal limits of stability (LOS). When an individual's limits of stability

are threatened due to external perturbations or pathology, a fall is imminent unless a corrective step is taken. Unfortunately, patient-specific limits of stability are not currently considered when determining one's postural stability and subsequent fall risk.

We explored the influence of an individual's personal limits of stability on their reported measures of postural sway. Limits of stability were determined from 60 healthy volunteers recruited into three age groups of young, middle-aged, and elderly. Individual measures of LOS were compared to the universal theoretical estimates of the limits of stability and found to be significantly lower. Implications for implementing an individual's personal LOS for determining postural stability will be discussed. Moreover, implications for determination of fall risk will be highlighted when accounting for an individual's personal limits, particularly in vulnerable populations such as vestibular disordered patients and the aging population.

Assessment of Vestibular Perception in Persistent Postural Perceptual Dizziness
Megan Kobel, *University of Arizona*

Individual Abstract: Vestibular reflexes and vestibular perception (i.e., perception of self-motion) represent distinct neurologic pathways. Recently, psychophysical methodology has been implemented to measure vestibular perceptual thresholds (i.e., whole-body direction recognition thresholds) in order to quantify vestibular perception and provide unique insights into how afferent vestibular information is processed by the brain. Past evidence has suggested that vestibular perceptual thresholds can quantify changes in processing of self-motion in patients with peripheral, and central vestibular disorders. However, evaluation of potential applications of vestibular thresholds in multiple patient groups is limited. Persistent postural-perceptual dizziness (PPPD) has been classified as a functional neuro-otologic disorder and while the underlying pathophysiology is incompletely understood, the primary processes are believed to, in part, reflect alterations in neurologic functions underlying spatial perception and orientation. As such, we aimed to assess vestibular perceptual thresholds in patients with PPPD to systematically quantify self-motion perception and determine if vestibular thresholds can identify changes in vestibular processing in functional neuro-otologic disorders. We assessed vestibular thresholds in patients with PPPD alongside age-matched healthy controls. All participants completed a vestibular threshold test battery designed to isolate perception predominantly mediated by the otoliths, semicircular canals, and canal-otolith integration. Global deficits in motion perception were not identified in patients with PPPD. However, localized changes in thresholds capturing saccular function, superior-inferior z-axis translations, and those capturing canal-otolith integration, head-centered roll-tilt, were identified. This effect was modulated by diagnosis of co-occurring vestibular migraine, a pathology previously identified to exhibit increased sensitivity to distinct motions. As both vestibular thresholds identified as exhibiting changes in PPPD are reliant on accurate perception of gravity, this may suggest that patients with PPPD exhibit impaired processing of graviceptive cues. Overall, this work adds to the body of work suggesting potential utility of vestibular thresholds in the diagnosis of patients with vestibular disorders.

Central Vestibular Processing After Peripheral lesions: Implications for Diagnosis
Faisal Karmali, *Harvard Medical School*

Individual Abstract: The vestibular organs are imperfect, yet the brain is able to synthesize a robust estimate of three-dimensional motion and orientation in most individuals. The mechanisms that perform this estimation process include velocity storage. In fact, the velocity storage time constant is an important clinical parameter which varies with age and peripheral damage. Furthermore, it has been suggested that some of the complex, non-intuitive characteristics of vertigo may arise from the three-dimensional central processing of vestibular signals. Importantly, mechanistic explanations for the relationship between vestibular damage and changes in these behaviors are lacking. It has been hypothesized that Bayesian optimal processing determines velocity storage dynamics based on the statistics of vestibular neural noise (i.e., variability) and experienced motion. Specifically, while a longer time constant would be advantageous because this would make the VOR accurate over a longer period of time, it has been argued that this would result in the accumulation of noise by the velocity storage mechanism, which would result in drift and make the VOR less precise (1). We have recently found evidence to support the hypothesis that the brain determines the time constant based on vestibular noise to determine the optimal tradeoff between being accurate and being precise. We found that age-dependent changes in the VOR are explained by a velocity storage model responding to death of motion-sensing hair cells (2). Specifically, we found that the temporal dynamics of the VOR as a function of age are predicted (r=0.93, p LESS THAN 0.001) by a model that determines the optimal behavioral output when the sensory signal-to-noise characteristics are degraded by death of the transducers. We also found that these models predict reduced velocity storage time constants for unilateral lesions. We have also developed models that predict the three-dimensional non-intuitive characteristics of vertigo that occur after unilateral damage, such as the reduction in illusory rotation when a patient lies on their side. Together, these results provide a conceptual understanding for common diagnostic criteria and could lead to a better understanding of vertigo.

(1) Karmali F. The velocity storage time constant: Balancing between accuracy and precision. Prog Brain Res 248, 269-276 (2019).

(2) Karmali F, Whitman GT, Lewis RF. Bayesian optimal adaptation explains age-related human sensorimotor changes. J Neurophysiol 119, 509-520 (2018).

(3) Madhani A, Lewis RF, Karmali F. Journal of the Association for Research in Otolaryngology 23.4 (2022): 551-566.

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Novel Kinematic Analyses for Detection of Intravenous Aminoglycoside-Induced Vestibular Loss

Angela Garinis*, Oregon Health and Science University*

Individual Abstract: Pharmacologic agents, such as aminoglycoside antibiotics, are a leading preventable cause of peripheral vestibular loss. The vestibular (balance) system is essential for maintaining the relationship between our body in space and the physical world. Vestibular loss commonly leads to dizziness, vertigo, elevated risk of falling, difficulties with spatial memory and higher cognitive function. Conventional test methods, such as the video head impulse (vHIT) test, are widely used clinical metrics for evaluating vestibular function. However, these tests lack

sufficient sensitivity to detect subtle but clinically important losses and correlate poorly with patient-reported outcomes.

Kinematic methods using body-worn inertial sensors represent a promising new modality to meet this challenge. Gait and balance depend on adequate vestibular input, meaning that kinematic changes during these activities, as measured by inertial sensors, could serve as sensitive indicators for changes in vestibular function. Additionally, compared to conventional vestibular testing, kinematic testing may be better tolerated, more portable, cheaper, and quicker. These methods are superior to conventional tests for detection of gait and balance deficits in patients with concussion and vestibular schwannoma, but have not been extensively explored in other clinical populations.

The purpose of this clinical investigation was to investigate the ability of kinematic methods to identify people with vestibular loss treated with intravenous aminoglycosides. The targeted aims were to compare the ability of kinematic testing to the widely used vHIT for identification of aminoglycoside-induced vestibular loss, and to explore if patient-reported symptoms relate to kinematic changes pre- and post-treatment with intravenous aminoglycosides. The outcomes of this research will improve our understanding of the clinical value of measuring kinematics during gait and balance tasks for detecting vestibular loss. Additionally, the findings will offer important insights into the different strategies for maintaining functional mobility.

Implementation of an Automated Triage System in Epic for Patients With Dizziness Using Machine Learning

Devin McCaslin*, University of Michigan*

Individual Abstract: Background

Failures of care coordination and overtreatment account for more than $175 billion per year in wasteful spending. One of the most challenging problems is finding the right provider for the right patient at the right time. However, managing the various causes of dizziness spans several healthcare specialties, including Otolaryngology, Neurology, and Psychiatry, among others. This creates a substantial challenge for patients and referring primary care providers, leading to inconsistency in the care, management, and referral pathways of dizzy patients. An effective triage system would reduce needless physician visits while producing considerable cost savings for medical centers nationwide.

Methods

We have developed a triage system for patients with dizziness symptoms coming to our medical center using a clinical informatics approach. The resulting system design and architecture can be applied to the triage of many other complex conditions or presenting complaints. The process starts with the patient contacting our department’s appointment office to request an appointment. The patient is then sent a questionnaire through the patient portal. This questionnaire, which constitutes the afferent limb of the system, includes approximately 50 questions about different

characteristics of symptoms (duration and timing, associated symptoms, previous medical history, etc.) using branching logic to reduce the burden on the patient.

After the patient completes the questionnaire, their responses are processed by a triaging algorithm housed in the Epic AI platform Nebula. The triaging algorithm, developed with a mixed approach using statistical modeling and clinical expertise, also takes into account the availability of appointments in different clinical departments. The list of appointments and symptom clusters generated by the model is then presented in a dashboard for triaging clinicians to review and approve.

Results

The system has shown a high acceptance rate among patients. Data collection is ongoing, and the outcomes to be discussed at the time of presentation include algorithm accuracy, clinician time saved during chart reviews, changes in the number of patients scheduled in neurotology, and clinician assessment of the value of appointments.

Conclusions

We developed an automated triage system for patients with dizziness in Epic, addressing a common complaint that spans multiple clinical specialties. The system requires an afferent limb that captures information, a processing core, and an efferent limb to communicate and schedule appointments.

Podium 4: From Hearing Loss to Functional Hearing

Moderators: Samira Anderson and Gal Nitsan

10:30 a.m. - 12:30 p.m.

Ocean Ballroom 5 - 8

Behavioral and Neurophysiological Signatures of Functional Hearing Difficulties in Blast Exposed Veterans

Jonathan Venezia*[1]

[1]*VA Loma Linda Healthcare System*

Background: Many blast-injured Veterans have listening difficulties that cannot be attributed to elevations in pure-tone thresholds, a condition termed “functional hearing difficulties” (FHDs). The existence of FHDs is well appreciated clinically, with 99.5% of audiologists reporting an FHD patient encounter in a recent survey. The empirical literature, while indicative of a reliable connection between self-reported FHD and objective measures of auditory processing, has yet to pin down the mechanisms involved. This presentation is the synthesis of a five-year project whose aims were to: (i) determine whether blast exposure is associated with changes in temporal processing and speech recognition in background noise (SIN) among Veterans with self-reported FHDs; and (ii) determine whether FHDs reflect a genuine auditory deficit as opposed to

generalized cognitive or psychological sequelae of blast exposure. Experiments were modeled on prior work showing auditory deficits in middle aged adults with normal hearing, thus casting blast exposure as a form of accelerated auditory aging.
Methods: A group of more than 80 Veterans (aged 25-60) with variable histories of blast exposure and other mild head injuries was recruited. All had pure-tone average thresholds (0.5, 1, 2, 4 kHz) of 35 dB HL or better. Subjects completed a battery of temporal processing, SIN, and cognitive tests, as well as diffusion tensor imaging (DTI) and a functional magnetic resonance imaging (fMRI) SIN task. A continuous measure of lifetime exposure to blast and other mild head traumas (the Blast and Blunt Trauma Index or BBTI) was obtained. Regression models examined the association between BBTI and the various outcome measures.
Results: BBTI was associated with poorer temporal processing and SIN performance. Additionally, BBTI was associated with widespread changes in DTI metrics, suggestive of spatially heterogenous reductions in brain white matter connectivity. However, DTI metrics in one brain region, the acoustic radiations linking subcortical and cortical auditory centers, were specifically associated with SIN performance. Finally, BBTI was associated with changes in the balance of activation in cortical networks recruited in response to background interference during the fMRI SIN task. Throughout, outcomes were controlled for audiometric thresholds, age, PTSD symptom severity, and cognitive ability. In many cases, additive (i.e., independent) or interactive effects of age and BBTI were observed.
Conclusions: FHDs in blast exposed Veterans are reflected as deficits in temporal processing and SIN that can be traced to changes in the white matter linking subcortical and cortical auditory centers. These deficits are not merely due to cognitive or psychological changes yet result in suboptimal recruitment of compensatory cognitive networks during speech processing, suggesting that cognitive resources may be "effectively" diminished in Veterans with FHD. Normal aging exerts similar effects, providing empirical support for accelerated auditory aging in blast exposure. Supported by VA RR and D IK2RX002702 to JHV.

Peripheral and Central Auditory Effects From Continuous Aircraft Carrier Noise Exposure at Moderate Sound Levels

Fernando Aguilera de Alba*[1], Isabella Huddleston[1], Elizabeth Jensen[1], Michael Heinz[1]

[1]*Purdue University*

Background: In 2024, the Veterans Benefits Administration reported that tinnitus and hearing loss are some of the most prevalent service-connected disabilities, accounting for 12% of all veteran compensations. Service members are often exposed to varying types of damaging sounds, which may be present continuously (e.g., aircraft carriers) or just briefly (e.g., improvised explosive devices, IEDs). It is imperative to understand how exposure to different sounds affect auditory processing, especially at moderate sound levels considered to be non-damaging.
Methods: Awake chinchillas (n = 18, 9 female) were exposed to noise mimicking aircraft-carrier conditions experienced by U.S. Navy service members. Animals were exposed for four consecutive weeks (40 hours/week) at 87.5 dBA using chinchilla middle-ear absorbance to determine sound-level weighting. Auditory assessment was performed at baseline and 1, 2, and 4 weeks from the start of noise exposure using the following measures: tympanometry, wide-band middle-ear muscle reflex (WB-MEMR), otoacoustic emissions (OAEs; swept distortion product,

DP; swept stimulus frequency, SF; transient evoked, TE), auditory brainstem response (ABR), and envelope frequency response (EFR) to modulated sounds. Sedated measures (ABR and EFR) were performed under anesthesia using xylazine (2 mg/kg) and ketamine (20 mg/kg) while awake measures (tympanometry, WB-MEMR, and OAEs) were collected with the animals restrained and fully conscious.
Results: DPOAEs: Reduced amplitudes at mid-to-high frequencies (5-20 dB shift at 2-12 kHz) as soon as 1-week post-exposure with no sign of recovery or worsening up to 4 weeks. SFOAEs: Progressive amplitude reduction at mid-to-high frequencies (5-15 dB shift at 3-8 kHz) with no recovery. TEOAEs: Mixed effects were observed across all frequencies. WB-MEMR: Absorbed power was progressively reduced up to 2 weeks post-exposure, but partially recovered by week 4. ABR: Hearing thresholds were elevated across all frequencies resulting in acute mild hearing loss with no signs of recovery. EFR: Reduced neural coding of middle harmonics was evident at 1-week post-exposure with no signs of recovery.
Conclusions: Our multi-metric auditory framework has highlighted potential hearing deficits and how these deficits develop over time due to continuous noise exposure up to 4 weeks. Peripheral and central effects appeared within one week of noise exposure and persisted up to 4 weeks. These findings will help elucidate the relative contributions of peripheral and central damage to the overall development of hearing loss due to continuous noise exposure.

Prophylactic Nimodipine for Hearing Preservation in Vestibular Schwannoma: A Retrospective Cohort Study

Clifford He*[1], Douglas Bennion[1], Abhishek Bhatt[1], Michael Brandel[1], Joshua Lee[2], Marc Schwartz[1], Rick Friedman[3]

[1]*University of California, San Diego,* [2]*New York Medical College,* [3]*University of California, San Diego Medical Center*

Background: Hearing loss affects millions worldwide, significantly diminishing quality of life and increasing the risk of social isolation, depression, and cognitive decline. Preserving hearing in patients undergoing treatment for conditions such as vestibular schwannoma remains a critical clinical challenge. Standard treatments often result in low rates of complete or partial hearing recovery, highlighting the need for innovative approaches.
Nimodipine, a calcium channel blocker traditionally used for subarachnoid hemorrhage, has shown potential in protecting cranial nerve function and preserving hearing during vestibular schwannoma surgery. This study aimed to evaluate the efficacy of prophylactic nimodipine in hearing preservation within a patient cohort at our single-center tertiary care hospital. Additionally, we explored the relationship between nimodipine dosage and hearing outcomes.

Methods: A retrospective analysis was conducted on a cohort of patients diagnosed with vestibular schwannoma who underwent hearing preservation surgery via retrosigmoid or middle fossa approaches from November 2017 to August 2024, excluding NF2 patients. The study included 68 control and 309 nimodipine-treated patients, matched for demographics, tumor characteristics, and pre-surgical hearing levels. Hearing outcomes were assessed using word recognition scores and pure tone audiometry. Statistical analyses, including logistic regression, were performed to determine the impact of nimodipine on hearing preservation and to assess dose-response relationships.

Results: We found that for patients treated with nimodipine starting immediately post-operatively and continued for any duration, the odds of preserving word recognition were increased by 1.5 (95% CI 0.9, 2.6), and the odds of preserving pure tone audiometry were increased by 1.2 (95% CI 0.7, 2), indicating that prophylactic nimodipine has a therapeutic effect on hearing preservation.
We then performed a dose-response analysis, revealing a positive correlation between higher nimodipine doses and improved hearing preservation. There was a significant (p LESS THAN 0.005) protective effect of nimodipine, with the multiple logistic regression analysis indicating a coefficient of 0.018 increase in WRS and a 0.012 increase in pure tone audiometric average as a function of increasing the number of doses. These results suggest that nimodipine administration may enhance hearing preservation rates in post-surgical vestibular schwannoma patients, potentially improving patient outcomes.

Conclusions: This study, the largest of its kind to date, provides compelling evidence for the efficacy of prophylactic nimodipine in preserving hearing in patients undergoing vestibular schwannoma surgery. While our results highlight nimodipine's potential clinical benefits, the retrospective design limits the ability to assess long-term hearing outcomes. Future prospective studies are necessary to confirm these findings and further elucidate nimodipine's role in otologic surgery. Our findings advocate for the consideration of nimodipine in clinical protocols aimed at preserving hearing and improving the quality of life for patients at risk of hearing loss.

Assessing the Cognitive Decline Post Hearing Loss

Sriram Hemachandran*[1], Jesus Maldonado[1], Anthony Ricci[2]

[1]*Stanford School of Medicine,* [2]*Stanford University*

Background: Hearing loss exacerbates cognitive decline in humans (Livingston et al., 2024). Drawing causal links with human data is difficult because there are multiple underlying etiologies for hearing loss and there are multiple factors that impact cognitive outcomes including genetics, social and environmental. Previous work developed a mouse model using the human diphtheria toxin receptor to selectively and uniformly target hair cells for ablation (Tong et al., 2015). Cognitive function was tested in mice using an 8-arm radial maze (8ARM) that has no auditory cues and found that deafened mice showed a dramatic deficit in working memory (Qian and Ricci, 2020). Despite this very uniform and complete deafening, there was a large variance in cognitive function loss (Qian and Ricci, 2020).
Methods: We are creating a battery of cognitive tests to better investigate the variance in deficit induced by hearing loss by allowing for repeated measurements in the individual animals. For cognitive assessment we are using Y-maze where we will measure spontaneous alternation (inherent tendency of the mice to explore new places, a kind of short-term memory) and Novel object recognition test (NORT), where mice will be exposed to a familiar object and then will be presented with a novel object. The mice tend to explore the novel object more than the familiar object it was used to. This experiment will test the recognition memory. The experiments were performed on 2-month-old c57BL6 mice (WT and Pou4f3DT). We selectively ablate the IHCs in the mice by injection DT through various modes of administration (IP and posterior semicircular canal (PSCC)). We perform the cognitive battery and measure the auditory brainstem response

(ABR) before and 1 month after DT administration. Finally, the mice go through 8ARM (Qian and Ricci,2020) and histology to evaluate the level of hair cell loss.
Results: 1. The mice injected with DT (IP and 5ng DT through PSCC) showed a significant decrease in alternation % (Y-maze) compared to sham surgery wild type mice.
2. The mice injected with DT (1ng and 5ng DT through PSCC) showed a significant decrease in discrimination % to novel objects compared to sham surgery and wild type mice.

Conclusions: The results clearly show a decrease in mice cognition post IHC ablation using DT. This result implicates an underlying biological cause for cognitive variance post DT-induced deafening. We are presently pursuing where the variance arises, is it genetic or environmental? Also, we are trying to identify markers, perhaps in the auditory evoked potential responses that might be predictive toward identifying the expected level of cognitive deficit.

Impact of Hearing Loss and Cochlear Implantation on Attentional Selection in Older Adults

Alex Tu*[1], Gennadiy Gurariy[2], Shannon Walsh[1], Kristin Kozlowski[1], Samiah Ziadeh[3], Sarah Mleziva[1], Adam Greenberg[3], Michael Harris[1]

[1]*Medical College of Wisconsin,* [2]*Ohio University,* [3] *Medical College of Wisconsin and Marquette University*

Background: Globally, the aging population is projected to double by 2050, increasing the prevalence of age-associated conditions such as cognitive decline and dementia. Hearing loss has been identified as a modifiable risk factor for dementia, and if addressed, has the potential to significantly reduce global incidence of dementia. However, the mechanism underlying this relationship remains unsubstantiated. Attention is fundamental to all neurocognitive processes and an excellent proxy by which to study cognition generally. This study aims to explore the impact of hearing loss and hearing rehabilitation through cochlear implants (CIs) on non-auditory attentional selection in older adults.
Methods: Thirty-three participants were recruited: 11 with normal hearing (less than 25 dB hearing loss), 12 with untreated post-lingual deafness (CI candidates), and 10 unilateral CI users within one year of implantation. Participants were excluded if they had known cognitive decline or dementia. Attention was assessed via the Attentional Network Test (ANT), a non-verbal assessment of attentional control in the visual domain that resolves attentional control into the key attentional sub-processes according to the three-network model of attention: alerting (temporal), orienting (spatial), and distractor filtering (executive control).
Results: In our sample, CI candidates and CI users utilized alerting (temporal) cues significantly less often than normal hearing controls, although this difference was only significant between CI candidates and controls. In contrast, CI candidates overutilized orienting (spatial) cues relative to normal hearing controls, although this difference was only significant between CI users and controls. No significant differences were noted across the three groups in distractor filtering (executive control).
Conclusions: Attentional selection and efficiencies differ between older adults with and without hearing loss. Differential utilization of spatial cues preferentially over temporal cues is demonstrated in participants with hearing loss with or without hearing rehabilitation compared to controls. Two hypotheses are discussed regarding the nature of these observations.

The Effects of Noise-Induced Hearing Loss on Auditory Decision-Making

Madeline Berns*[1], Genesis Nunez[1], Xingeng Zhang[1], Anindita Chavan[1], Klavdia Zemlianova[2], Marissa Calvano[1], Todd Mowery[1], Justin Yao[1]

[1]*Rutgers University,* [2]*Columbia University*

Background: Loud noise exposure is a leading cause of permanent hearing loss. Individuals with noise-induced hearing loss (NIHL) suffer from speech comprehension deficits and experience impairments to cognitive functions such as attention and decision-making. Here, we tested whether a specific sensory deficit, NIHL, can directly impair auditory cognitive function. We additionally assessed whether NIHL-related impairments could be reflected by auditory cortical (AC) processing deficits.

Methods: Adult gerbils (N=7) were trained to perform an auditory decision-making task. Gerbils initiated each trial by nose-poking at one end of a test cage and then discriminated between slow (LESS THAN 6.25-Hz) and fast (GREATER THAN 6.25-Hz) presentation rates of amplitude-modulated (AM) noise by approaching one of two food trays. Permanent hearing loss was induced by exposing the gerbils to loud noise (~120 dB SPL) during a 2-hour session. Hearing sensitivity was measured by recording auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs). After inducing NIHL, task performance was tested at sensation levels comparable to those before noise exposure. Performance was analyzed using a drift diffusion model (DDM) to assess the impact of NIHL on auditory evidence accumulation. Neural activity was recorded from the AC while gerbils performed the task. AC sensitivity was assessed with single-unit and population-level decoding analyses based on stimulus identity (AM rate), perceptual categories (slow vs. fast AM rates), and choice (left vs. right).

Results: Loud noise exposure permanently decreased hearing sensitivity by elevating ABR and DPOAE thresholds ~40 dB SPL for clicks and tones (p LESS THAN 0.0001). We found that following NIHL, gerbils exhibited diminished perceptual acuity (p LESS THAN 0.0001), impaired attentional focus (p LESS THAN 0.0001), altered choice bias (p LESS THAN 0.05), and reduced evidence accumulation speed (p LESS THAN 0.0001) and non-decision time (p LESS THAN 0.05). Video tracking analysis demonstrated that NIHL also altered decision-related motor responses (p LESS THAN 0.0001). Preliminary analyses of neural recordings showed that after NIHL, AC neurons could accurately distinguish AM rates and represent perceptual categories of slow versus fast AM rates.

Conclusions: Our results suggest that NIHL impairs auditory decision-making by decreasing the speed at which sensory evidence is accumulated and increasing the time required to encode the stimulus and execute a choice-related motor response. We also found that sensory and categorical variables are strongly represented in the AC even after NIHL, which is in stark contrast to decreased task performance following NIHL. Altogether, these results suggest that NIHL disrupts the cognitive processes underlying sensory decision-making in a manner that cannot be attributed solely to AC function.

Using Large-Scale Brain Recordings and Deep Learning to Engineer Optimal Hearing Aids

Fotios Drakopoulos*[1], Lloyd Pellatt[1], Yiqing Xia[1], Shievanie Sabesan[1], Andreas Fragner[2], Nicholas Lesica[1]

[1]*University College London,* [2]*Perceptual Technologies*

Background: While the auditory system is highly non-linear and the effects of hearing loss are complex, the design of current hearing aids is based on simplistic notions of auditory function and dysfunction. As a result, standard amplification strategies are of little benefit in many important real-world contexts such as understanding speech in adverse conditions or listening to music.
Methods: We recently used large-scale neural recordings from the inferior colliculus (IC) of normal-hearing and noise-exposed gerbils to develop deep neural network (DNN) models of central auditory encoding (ICNets). Our models provide highly accurate predictions of high-resolution neural activity across hundreds of units in response to a wide range of sounds. Here, we use ICNets to train sound-processing DNN models (AidNets) that restore impaired neural activity back to normal. Since our ICNets accurately describe neural activity before and after hearing loss, AidNets learn to compensate for all peripheral and central auditory changes (up to the level of the IC) that take place after noise exposure, without having to rely on any prior assumptions.
Results: We train and evaluate AidNets that target noise-exposed gerbils with a range of different hearing loss profiles. We show that our AidNets outperform state-of-the-art hearing aid strategies in restoring the neural activity of individual noise-exposed gerbils back to normal, when assessed by a range of metrics such as correlation and log probability. We also show that our AidNets outperform the state-of-the-art in restoring simulated speech recognition back to normal levels.
Conclusions: These initial results demonstrate the potential for using large-scale brain recordings and deep learning to develop improved hearing aid processing strategies. We aim to evaluate the developed strategies with hearing-aid users who are not satisfied with the existing strategies (moderate and severe hearing loss) as well as listeners who are not yet served at all (mild or hidden hearing loss).

Work funded by EPSRC EP/W004275/1 and MRC MR/W019787/1.

Neurotransmitter Concentration Levels in the Auditory Cortex Correlate With Subjective Hearing Impairment in Age-Related Hearing Loss

Stephanie Rosemann*[1], Christiane M Thiel[1]

[1]*Carl von Ossietzky Universität Oldenburg*

Background: Gamma-aminobutyric acid (GABA) and glutamate are major inhibitory and excitatory neurotransmitters in the mammalian brain, respectively, and play important roles in the auditory system. Previous research using magnetic resonance (MR) spectroscopy in humans demonstrated decreased GABA concentrations in auditory cortex in older compared to younger healthy individuals. Further, decreased GABA and glutamate concentrations in the auditory cortex were correlated with age-related hearing loss as well as with speech-in-noise perception. The aim of the current study was to investigate the relationship between age, neurotransmitter

concentrations (GABA, glutamate) in the auditory cortex and both objective and subjective hearing measures.
Methods: The study will consist of two participant groups. Young participants (aged 18-35 years) with normal hearing abilities (defined as 20 dB HL or better for all frequencies between 125-8000 Hz) and older participants (aged 50-75 years) with normal hearing abilities and varying degrees of age-related hearing loss. Exclusion criteria for participation are left-handedness, asymmetrical hearing abilities, low-frequency hearing loss (LESS THAN 1kHz), tinnitus, prior use of hearing aids, previous or current psychiatric or neurological disorders. Our final sample size aims at n=60 older participants and n=20 younger participants. Those individuals undergo MR spectroscopy, pure-tone audiometry, speech-in-noise perception (Oldenburg sentence test) and fill in questionnaires assessing daily life listening effort and subjective hearing impairment (Hearing Handicap Inventory).
Results: Data collection is still ongoing for the older participants. Preliminary data analysis on n=22 young participants and n=27 older adults indicate significant correlations between both GABA and glutamate concentration levels in the auditory cortex and subjective hearing impairment (corrected for age). The correlation with GABA concentration levels was negative while the correlation with glutamate concentration levels was positive.
Conclusions: The results indicate that subjective hearing impairment is associated with inhibitory and excitatory output of the auditory cortex to other brain regions. Those relationships were found in young and older adults and might serve as an early diagnostic marker for mild hearing loss. The underlying neurotransmitter imbalance in the auditory cortex might have further implications for neural activity as well as connectivity with other brain regions. Findings may also be relevant for a potential treatment in age-related hearing loss.

Podium 5: Immunology: Function, Dysfunction, and Treatment

Moderators: Hainan Lang and Gisselle Jimenez

10:30 a.m. - 12:30 p.m.

Ocean Ballroom 9 - 12

Bulk RNA and ScRNA-Seq Analyses Identify Macrophages as Major Effectors of Fibrosis After Cochlear Implantation in Rat

Frederic Venail*[1], Mélissa Urbain[2], Jholy de la Cruz Talaverano[3], Jerome Bourien[4], Adrien Caplot[2], Farida Djouad[3], Jean Luc Puel[5]

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Background: Cochlear implantation often leads to fibrosis, particularly due to immune reactions and macrophage involvement. Fibrotic responses are linked to trauma from electrode insertion, foreign body reactions, and electrical stimulation. Understanding the role of gene expression and

macrophage behavior is crucial for developing targeted treatments to mitigate fibrosis and improve cochlear implant outcomes.
Methods: This study utilized RT-qPCR and scRNA-seq to analyze gene expression and immune cell activity in cochlear tissues following implantation in a rat model. Key experiments focused on assessing macrophage activity and gene expression over time, comparing insertion trauma, foreign body reaction, and electrical stimulation conditions.
Results: RT-qPCR data revealed significant upregulation of inflammation-related genes, particularly those associated with macrophages, in response to electrical stimulation. scRNA-seq identified increased macrophage infiltration and activation in the fibrotic tissues, with specific pro-inflammatory markers. Macrophage depletion and modulation of their function significantly reduced fibrosis.
Conclusions: Macrophages play a pivotal role in the development of cochlear fibrosis post-implantation. Targeting macrophage activity or altering their inflammatory response offers a promising strategy to prevent fibrosis, which could enhance the efficacy of cochlear implants and preserve residual hearing.

Complement Component C3 Accumulates in the Cochlea of CBA/CaJ Mice With Noise-Induced Hearing Loss

Zixu Guo*[1], Benjamin Seicol[2], Katy Garrity[1], Mina Shenouda[1], Shengyin Lin[1], Ruili Xie[1]

[1]*The Ohio State University,* [2]*Johns Hopkins Medicine*

Background: Noise-induced hearing loss (NIHL) is characterized by dysfunction and pathological changes of the cochlea, including the loss of outer hair cells (OHC). Clearance of damaged OHCs is thought to be carried out by supporting cells and by cochlear macrophages. However, the underlying mechanisms that regulate OHC clearance remain largely unclear. The complement system is part of the innate immune system and plays important roles in removing cellular debris and apoptotic cells throughout the body, however, complement activation in the cochlea remains understudied. Complement component C3 can initiate the alternative complement cascade resulting in the phagocytosis of dead or dying cells. Recent studies suggested a potential role of the complement system in the proper functioning of the auditory system. Additionally, C3 gene expression increases in mouse cochlea during aging. Therefore, we sought to investigate whether C3 protein exists in the cochlea and if it increases after noise damage.
Methods: To understand whether C3 protein is present or elevated in NIHL, we investigated C3 using immunohistochemistry in the cochlea of CBA/CaJ mice that were noise exposed to an octave band noise (8–16 kHz) at 112 dB SPL at 9 weeks of age. Mice were sacrificed and examined at various post-exposure times. Cochleae were whole mount prepared or cryo-sectioned, and immunostained using antibodies against C3, along with additional markers to probe the potential mechanisms by which complement activation is impacting sensory hair cells.
Results: We found that C3 protein colocalized with varies noise-damaged structures in Organ of the Corti in mice after noise exposure, especially in the OHC area. Importantly, the accumulation of C3 aggregates appeared in the OHC layer where OHCs were lost.
Conclusions: Our results suggest that C3 might be involved in clearing damaged OHCs and OHC synapses potentially by supporting cells. We conclude that the complement system plays important roles in the clearance of damaged tissue in the cochlea after noise trauma.

Dectin-1 Dysregulation: A Potential Contributor to Age-Related Demyelination in the Auditory Nerve

Shelby Payne*[1], Jamie L Barth[1], Hainan Lang[1]

[1]*Medical University of South Carolina*

Background: Age-related hearing loss (ARHL) is a growing public health concern, with two-thirds of adults over 65 experiencing some hearing difficulty (e.g., poor speech recognition in noise). One contributing factor to ARHL is auditory nerve (AN) dysfunction, including age-related demyelination. Myelin, which ensheathes the AN fibers, ensures efficient saltatory conduction along the AN and preserves the synchrony of action potentials required for auditory processing. While previous studies show pathological alterations in myelin structure and function in the aged brain, the mechanisms behind age-related demyelination of the AN are still unclear. Additionally, macrophage/microglia activation has been linked to demyelination in the aged brain. In demyelinating conditions, disease-associated microglia (DAMs) may be associated with an activated and inflammatory phenotype contributing to pathogenesis. A potential contributor to this demyelinating phenotype is dysregulation of Dectin-1 (Clec7a), a C -type lectin receptor, which controls macrophage/microglia activation and phagocytosis in the brain, particularly in neurodegenerative diseases. Our preliminary data show that in aged human AN, where there is evidence of demyelination, there are increased numbers of macrophages/microglia, suggesting that inflammation may play a role in the progression of demyelination and AN functional declines. Here, this study aims to address the hypothesis that increased activation of Dectin-1-expressing macrophages/microglia contributes to age-related AN demyelination and ARHL.
Methods: We used bulk-RNA sequencing of the AN in young and aged CBA/CaJ mice to identify age-related changes in expression of genes related to immune system function, particularly macrophage/microglia activity. Significantly upregulated genes were validated with immunohistochemistry in young and aged CBA/CaJ mice and aged humans ANs to characterize their activity in the context of age-related AN demyelination.
Results: RNA sequencing revealed significant upregulation of a cluster of DAM-linked genes, including Clec7a, Lgals3, Cst7, Itgax, and Apoe. We validated the presence of Dectin-1 (Clec7a) in macrophages/microglia of the AN in mice as well as humans. Additionally, a qualitative assessment suggested an increased presence of Dectin-1-expressing macrophages/microglia in the AN in aged mice compared to young mice, though analysis is still ongoing.
Conclusions: The identification of an upregulation of the DAM molecule Dectin-1 in the aged AN suggests that a subset of macrophages/microglia with a specific activation state may be an important contributor to myelin degeneration and auditory functional declines in ARHL.

Endothelial Cells and Iba1-Positive Cells Under the Organ of Corti Constitute the Immunological Unit for Cochlear Hair Cells

Yushi Hayashi*[1]

[1]*Nippon Medical School*

Background: The immune function of the cochlea has been received less attention because of the characteristic environment created by the blood-labyrinthine barrier, to which systemic

immune cells have limited access. However, recent studies have revealed that cochlear resident macrophages are distributed throughout various regions of the cochlear duct, except for the organ of Corti. This led to the assumption that the organ of Corti was immunologically vulnerable, but we have shown that cochlear supporting cells play a significant immunological role in affecting cochlear hair cells (Hayashi et al., 2013, 2020, 2021, 2024). Here, we investigate whether immunological systems other than supporting cells function in the organ of Corti.
Methods: Given the existence of a limited number of ionized calcium-binding adapter molecule 1 (Iba1)-positive cells under the organ of Corti (Hayashi et al., 2020), we evaluated the precise anatomical location of these cells using immunohistochemistry. Also, we used explant culture system of the postnatal mouse organ of Corti. After dissected out of P2 mice, the organ of Corti was incubated overnight for stabilization. Then aminoglycoside was administered to the explants. Aminoglycosides are known to harm hair cells selectively. The explants were incubated for up to 24 h, followed by histological or gene expression analysis.
Results: The immunohistochemistry data showed that Iba1-positive cells were located along the vessel running under and along the organ of Corti from the base to the apex. The distances between the vessel and Iba1-positive cells got smaller when aminoglycoside was administered to the organ of Corti, indicating the interaction between these cells and endothelial cells constituting the vessel. Also, incorporation of aminoglycoside by Iba1-positive cells and endothelial cells was observed, which affected hair cell death induced by the aminoglycoside. Based on these results, we performed pharmacological depletion or activation of Iba1-positive cells and observed phenotypical changes in endothelial cells.
Conclusions: It is conceivable that endothelial cells and Iba1-positive cells under the organ of Corti constitute the immunological unit for cochlear hair cells, which functions in an emergency state such as exposure to ototoxic aminoglycoside. We are currently analyzing the mechanism of interaction between endothelial cells and Iba1-positive cells under the organ of Corti.

Role of Immune Cell Trafficking via Cochlear Blood Vessels and the Newly Discovered Cochlear Lymphatics in the Foreign Body Response Following Cochlear Implantation

Muhammad Rahman*[1], Md Ibrahim Razu[1], Shakila Mahmuda Fatima[1], Md Fahad Hossain[1], Md Shuaib Akhter[1], Bryce Hunger[1], Alexander Claussen[1], Young-Kwon Hong[2], Marlan Hansen[1]

[1]*The University of Iowa,* [2]*Keck School of Medicine, University of Southern California*

Background: The inflammatory foreign body response (FBR) following cochlear implantation (CI) has potential negative impacts on CI outcomes, including increased electrode impedances, accelerated loss of residual acoustic hearing, reduced battery life, and in extreme cases, extrusion of the implants. Immune cells including macrophages and lymphocytes have been reported to contribute to the FBR post-CI. In most organs, the immune cells are trafficked to the tissue via blood vessels and return to the bloodstream via the lymphatic vessels forming a classical 'immune surveillance system'. This study aims to investigate the role of immune cell trafficking via cochlear blood vessels and recently discovered cochlear lymphatic network in the FBR post-CI.
Methods: We used four mice strains to investigate the response of cochlear immune cells, blood vessels, and lymphatics to CI: CX3CR1+/GFP (macrophage reporter), Prox1tdTomato-CD11c-

GFP (lymphatic-dendritic cells dual reporter), Prox1tdTomato-CX3CR1+/GFP (lymphatic-macrophage dual reporter) and Prox1tdTomato-PECAM1GFP (lymphatic-blood vessel dual reporter) mice. The left ears of 10-12-week-old mice models were implanted with standard non-functional CI. Following the euthanasia of mice at 28 days post-operatively, harvested cochleae fixed with 4% PFA were processed and cryosectioned at 30μm parallel to the mid-modiolar plane. Sections labeled with antibodies against appropriate markers were used to identify different cell types: CD45, CD3e, MHCII, anti-GFP (to enhance GFP), and PDPN. Confocal images were analyzed using IMARIS image analysis software. Outlines of the scala tympani, Rosenthal canal, and lateral wall for each turn were used to measure the volume of each area. The density of CX3CR1+ macrophages, CX3CR1+MHCII+ (APC), CX3CR1-CD45+ (non-macrophage immune cells), CX3CR1-CD45+ CD3e+ (T lymphocytes), CD11cGFP+ (dendritic cells) were calculated. The volume of blood vessels (PECAM1+) and lymphatic vessels (Prox1tdTomato+, PDPN+) was measured within the scala tympani of the base of the cochlea. The volume of blood vessels and lymphatic vessels were divided by the volume of scala tympani to quantify the blood vessel and lymphatic vessel response to CI. Intra-lymphatic immune cell density was quantified by creating mask for lymphatic vessels and the immune cells.
Results: Post-CI, macrophages, and blood-derived, non-macrophage immune cells are recruited. MHCII+APCs, dendritic cells, and T lymphocytes are recruited suggesting adaptive immune system activation. Lymphatic vessels proliferate into the scala tympani of the implanted cochlea surrounding the implant site and traffic infiltrated dendritic cells. Blood vessels proliferate around the cochlear implant potentially trafficking the blood-derived immune cells.
Conclusions: Our data suggests that following cochlear implantation, lymphangiogenesis, and neoangiogenesis within the cochlea. This is associated with an infiltration of blood-derived non-macrophage immune cells into the cochlea and the trafficking of dendritic cells via the proliferated lymphatics. Two-way traffic of immune cells to (via blood vessels) and away (via lymphatics) from the cochlea supports the involvement of the cochlear 'immune surveillance system' in FBR post-CI.

Radiation Alters Secretion of Proinflammatory Cytokines From Primary Vestibular Schwannoma Cells

Mikhail Marasigan*[1], Olena Bracho[1], Bryan Sousa[1], Michael Ivan[1], Fred Telischi[1], Cristina Fernandez-Valle[2], Christine Dinh[1]

[1]*Miller School of Medicine, University of Miami,* [2]*Burnett School Biomedical Sciences, College of Medicine, University of Central Florida*

Background: Vestibular Schwannoma (VS) is a benign intracranial tumor arising from Schwann cells of the vestibulocochlear nerve. VS develop after inactivating mutations of the NF2 gene and cause hearing loss and dizziness. Recent studies suggest that VS can secrete pro-inflammatory factors that may contribute to tumor growth and hearing loss. However, the VS secretome and impact of radiation on secreted cytokines are largely unknown. In this study, we measure the effect of radiation on the secreted cytokine profiles in NF2-mutant Schwann cells and 3 primary VS cultures.
Methods: NF2-mutant Schwann cells (HS01) and 3 primary VS were cultured in T25 flasks until 90% confluent. Subsequently, Schwann proliferative media was exchanged for maintenance media. The cells were subjected to single dose radiation (0 Gy or 12 Gy) and cultured at 37□C

and 5% CO2 for 48 hours. The supernatant was then collected and analyzed using an 80-cytokine array, per manufacturer's protocol. Chemiluminescence imaging was performed, and protein expression was determined using ImageJ software with microarray plug-in. Expression levels were normalized to positive controls. Cells were also cultivated on 96-well plates at 10,000 cells per well in maintenance media, and viability assays were performed at 48 hours post-irradiation. Data was analyzed using Mann Whitney U tests with Bonferroni correction for multiple comparisons.
Results: HS01 cells secreted high levels of interleukin (IL) 6, IL8, monocyte chemotactic protein-1 (MCP1), brain-derived neurotrophic factor (BDNF), macrophage inflammatory protein-3-alpha (MIP3a), and osteoprotegerin. In HS01 cells, radiation significantly increased growth regulated protein alpha (GROa) and reduced the expression of MCP1, when compared to control. Primary VS secreted high levels of GRO, GROa, IL6, IL8, MCP1, germination-specific cysteine protease 1 (GCP1), insulin-like growth factor binding protein-2 (IGFBP-2), MIP3a, and tissue inhibitor of metalloproteinase (TIMP) 1, and TIMP2. In primary VS, radiation significantly reduced expression of IL8, MCP1, and TIMP2, when compared to control. In HS01 and 3 primary VS, radiation significantly reduced total cytokine expression, when compared to control. We will describe the effect of radiation on the viability of cells at 48 hours.
Conclusions: Both NF2-mutant Schwann and primary VS cells secreted IL6, IL8, MCP1, and MIP3a, which are pro-inflammatory cytokines known to facilitate monocyte recruitment and polarization toward M1 and M2 macrophages. Radiation caused significant reduction in total cytokine expression among the 4 cultures, which may be related, in part, to effect of radiation on cell viability. When analyzing individual cytokines, radiation significantly reduced expression of cytokines, particularly IL8 and MCP1. These findings suggest that VS secrete pro-inflammatory cytokines, and radiation can modulate the secretion of cytokines that regulate the tumor immune microenvironment. Further investigations into the VS secretome and impact of radiation on the tumor immune microenvironment can reveal novel targets for patients with VS.

Spatial Organization of Cochlear Lymphatic Vessels in Murine Model

Md Ibrahim Razu*[1], Muhammad Rahman[1], Shakila Mahmuda Fatima[1], Md Fahad Hossain[1], Md Shuaib Akhter[1], Bryce Hunger[1], Young-Kwon Hong[2], Alexander Claussen[1], Marlan Hansen[1]

[1]*The University of Iowa,* [2]*Keck School of Medicine, University of Southern California*

Background: The lymphatic system circulates lymph, from tissue the circulatory system. The system consists of lymphatic vessels and plexuses, lymph nodes and associated organs; it collects interstitial fluid and immune cells order to recirculate them in the bloodstream. It plays a pivotal role in fluid homeostasis and the 'immune surveillance system'. Although the nervous system has been considered devoid of any functional lymphatic system for many years, recent discoveries of brain lymphatics and lymphatic drainage of cerebrospinal fluid have shifted the paradigm. Lymphatics of some sensory systems, like visual systems, have also been described. Although there is sufficient evidence that the inner ear, or cochlea, harbors a lymphatic vascular system draining interstitial fluids and immune cells, cochlear lymphatics have not been described in detail. The current study is aimed at exploring and describing the possibility of lymphatics in the cochlea.
Methods: 0-12 weeks-old C57B6 and CBA-J backgrounds were used to identify the cochlear lymphatic vessels. To differentiate lymphatics from spiral ganglion neurons (SGNs), Thy1+/YFP

mice, and to differentiate lymphatics from cochlear blood vessels, PECAM1-EGFP mice on C57B6 mice were used. Mice were euthanized, cochlea harvested, and fixed with 4% PFA overnight at 4°C. Excess PFA was rinsed with PBS, cochleae were decalcified with 0.12M EDTA for 3 days on a rocker at 4°C. Decalcified cochleae were used for either cryosections or whole mounts. For cryosections, decalcified cochleae were cryopreserved using increasing concentrations of sucrose (10%-30%), embedded in OCT, and sectioned at 30µm parallel to the mid-modiolar plane. Whole mounts were prepared from decalcified cochlea following protocols previously described. Cochlear cryosections and whole mounts were labeled with anti-podoplanin/anti-PDPN (lymphatic endothelial marker), anti-GFP (to amplify PECAM-EGFP signal), and Anti-myosin VIIa (hair cell marker) antibodies and imaged using a confocal microscope.
Results: A robust lymphatic vascular network, labeled with anti-PDPN was observed in the cochlea: in spiral ganglia, stria vascularis, spiral ligament, along the margins of scala tympani and scala vestibuli and modiolus of cochlea. In the organ of Corti, PDPN+ lymphatic vessels were observed underneath the Myosin VIIa hair cells. Within the bony spiral lamina, PDPN+ lymphatics could be distinguished from Thy1+ SGN fibers. Similarly, lymphatics in the stria vascularis, spiral ligament, and margin of the modiolus could be distinguished from PECAM1+ blood vessels.
Conclusions: Our results suggest the presence of a cochlear lymphatic network in adult murine cochlea. Expectedly, cochlear lymphatics are adjacent to the cochlear blood vessels and located in strategic locations suitable for draining major sensory structures and cochlear fluid. Further investigations using different lymphatic markers is necessary for anatomical and molecular characterization of cochlear lymphatics.

The Time Course of Monocytes Infiltration after Acoustic Overstimulation

Seong Hoon Bae*[1], Seung Ho Shin[1], HaengRan Park[1], Sung Huhn Kim[1], Jinsei Jung[1]

[1]*Yonsei University College of Medicine*

Background: Cochlea macrophages are believed as a regulator of cochlea inflammation and may harbors the potentials to protect hearing functions from many types of injury including acoustic overstimulation. Researchers observed cochlea macrophages increased in 3 – 7 days after acoustic stimulation. However, the exact time course of infiltration and maturation of macrophages from the inflammatory monocytes was not yet concretely discovered. Further, recent studies revealed that not only monocytes but also neutrophils are involved in this process. Therefore, we tried to investigate the time-dependent immune cell infiltration, their transformation, and the involvement of neutrophils in this process.
Methods: Flow cytometry was serially conducted in Cx3cr1+/GFP mice after acoustic overstimulation (control, 1, 2, 3, and 5 days after 120dB/1hr noise exposure) to identify inflammatory monocytes in the cochlea. Immunofluorescence study was conducted to support the results of flow cytometry. RNA-sequencing and quantitative PCR were performed to reveal what kind of genes were involved in this early response.
Results: Inflammatory monocytes infiltrated into the lower portion of the lateral wall within 2 days after acoustic overstimulation (dpn) followed by transformation into macrophages in 3 – 5 dpn by increasing Cx3cr1 expression and losing Ly6C expression. In addition, inflammatory monocytes were aggregated inside the collecting venule only in 1 dpn. Neutrophils were not

observed at any time points. Ccl2 gene is significantly up-regulated as early as 3 hours after acoustic overstimulation.
Conclusions: Inflammatory monocytes, but not neutrophils infiltrated briefly after acoustic overstimulation. From infiltration to transformation into macrophages are finished within 5 dpn. Chemokine up-regulation responds even earlier, within 3 hours after acoustic stimulation.

Poster Session II

1:30 p.m. - 3:00 p.m.

Peninsula Ballroom

SU1. Quantifying Cross-Hearing in Mice FOLLOWING Induction of Unilateral Deafness via Ossiculectomy

Jesus Maldonado*[1], Anthony Ricci[2], Yuxuan Xu[3]

[1]*Stanford School of Medicine,* [2]*Stanford University School of Medicine, Stanford University,* [3]*Hamilton College*

Category: Auditory Nerve
Background: Auditory brainstem response (ABR) signals measure the synchronized activity of the cochlea and brainstem in response to targeted sound stimulation. ABRs are vital for diagnosing hearing impairments. This study investigates the extent and sources of cross hearing in mice. Cross-hearing is when sound presented to one ear is detected by the opposite ear, potentially skewing unilateral testing results. Sources of cross hearing include air or bone conduction, electrical spread, anatomical crossover, and technical issues such as electrode placement or cross talk between channels. Understanding the contributors to cross hearing will improve our ability to interpret and quantify the ABR results.
Methods: We analyzed ABR click data from eight mice, measuring threshold as the appearance of wave 1, wave 1 amplitude and latency, and we performed cross-correlation analysis between L and R ear waveforms in both open-field (OF) and closed-field (CF) conditions. Analysis of amplitude, cross correlation, and latency were done on waveforms recorded at 70 dB. Pre- and post-ossiculectomy removal recordings were compared to assess the influence of cross hearing on each measured parameter.
Results: Wave 1 Threshold Shift: Opposite ear CF ABR recordings showed comparable threshold shifts (30 ± 3 dB to 33 ± 3dB p=0.033) post-ossicle removal compared to OF (33 ± 3 dB to 36 ±4 dB, p=0.002)
Wave 1 Amplitude: OF recordings displayed larger amplitude changes post-ossicle removal (5.1± 1.9 to 3.6 ± 1.2 μV, p=.013), suggesting they are more affected by cross hearing than CF recordings (2.7 ± 0.8 to 2.7 ± 0.9 μV, p=0.985).

Cross-Correlation of Whole Waveform: L and R waveforms exhibited stronger cross-correlation pre-ossiculectomy in OF conditions (0.94 ± 0.06 to 0.77 ± 0.1 , p=0.0001) compared to CF conditions (0.71 ± 0.09 to 0.64 ± 0.14, p=0.211).

Wave 1 Correlation: OF Wave 1 cross-correlation exhibited a significant decrease post-ossiculectomy (0.96 ± 0.03 to 0.83 ± 0.09, p=.0001) compared to CF recordings (0.77 ± 0.16 to 0.81 ± 0.13, p=0.603).

Post-Wave 1 Correlation: OF post-Wave 1 cross-correlation decreased significantly post-ossiculectomy (0.92 ± 0.11 to 0.69 ± 0.13, p=.001), while CF recordings showed no significant changes (0.67 ± 0.16 to 0.56 ± 0.24, p=0.236).

Wave 1 Latency: A significant latency change was observed in OF recordings post-ossiculectomy (1.96 ± 0.07 to 2.1 ± 0.13 ms, p=0.016), but not in CF recordings (1.98 ± 0.09) to 2.1 ± 0.19 ms, p=0.066).

Conclusions: Cross-hearing more prevalent in OF than CF testing
Cross-hearing more prevalent with louder stimulations and has little impact on threshold

Post wave 1 sensitivity implies the anatomical cross-over is less relevant than bone conduction.

Acknowledgements:

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SU2. Auditory Nerve Response Synchrony of Mutant Mice and Diagnosis of Hearing Loss Pathology

Neil Ingham*[1], Clarisse Panganiban[1], Carolyn McClaskey[2], Kelly Harris[2], Karen P. Steel[1]

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Category: Auditory Nerve
Background: As the search for treatments for hearing impairment gathers pace, it is increasingly important to develop reliable tools to diagnose the underlying pathology so that the appropriate treatment method may be delivered. We are using a range of mutant mice to develop non-invasive physiological tests that may be transferrable into a clinical setting. The mice carry different single gene mutations and have known sites of lesion covering the 3 major categories of sensorineural hearing impairment described by Schuknecht and Gacek (1993); Sensory (hair cell dysfunction), Metabolic (stria vascularis dysfunction), and Neural (auditory neuron defects). In our previous work presented at this meeting, we have highlighted the difficulty in distinguishing strial hearing impairment from other pathophysiology.
Methods: Following recordings of auditory brainstem responses (ABR) from ketamine/xylazine-anaesthetised hearing-impaired mutant and normal hearing littermate control mice, we have analysed single trial responses to generate Inter-Trial Coherence (ITC) of the ABR (McClaskey et al 2020), a measure of neural synchrony over the time course of the response. Following extraction of the maximal ITC value in the ABR wave 1 time window, reflecting the neural synchrony of the auditory nerve, and measurement of peak amplitude of the first positive peak of

the grand average ABR, we have used an unsupervised machine learning approach to differentiate responses from mice with different primary sites of hearing loss pathology.
Results: ITCmax and peak amplitude measurements from ABRs evoked by 12, 18 and 24 kHz tone pips, presented at 90 dB SPL, were split into clusters using k-means and visualised by principal component analysis (implemented in Matlab R2023a). A relatively simple 2 parameter (ITCmax, Wave 1 amplitude), 2 cluster analysis proved to give a good separation of responses from mice carrying a metabolic hearing impairment from those with a sensory or neural deficit. Over 90% of responses from mice with a sensory or neural hearing impairment fell into the same k-means cluster #1. Interestingly, mice with normal hearing clustered along with the strial dysfunction animals, suggesting that despite their elevated ABR thresholds, mice with a metabolic hearing impairment may maintain good auditory nerve synchrony and wave 1 peak amplitude. Over 79% of control responses from normal hearing mice, and over 81% of responses from mice carrying a strial / metabolic hearing impairment fell into the opposite k-means cluster #2. Inclusion of additional parameters (such as wave 1 peak latency, or ABR threshold) or more clusters did not improve the success rate in differentiating strial pathology from neural or sensory pathology.
Conclusions: Inter-Trial Coherence measures of auditory nerve discharge synchrony may prove to be a useful non-invasive measurement tool to aid in differential diagnoses of hearing loss pathology.

SU3. Myelin Ultrastructure in Mice With Targeted Deletion of Esrrg in the Inner Ear

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Category: Auditory Nerve
Background: Estrogen-related receptor gamma (ESRRG) is one of three highly homologous genes that constitute the NR3B subset of the nuclear receptor superfamily. Previously, we showed that genetic variation in ESRRG is a sex-specific risk variant for susceptibility to age-related hearing loss in women. In recent investigations from our laboratory, we have shown that targeted disruption of Esrrg in mouse inner ear (Esrrgtm1d/tm1d/Sox10-Cre) leads to an early onset hearing impairment and a phenotype characteristic of an auditory neuropathy. A key feature of the auditory neuropathy is the appearance of truncated myelinated nerve fibres. Here, we describe a detailed investigation conducted to characterise the myelin ultrastructure in Esrrg-mutant mice.
Methods: 16 mice aged between postnatal day 57-65 were used in this study. There was a total of 7 Esrrg-mutant mice (females n = 4, males n = 3) and 9 littermate controls (females n = 4, males, n = 5). Cochleae were fixed in 2% PFA, 2.5% glutaraldehyde in 0.1M cacodylate buffer (with added calcium) for 2 hours at room temperature. Fixed cochleae were then decalcified in 4% EDTA (pH7.4) in 0.1M cacodylate buffer for 48 hours at 4°C, and post-fixed in 1% buffered osmium tetroxide. After post-fixation, cochleae were embedded in TAAB 812 hard resin and prepared for TEM imaging on a JEOL 1400Flash operating at 120kV.
Semi-thin sections at 500nm were taken on an ultramicrotome to clarify the depth and appearance of the mid-modiolar cut. Samples that did not display the classic mid-modiolar

orientation with intact basal, mid and apical coils were excluded. TEM images of the myelinated auditory nerve fibres were acquired in the osseous spiral lamina in the apical coil at the closest available region to the habenula perforate.

Metrics for g-ratios and myelin thickness were collected for each sample using Fiji software. The thickness of the myelin sheath was defined by measuring the width of the myelin layer perpendicular to the myelin lamellae. The number of individual myelin lamella layers was also recorded.

Results: 5 of the 16 samples did not meet our inclusion criteria and were excluded from further analysis.
In the remaining samples, we found axon diameters were significantly reduced in Esrrg-mutant mice compared to littermate controls. Esrrg-mutant mice also displayed slightly reduced g-ratios suggesting the presence of thicker myelin sheaths. However, measuring the width of the myelin thickness at greater magnification and counting the individual myelin lamella layers revealed a significant reduction in myelin thickness in Esrrg-mutant mice in conjunction with a reduced number of myelin sheets.

Conclusions: Esrrg-mutant mice display thinner myelin sheaths and counting the number of individual myelin lamella layers in conjunction with measuring the width of the myelin layer may be more sensitive to small changes in myelin thickness compared to g-ratios.

SU4. Ouabain Ototoxicity as a Model for Auditory Neuropathy in Guinea Pigs

Diane Prieskorn[1], Lisa Beyer[1], Rami Skaliter[2], Dana Hayoun Neeman[2], Ofer Wiser[2], Olga Mizrahi[2], Jennifer Bahr-Davidson[2], Teresa Jones[2], Yehoash Raphael*[1]

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Category: Auditory Nerve
Background: Animal models for auditory neuropathy are useful for testing cell-based therapies for replacing lost spiral ganglion neurons. In models where inner hair cells are preserved, the contribution of transplanted cells can be tested using acoustic stimulation, whereas models with no hair cells are useful for testing function with a cochlear implant. Guinea pigs are of interest because they are commonly and successfully used for auditory research and cochlear implant research. The goal of this on-going project is to compare two ouabain deafening paradigms and their effect on hair cell and auditory nerve survival in guinea pigs.
Methods: Hartley albino guinea pigs were given 5 µl of 7.5 mM or 10 mM ouabain, unilaterally, infused through the round window membrane into the perilymph of the scala tympani. Ouabain-soaked gelfoam was then placed on the round window. In the low dose group, a baseline pure tone auditory brainstem response (ABR) of the left ear was measured at 4, 8 and 16 kHz. Two weeks post-ouabain, a post-deafening ABR was measured with simultaneous 50 dB white noise masking of the contralateral ear.
The high dose ouabain group was processed for histology 1 month after the ototoxic insult. The lower dose animals were processed at the 17-day timepoint. Temporal bones were fixed in

paraformaldehyde, decalcified, embedded in resin and sectioned for light microscopy at a near-mid-modiolar plane.

Results: Animals treated with both doses of ouabain developed vestibular signs such as brief nystagmus and torticollis. In the high dose group, histology showed a near complete loss of all hair cells and a near complete loss of auditory nerve throughout the cochlea. Histology in the lower dose group showed that many outer hair cells were missing, but inner hair cells were present in all cochlear turns. While many spiral ganglion neurons were missing, several cells remained in every turn. These data corroborate ABR results, which showed that in this lower dose group, threshold shifts from baseline averaged 45 dB or greater across all frequencies.
Conclusions: We determined that infusion of ouabain into perilymph of guinea pig at a concentration of 7.5 mM is useful for auditory neuropathy studies involving implantation of neural progenitors, which will be the next step of this study. The lesion leaves behind a small number of neurons that can provide a scaffold for path finding into the cochlear nucleus and also towards surviving inner hair cells. The higher dose appeared more suitable for studies where cochlear implants are combined with cell transplantation, because inner hair cells do not survive.

Support: Lineage Cell Therapeutics, Inc.

SU5. Peripheral Auditory Impacts of Alzheimer's Disease in Cochlear-Implanted Transgenic Mice

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Category: Auditory Nerve
Background: Alzheimer's disease (AD) is the most common form of dementia and one of the greatest public health concerns worldwide. While no cure exists, hearing loss represents the largest modifiable risk factor. Cochlear implants (CIs) are one option for hearing rehabilitation, though dementia may complicate outcomes due to potential cognitive decline or peripheral neural involvement. Few studies have explored the impacts of AD on the peripheral auditory system with cochlear implants and have often been confounded by coincident sensory hearing loss. Therefore, this study aims to elucidate the role of AD in peripheral auditory status using transgenic AD mouse models that are less susceptible to sensory hearing loss. We hypothesize that AD mouse models will demonstrate more pronounced degeneration of spiral ganglion neurons compared to controls, resulting in impaired neural responses to acoustic and electrical stimulation before and after cochlear implantation.
Methods: Three mouse strains were utilized in this experiment, including a CBA/J wild-type and AD-Tau and 5XFAD transgenic models. Acoustically-evoked auditory brainstem responses (ABRs) and distortion product otoacoustic emissions were elicited with 8k/16k/32k tones for threshold determination. These tests were conducted 3-7 days before implantation to elucidate auditory nerve and outer hair cell function, respectively. Mice were unilaterally implanted and electrically stimulated intraoperatively. Electrically-induced compound action potentials (eCAPs) were elicited using charge-balanced, biphasic square-wave pulses with an interphase

gap (IPG) of 7μs and 42μs to assess auditory nerve function. Input/output (I/O) functions for each IPG were obtained by incrementally adjusting current level to estimate eCAP threshold, N1 latency, maximum amplitude, amplitude-growth function (AGF) slope, and IPG effects for each metric. Refractory recovery functions were also obtained by incrementally adjusting masker-probe interval within a forward masking paradigm, allowing for estimates of absolute and relative refractory periods. I/O functions were constructed by considering peak-to-peak amplitudes against current level. Researchers were blinded during data collection and subjective data analysis (visual detection, peak marker placement).
Results: Eleven mice (1 CBA-J, 6 AD-Tau, 4 5XFAD) between ages 8-123 weeks have been implanted to date. Preliminary analysis suggests a positive relationship between ABR threshold and age within and between transgenic groups. ABR threshold and DPOAE threshold are also positively correlated across the entire cohort. Maximum eCAP amplitude and AGF slope demonstrate negative relationships with age within and between transgenic groups. There do not appear to be substantial sex differences across measurements. Data collection is ongoing and will be presented more robustly in person.
Conclusions: While our present sample is limited, several notable trends across acoustic and electrical measures have been identified, suggesting relationships between Alzheimer's disease and peripheral auditory function in the setting of cochlear implantation. Further data collection to increase sample size, histopathology, and future longitudinal studies will clarify important mechanistic links and potential applications to patient care.

SU6. Middle Latency Responses Assist Hearing Threshold Detection Using Parallel ABR Stimuli

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Category: Auditory Nerve
Background: Frequency-specific auditory brainstem responses (ABR) are used to objectively evaluate hearing thresholds in cases where behavioral methods cannot be completed. A complete set of thresholds can take a long time to record serially in the clinic, but can be sped up by simultaneously presenting all frequencies to both ears using the parallel ABR (pABR) paradigm. The pABR's randomized stimulus timing allows for the simultaneous presentation of stimuli, but has the additional benefit of extending the analysis windows to visualize more of the waveform, such as the middle latency response (MLR). Viewing both the ABR and MLR together may assist threshold selection for waveforms with poorer morphology, such as the broader low-frequency responses. This study aimed to characterize the number of participants with hearing loss who have a visible MLR and to evaluate the usefulness of the MLR for aiding threshold detection during pABR measurement.
Methods: Two-channel ABRs were collected from 70 adults (40 females, 30 males, ages 18-70 years) with various behavioral audiogram threshold configurations who listened to pABR stimuli (0.5, 1, 2, 4, and 8 kHz tonebursts in both ears) at a mean stimulation rate of 40 stimuli / s and at various intensities to find the threshold. ABR-estimated thresholds for each participant were chosen three ways, based on: 1) only wave V presence, similar to what is currently available in

clinical systems; 2) only MLR Na-Pa component presence; and 3) using all information available with the pABR extended window (i.e., both waves V and Na-Pa).
Results: The MLR Na-Pa component was present in the vast majority of participants, particularly for the lower frequency responses (500 and 1000 Hz). For these frequencies, Na-Pa was larger – and consequently more visible – at threshold than wave V. Correlations between ABR-estimated and behavioral thresholds improved when both ABR and MLR were considered together compared to each alone.
Conclusions: The MLR provided additional useful information to more accurately estimate hearing thresholds, especially for harder-to-visualize waveforms at or near threshold. Presence of the later and larger MLR components may also facilitate faster detection of responses, which could provide additional speedup benefits.

SU7. Validating a Novel Online Tool for Non-Stationary Fluctuation Analysis of AMPA Receptor Properties

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Category: Auditory Nerve
Background: AMPA receptors are concentrated in specific regions of synapses called postsynaptic densities (PSDs) where they activate and mediate fast neurotransmission. For spiral ganglion neurons (SGNs) contacting inner hair cells (IHCs), heterogeneity of the composition of PSDs may in part determine their sound response properties. However, the number of AMPA receptors in single SGN PSDs, how they are organized spatially, and how they may differ from one another molecularly within a single synapse are currently unknown.
Nonstationary fluctuation analysis (NSFA) is a statistical process that has commonly been used to infer single channel current and postsynaptic receptor counts in synapses within the central nervous system (CNS). By recording excitatory postsynaptic currents (EPSCs), NSFA method can be used to characterize AMPA receptor properties for SGN synapses. However, though the process is well-defined, NSFA remains a computationally cumbersome task with no known tools allowing for multi-recording analysis of EPSCs.

Here, we report on the design and validation of an open-source online platform designed to run and standardize NSFA for batch usage. Using the NSFA webtool, we also report on the preliminary results of analyzing and comparing the EPSCs of cells under different conditions: spontaneous EPSCs (sEPSCs), EPSCs evoked by glutamate uncaging (uEPSCs), and EPSCs of cells treated with a blocker of calcium permeable AMPA receptors, IEM 1460 and IEM 1925.

Methods: The NSFA webtool was built using the Streamlit open-source library in Python. Recordings of simulated EPSCs with known channel counts and properties were used to validate the utility of the tool. Data from published studies using NSFA was also analyzed with the webtool and compared to original published results. For analysis of PSDs within IHC to SGNs synapses, explants of the organ of Corti from C57/BL6 mice of 15-17 days of age were used. A

glutamate photolysis method was implemented on an upright microscope within an electrophysiology set up to gather the date for the uncaging EPSCs.
Results: Parameters for the simulation were 200 AMPA receptors, half with a single channel current of 0.6 pA, and half with single channel current of 1.4 pA. The mean-variance analysis from the webtool recorded a result of .84pA single channel current and 139 AMPA receptors. For experimental data, NSFA of EPSCs from cells treated with calcium-permeable channel blockers IEM 1460 and 1925 show significant reduction of channel availability, as expected. Spontaneous EPSCs will be compared with those evoked by glutamate uncaging.
Conclusions: The current NSFA webtool is sufficiently accurate to existing methods and has potential to greatly increase the speed of nonstationary fluctuation analysis for large batches of electrophysiological data. Preliminary results of its use on EPSCs of cells under varying conditions will be shared.

SU8. Unraveling the Role of Mitochondrial Protein ACO2 in Hearing Loss

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Category: Auditory Nerve
Background: Hearing relies on the proper function of cochlear hair cells that detect sound vibrations, and auditory nerves that transmit this information to the brainstem. Mitochondrial dysfunction disproportionately affects cells requiring large amounts of energy such as neurons. Mutations in mitochondrial DNA or nuclear genes encoding mitochondrial proteins can affect mitochondrial biogenesis or energy metabolism, impacting auditory function. Mutations in the nuclear-encoded mitochondrial enzyme aconitase hydratase 2 (ACO2) are associated with severe neurological disorders and deafness in children. ACO2 is a tricarboxylic acid cycle (TCA) enzyme that catalyzes the interconversion of citrate into isocitrate. The cellular mechanisms underlying ACO2-associated disease remain poorly understood due to the lack of useful animal models.
Methods: Using whole genome sequencing, we identified an arginine to leucine substitution in Aco2 in a mouse stock showing circling behavior at The Jackson Laboratory. Interestingly, Aco2R56L animals are viable and fertile as homozygotes whereas an Aco2 knock-out strain is lethal before birth. We measure ABR and DPOAEs to test auditory function and use immunofluorescence and confocal microscopy on whole and cryosectioned samples to assess cochlear structure. Mitochondria are genetically labeled with a cell-specific, sparse Cre driver using the Mito-Dendra2 reporter. We use organotypic culture of the cochlea to test metabolic supplementation approaches that can be next used in vivo to rescue or delay ACO2 dysfunction.
Results: Homozygous Aco2R56L mutants have elevated ABR thresholds compared to wild-type and heterozygote littermates with shifts worsening between postnatal (P) day 28 and P47. DPOAEs are normal and comparable between all genotypes at P56, when harvested inner ears show normal OHC and IHC apical morphology (F-actin labeling). SGN quantifications suggest a normal spiral ganglion neuron (SGN) count at 3 weeks, a 17-23% reduction at the apical and mid-cochlear turns at 4 weeks, and a 33-60% reduction affecting all positions at 8 weeks. Ongoing work aims to visualize and quantitatively assess the mitochondrial network in SGNs before and during degeneration. We hypothesize that mitochondrial dysfunction leads to SGN-

inner hair cell synapse loss and/or peripheral dendrite defects, which in turn cause SGN loss. We are currently culturing the cochlea including SGNs to apply TCA metabolites and antioxidants with the goal of preventing SGN loss and earlier defects in Aco2 mutants.
Conclusions: The Aco2R56L strain is a unique new model to investigate the role of ACO2 in auditory function and disease. Normal DPOAEs, largely intact hair cells and SGN degeneration suggest that Aco2R56L mutants and ACO2 patients suffer from early onset, rapidly progressing auditory neuropathy. Our objective is to use compounds first validated in cochlear explants for supplementation in vivo to rescue or slow down auditory dysfunction. The Aco2R56L strain and metabolic supplementation also have the potential to model and mitigate other ACO2 neurological disorders including cerebellar-retinal degeneration.

SU9. Evidence for the Auditory Nerve Generating Envelope Following Responses When Measured From Eardrum Electrodes

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Category: Auditory Nerve
Background: Steady-state auditory evoked potentials are useful for studying the human auditory system and diagnosing hearing disorders. Identifying the generators of these potentials is essential for interpretation of data and for determining appropriate clinical and research applications. Here we infer putative generators of a steady-state potential measured from an electrode on the eardrum and compare this potential with the traditional envelope following response (EFR) measured from an electrode on the high forehead. We hypothesized that responses from the eardrum electrode would be consistent with an auditory nerve (AN) compound action potential (CAP) evoked by each cycle of the stimulus envelope, resulting in a potential we call CAPENV.
Methods: Steady-state potentials were evoked by a 90-dB peSPL, 3000-Hz puretone carrier whose envelope was modulated by a tone sweep with frequencies from 20-160 Hz or 80-640 Hz. We calculated group delay to infer potential generators. We also compared the empirically measured CAPENV with simulated CAPENV from a humanized model of AN responses.
Results: Response latencies and model simulations support the interpretation that CAPENV is generated by the AN rather than hair cell or brainstem generators for all modulation frequencies tested. Conversely, latencies for the traditional EFR were consistent with a shift from cortical to brainstem generators as the modulation frequency increased from 20 – 200 Hz.
Conclusions: We propose that CAPENV may be a fruitful tool for assessing AN function in humans with suspected AN fiber loss and/or temporal coding disorders.

SU10. Cell Type-Specific Assessment of Synaptic Drive Onto Principal Neurons of the Mouse Lateral Superior Olive

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Category: Brainstem: Structure & Function

Background: Principal neurons of the lateral superior olive (LSOPNs) are a critical component of brainstem circuits that compare information between the ears to extract location-related cues. Previously we found that inhibitory and excitatory LSOPNs extensively differed in their membrane properties (Haragopal and Winters,2023). Inhibitory LSOPNs exhibited lower activation threshold, however, differences in synaptic drive might accentuate or offset differences in membrane excitability. We also previously showed that excitatory LSOPNs have more complicated dendritic arbors that could house more synaptic inputs. Here we examine the glycinergic and glutamatergic synaptic inputs onto LSOPN types to address these questions.

Methods: To target specific LSOPN subtypes, we used knock-in reporter mice on C57BL/6J background that co-express the fluorescent protein tdTomato with vesicular glutamate transporter 2 (vGlut2,P26-49). We assessed synaptic drive onto LSOPN types ex vivo in voltage-clamp mode using spontaneous events and electrical stimulation. Pharmacologically isolated glutamatergic inputs were stimulated near the 7th nerve root while glycinergic inputs were stimulated between LSO and medial nucleus of the trapezoid body. Minimal stimulation had failure rates GREATER THAN 12.5%(53.4±2.8%). Broad range stimulation was from 20µA to 10mA. Nickel DAB staining for biocytin was used to recover morphology.

Results: Spontaneous excitatory post synaptic currents (sEPSCs) exhibited larger amplitudes in inhibitory LSOPNs (E:20.9±1.6pA,I:26.66±2pA,t-test,p=0.04), but frequency and kinetics were similar (frequency:E:10.9±3.2Hz,I:10.3±1.9Hz;rise:E:0.16±0.004ms,I:0.15±0.008ms;halfwidth:E:0.73±0.04ms,I:0.69±0.046ms;decay:E:0.76±0.05ms,I:0.75±0.08ms). Minimal evoked EPSCs (eEPSCs) had similar amplitude and kinetics between LSOPN types (amp:E:120.2±66.1pA,I:233.3±118.9pA; rise:E:0.36±0.03ms,I:0.32±0.02ms;halfwidth:E:1.36±0.15ms,I:1.29±0.17ms;decay:E:1.27±0.17ms,I:1.22±0.2ms). The paired-pulse ratio of minimal eEPSCs were also similar (5ms:E:1.3±0.07,I:1.4±0.2).

Spontaneous inhibitory PSCs (sIPSCs) had similar average frequencies and amplitudes (frequency:E:44.8±8.9Hz,I:40.3±6.9Hz;amplitude:E:96.8±11.4pA,I:106.5±10.3pA), however the cumulative probability tended toward more large events in inhibitory LSOPNs (K-S-test,D=0.21,p LESS THAN 0.0001). We also found that sIPSCs recorded in inhibitory LSOPNs had slower decay kinetics (halfwidth:E:0.94±0.05ms,I:1.2±0.07ms,t-test,p=0.0053;decay:E:0.93±0.05ms,I:1.28±0.09ms,t-test,p=0.0008). We did not observe differences in eIPSC minimal size or paired-pulse-ratio between LSOPN types (amp:E:389.3±94.94pA,I:1003±448.8pA;5ms:E:0.91±0.1,I:1.2±0.2), however, eIPSCs also had slower kinetics (halfwidth:E:1.54±0.11ms,I:2.2±0.17ms,t-test,p=0.002;decay:E:1.5±0.11ms,I:2.32±0.21ms,t-test,p=0.0007).

Broad range stimulation suggested that inhibitory and excitatory LSOPNs receive similar numbers of input fibers (glutamatergic:E:3.1±0.5,I:2.7±0.6;glycinergic:E:3±0.5,I:3.1±1.2).

We reconstructed the dendritic arbors of a subset of LSOPNs from which we recorded sEPSC. We found that inhibitory and excitatory LSOPNs had similar dendritic complexity (primaries:E:2.5±0.4,I:2±0.2;tips:E:4.8±0.97,I:3.3±0.6;branch-points:E:2.2±0.6,I:1.3±0.4;total-length:E:284.3±61.7µm,I:205.2±33.7µm). We also found that the frequency of sEPSCs were not

correlated with total dendritic length (Spearman[r]=0.14,p=0.57) and frequency of sIPSCs were not correlated with soma area (Spearman[r]=0.18,p=0.43).

Conclusions: We did not find evidence that synaptic drive offsets intrinsic excitability differences between LSOPN types. This supports the hypothesis that inhibitory LSOPNs may be a source of leading inhibition observed in the inferior colliculus. Counter to our previous findings using 2-photon reconstructions, we did not see differences between LSOPN types in dendritic complexity nor did we observe differences in sEPSC frequency suggesting that excitatory LSOPNs do not have greater integrative potential regarding synapse number. Our wide-range stimulation results likewise suggest LSOPN types do not integrate overtly different numbers of independent inputs.

SU11. Dopamine Receptor Expression in the Mouse MNTB

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Category: Brainstem: Structure & Function

Background: The medial nucleus of the trapezoid body (MNTB) has been well studied as the primary source of contralaterally derived inhibition to the brainstem auditory circuitry. MNTB-derived inhibition plays a critical role in the computation of sound location as the temporal aspects of sounds are precisely conveyed through the calyx of Held/MNTB synapse. Neuromodulators, like acetylcholine are known to play a role in mediating MNTB responses. In addition to cholinergic input, dopaminergic projections to the MNTB have been documented (Nevue et al. 2016). However, there have been no studies demonstrating expression of dopaminergic receptors or physiological modulation by dopamine (DA) in MNTB neurons. While dopaminergic neurons compose a small population in the brain, they have potent and widespread impacts on targets throughout the nervous system. Partially mediating this diverse array of effects are DA receptor subtypes 1-5 which are members of the G protein-coupled receptor family and divided into two classes. D1 and D5 belong to the D1-like family while D2, 3, and 4 are members of the D2-like family. Each of these families of DA receptor subtypes initiate unique downstream cascades.

Methods: . To investigate the potential auditory processing function(s) of the dopaminergic input to the MNTB, we first used immunohistochemistry to describe the expression patterns of DA receptor subtypes 1, 2, 4, and 5 in mouse MNTB neurons. Mice aged P24 were perfused with paraformaldehyde, and fixed brains were prepared with coronal sections through the auditory brainstem. Antibodies directed against D1, D2, D4, and D5 receptors were used to examine expression in the mature MNTB.

Results: We demonstrate that all four DA receptor subtypes are differentially expressed in the mouse MNTB. D1 appears to have strong postsynaptic expression while D2 and D4 exhibit lower postsynaptic expression. Preliminary evidence suggests that D5 receptors are predominantly expressed presynaptically.

Conclusions: Our results show for the first time that DA receptor subtypes 1, 2, 4 and 5 are expressed in the mature mouse MNTB with unique expression profiles. These results strongly suggest that DA is likely to provide neuromodulatory signaling in the MNTB.

SU12. Optimizing Frequency-Specific Auditory Brainstem Responses to Continuous Speech Using Different Chirp-Phase Profiles

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Category: Brainstem: Structure & Function

Background: Auditory brainstem responses (ABR) are used to identify hearing loss across multiple frequency bands in young children. ABRs typically use brief tonebursts or narrowband chirps, but we recently created a "peaky" speech method that uses audiobooks to evoke multiband ABRs—an engaging stimulus that 1) may facilitate testing in infants and toddlers who cannot nap, sit still, or participate in behavioral testing, and 2) may be played through hearing aids to assess neural responses to amplified speech. Previous work has shown that ABRs to multiband peaky speech are larger and faster to record by compensating for ear timing delays (chirp-phase) to promote synchronous neural activity. However, the applied chirp-phase in previous work seemed to overcompensate the delays based on wave V latencies. Other chirps may provide better synchrony and larger ABRs to speech. This study aimed to determine which chirp-phase profile of multiband peaky speech evokes the largest ABRs in the fastest recording time, which will facilitate testing in young children.

Methods: Two experiments were conducted with speech presented at 65 dB SPL: the first with 16 adults and the second with 18 adults, all with normal hearing. In the first experiment, multiband speech ABRs (10: 5 bands centered at .5, 1, 2, 4, and 8 kHz in 2 ears) were simultaneously recorded to 30 minutes each of four chirps: CE, 60-dB and 65-dB level-dependent chirps, and a peaky-speech (PS1) chirp created based on data from four previous studies using multiband peaky speech. In the second experiment, multiband ABRs were recorded to 40 minutes each of three chirps: CE, PS1 and a second peaky speech chirp (PS2) based on a new experiment using optimized parameters for multiband peaky speech. ABRs were compared across chirps using wave V latency and amplitude and the time to reach a 0 dB signal-to-noise ratio (SNR).

Results: ABRs were similarly sized for the 2-8 kHz bands across all chirps, but largest for the CE, PS1 and PS2 chirps for the .5-1 kHz bands. The CE and two peaky speech chirps evoked comparable responses, although the CE-chirp SNRs were a bit higher, and thus reached a 0-dB SNR sooner, in more participants. Low-frequency band ABR wave V latencies were earlier for the CE-chirp, suggesting that the delays were overcompensated, but this did not affect wave V amplitudes or SNRs.

Conclusions: Overall, the CE and peaky speech chirps provided ABRs with good SNRs in similar recording times but the level-dependent CE chirps evoked the smallest ABRs. Creating multiband peaky speech stimuli with a CE-chirp is as effective as the speech-based chirps at evoking large multiband ABRs to facilitate efficient audiobook-based ABR testing.

SU13. L-Stellate Cells are Differentially Activated by the Auditory Nerve and T-Stellate Cells Within the Ventral Cochlear Nucleus

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Category: Brainstem: Structure & Function

Background: In the ventral cochlear nucleus (VCN), feedforward inhibition from glycinergic cells refines auditory processing by principal excitatory neurons. Glycinergic inputs onto excitatory bushy and T-stellate cells enhance acoustic tuning properties, improve temporal precision, and stabilize neuronal firing responses to acoustic stimuli. Within the VCN, two types of glycinergic cells, D-stellate and L-stellate cells, provide feedforward inhibition onto excitatory principal cells. We previously showed that L-stellate cells receive monosynaptic excitatory inputs from the auditory nerve (AN) fibers and disynaptic inputs from a local excitatory neuron within the VCN. This study investigated the source of feedforward excitation and the response of L-stellate cells to these dual excitatory inputs.

Methods: We utilized GlyT2-EGFP mice to identify L-stellate cells in the VCN. Parasagittal slices of the cochlear nucleus from postnatal day 16-31 mice were prepared in an ice-cold sucrose solution. Patch-clamp recordings were made from L-stellate cells using K-gluconate-based internal solutions. A bipolar electrode was positioned in the AN root to deliver brief electric shocks to AN fibers. For T-stellate cell activation, retrograde excitatory channelrhodopsin-expressing virus was stereotactically injected into the inferior colliculus.

Results: In voltage-clamp, AN root stimulation evoked EPSCs with varying latencies in L-stellate cells. The initial EPSC exhibited a consistent synaptic delay of less than 1 ms, indicative of monosynaptic inputs, whereas subsequent EPSCs showed delays of 2 ms or more, consistent with disynaptic inputs. This suggests that AN fibers provide monosynaptic inputs, while a local excitatory cell delivers feedforward disynaptic inputs. Previous studies have reported that excitatory T-stellate cells make local collaterals in the VCN. Activation of Chr2-expressing T-stellate cells with light reliably induced EPSCs in L-stellate cells. In current-clamp, AN-stimuli or light-driven spikes in T-stellate cells were able to elicit action potentials in L-stellate cells. In pharmacological experiments to identify receptor subtypes mediating these two sources of synaptic activity, we found that AN inputs primarily activate AMPA receptors, while T-stellate inputs engage both AMPA and NMDA receptors in L-stellate cells.

Conclusions: We demonstrated that L-stellate cells receive powerful monosynaptic inputs from both AN fibers and disynaptic inputs from local T-stellate cells. This obligatory feedforward excitation from T-stellate cells can offer an additional target of modulation to regulate the firing of L-stellate cells. Moreover, depending on how T-stellate axons are distributed in VCN, feedforward excitation may also determine the shape of inhibitory sidebands. Finally, differential receptor activation suggests distinct functional roles for these inputs in shaping synaptic efficacy of L-stellate cells.

SU14. Age-Related Ultrastructural Changes in the Dorsal Cortex of the Inferior Colliculus in Fischer Brown Norway Rats

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Category: Midbrain: Structure & Function

Background: It has been demonstrated that GABAergic and excitatory synapses in the lemniscal inferior colliculus (IC) decline with age1. We sought to determine whether such synaptic declines also occur in the non-lemniscal dorsal cortex of the IC (ICd). We assessed Fischer Brown Norway rats at three ages: 3-months, 19-months and 28-months.
Methods: We used immunogold transmission electron microscopy to characterize GABAergic and excitatory synapses, their post-synaptic targets, presynaptic mitochondria, and the presence of dense core vesicles (DCV). Ultrathin sections (~50 nm) were placed on 300 Ni mesh grids (eight per case), reacted for anti-GABA immunochemistry and stained with uranyl acetate. A random 400 μm2 montage was taken with SerialEM from each grid. GABAergic synapses were identified as having pleomorphic vesicles, symmetric synaptic junctions, and GABA-positive presynaptic boutons. Postsynaptic targets comprise somas, dendrites of three calibers (LESS THAN 0.05 μm, between 0.5 and 1.5 μm and GREATER THAN 1.5 μm) and spines. Lastly, the diameter and location of each DCV was recorded. A total of 1,956 (491 GABAergic, 1,465 excitatory) synapses were characterized.
Results: Broadly, ~70% of presynaptic profiles at 3-months were classified as excitatory. Aging reduced the density of GABAergic synapses by 25% between 3- and 19-months; and 34% between 3- and 28-months. Interestingly, there was no significant age-related change in the density of excitatory synapses. We found that the average synaptic GABAergic bouton size decreased from 0.63 μm2 at 3-months to 0.48 μm2 at 28-months. This decrease underscored that many of the lost synaptic boutons were between 0.5 μm2-0.99 μm2. There was no change in excitatory presynaptic areas (young, 0.48 μm2; middle, 0.5 μm2; old, 0.47 μm2). At 28-months both synapse types targeted small caliber GABAergic and non-GABAergic dendrites with greater (+20-30%) frequency, while targeting medium caliber dendrites less frequently. The number of DCVs in excitatory cells more than doubled from 3-month to 28-month. This increase occurred in the presynaptic terminals and dendrites.
Conclusions: We conclude that in the ICd there is an age-related loss of GABAergic synapses at middle and old age. Perhaps the most interesting finding was the lack of excitatory synaptic loss, as significant age-related excitatory loss has been reported in the central IC and the lateral cortex of the IC. As the ICd is broadly defined as receiving dense inputs from auditory cortex, perhaps ascending and descending inputs to the IC are differentially affected by aging. Although excitatory synapses were not downregulated, the dramatic increase in DCV suggests that neuromodulators, neuropeptides and/or neurotrophic factors play an increasing role in the aging ICd. Taken together, the ICd undergoes several age-related changes to both GABAergic and excitatory synapses, however these changes are not uniform across GABAergic and excitatory neurotransmission.

SU15. Ultrastructural Evidence for Excitatory and Inhibitory Cholinergic Synapses in the Inferior Colliculus

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Category: Midbrain: Structure & Function
Background: Acetylcholine (ACh) can modulate the responses of most neurons in the inferior colliculus (IC) by acting on a variety of nicotinic and muscarinic cholinergic receptor types.

However, the cellular mechanisms have been unclear; in fact, whether ACh acts through traditional synapses or volume transmission in the IC is unknown. In this study, we labeled cholinergic neurons in the pontomesencephalic tegmentum (PMT), the largest source of cholinergic input to the IC. We then examined the ultrastructure of the labeled cholinergic axons in the IC.
Methods: We injected AAV carrying Cre-dependent peroxidase genes (HRP or dAPEX2) into the PMT in 9 month old, normal-hearing ChAT-Cre mice. After 2 weeks, the brain was fixed and processed for transmission electron microscopy. Cholinergic axons were stained with DAB. Depending on the specific vector used, the DAB filled the cytoplasm or, with different vectors, the DAB was confined to mitochondria or synaptic vesicles. The restricted label of the latter methods allowed unambiguous identification of labeled boutons while greatly facilitating examination of the ultrastructure. Labeled cholinergic boutons in lateral cortex, dorsal cortex and the central nucleus of the IC were imaged at 50k and 80k magnification.
Results: We identified 82 cholinergic synapses in the IC; 52 synapses were in the IC ipsilateral to the labeled PMT neurons and 30 synapses in the contralateral IC. The synapses included clear synaptic vesicles clustered adjacent to a synaptic junction that formed most often with dendrites and occasionally with spines or somas. We observed asymmetric synapses, characterized by a prominent postsynaptic density and typically associated with excitatory postsynaptic effects, as well as symmetric synapses, generally associated with inhibitory effects. Overall, asymmetric synapses predominated (50/82 = 61%). Both synapse types were found in each IC subdivision on both ipsilateral and contralateral sides.
Conclusions: We conclude that cholinergic projections from the PMT can form traditional synapses in the ipsilateral and contralateral IC. The ultrastructural morphology is consistent with both excitatory and inhibitory effects in each of the three major IC subdivisions. The majority of synapses were formed with dendrites, consistent with light microscopic observations of cholinergic boutons in the IC (Noftz et al. 2024, 10.1016/j.jchemneu.2024.102443). Cholinergic neurons of the PMT have been associated with modulation related to attention, reward, the sleep-wake cycle, and cortically-driven synaptic plasticity. We showed previously that cholinergic PMT neurons project throughout the IC, and suggested that these projections could modulate each of the parallel pathways that ascend from the IC to the auditory thalamus. The present results suggest that cholinergic modulation from the PMT relies at least in part on conventional synapses with excitatory or inhibitory effects on IC neurons. Supported by NIH DC004391.

SU16. Cortical Contribution to Task-Relevant Activity in the Inferior Colliculus

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Category: Midbrain: Structure & Function
Background: Descending projections from the neocortex to subcortical stations are ubiquitous across sensory systems. These feedback pathways are important for sensory processing, as they provide a route for high level activity to influence ascending information in a predictive or feedback manner. In the auditory system, the descending projection from auditory cortex to inferior colliculus (IC) famously controls IC neuron selectivity and some forms of perceptual

learning, but the underlying mechanisms remain poorly understood. We previously showed that in mice performing an appetitive sound discrimination task, auditory cortico-collicular neurons transmit not only auditory information but also high-level, non-auditory signals that correlate with the outcomes of behavioral trials (Ford/Czarny et al., 2024). More recently, we found conspicuously similar trial outcome-related activity in the IC (Quass et al., 2024). Collectively our published results support a working hypothesis whereby auditory cortex causally contributes to behaviorally relevant activity in the IC, though the extent to which is unknown.
Methods: We used transgenic mice, viral vectors, optogenetics, and two-photon Ca2+ imaging to record from GCaMP8s expressing IC neurons, as head-fixed mice engaged in the GO/NOGO sound discrimination task employed in Ford/Czarny et al., 2024. We optogenetically silenced the ipsilateral auditory cortex on 30% of trials by activating cortical GABAergic interneurons via a red-shifted excitatory opsin ChrimsonR. We also used sub-cellular Ca2+ imaging of auditory cortico-collicular axons in the IC to quantify task-related dynamics of cortical feedback, and to confirm the extent to which our optogenetic method reduces descending activity.
Results: Preliminary analyses reveal substantial task-related activity in auditory cortico-collicular axons, with a subset of axons responding to both auditory and non-auditory task variables. Optogenetic activation of cortical GABAergic interneurons largely abolished task-related activity in auditory cortico-collicular axons, thereby confirming the efficacy of our silencing method. IC neuron activity similarly correlated with auditory and non-auditory task epochs, with substantial neural activity occurring after reward consumption; this latter observation suggests a retrospective outcome evaluation signal. Auditory cortical silencing could reduce, but generally did not abolish task-related activity compared to control trials. Additionally, cortical silencing bidirectionally modulated sound responses, resulting in both increases and decreases in sound evoked activity across distinct IC neurons. Our in progress analyses are training linear classifiers to decode trial outcomes from IC fluorescence data, and testing if decoding accuracy is reduced on cortical silencing compared to control trials.
Conclusions: Our data indicate that descending signals from auditory cortex contribute to auditory and non-auditory activity in the IC of behaving mice. However, task-relevant and putatively non-auditory activity patterns nevertheless persist during auditory cortical silencing. Collectively these data argue that auditory cortex is but one of several sources of high-level information for IC neurons.

SU17. Simultaneous Encoding of Features of Frequency-Modulated Sweeps in Individual Inferior Colliculus Neurons

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Category: Midbrain: Structure & Function
Background: Rapid changes in sound frequency known as frequency-modulated (FM) sweeps are an important component of both human and animal vocalizations. The inferior colliculus (IC) is a hub of auditory processing, and some neurons in the IC respond selectively to particular FM sweep directions. However, how selectivity is shaped by other sweep features, such as sweep speed, intensity, and frequency range has not been explored. In addition, whether and how IC neurons encode these other FM sweep features remains poorly understood.

Methods: To investigate how specific FM sweep parameters shape FM direction selectivity in IC neurons, we performed in vivo juxtacellular recordings from IC neurons in awake, head-fixed mice while playing FM sweeps of different speeds (10 – 200 octaves/second), directions (up, down), frequency ranges (1 octave, 2 octaves, and 4 octaves at intervals between 4 – 64 kHz), and intensities (10 – 70 dB SPL). We also assessed the frequency tuning of each neuron using 4 – 64 kHz tone bursts played at intensities ranging from 0 – 70 dB SPL, and assessed vocalization responses using ultrasonic pup retrieval calls and lower frequency pup calls. Selectivity was analyzed with both the direction selectivity index (DSI) and a machine learning model that was trained to decode the direction of the sweep and other sweep parameters from neuron spike times.
Results: We found that in most IC neurons, four-octave FM sweep direction can be decoded from spike train data across varying sweep speeds. We also observed that IC neurons encode frequency range, speed, and to a lesser extent, direction of one- and two-octave FM sweeps. In addition, we found that spike timing plays a critical role in encoding these sound features, particularly sweep direction and speed. We also investigated decoding over time, and found that individual IC neurons often encode multiple features of a stimulus, only at different times relative to sound onset. Accordingly, we found that encoding of multiple sound features can be performed with high accuracy using small populations of IC neurons. Lastly, our findings indicate that the selective responses of IC neurons to different vocalization classes are not influenced by the direction of frequency change; instead, these responses may be better predicted by the neuron's receptive field for stimulus frequency and intensity.
Conclusions: These findings indicate that IC neurons simultaneously encode multiple sound features, highlighting a mechanism IC neurons may use to build representations of complex auditory inputs.

SU18. Exploring Analytical Procedures and Short Channels in Auditory Fnirs Brain Imaging

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Category: Auditory Cortex and Thalamus: Human Studies
Background: Functional near-infrared spectroscopy (fNIRS) is now a common tool in neuroscience research especially in developing children due to its weak sensitivity to head movements and in cochlear implanted recipients due to its lack of lack of sensitivity to implant artifacts. However different analytical methods are now available and should be compared to determine the best approach for data analysis.
Methods: We investigated the effect of short channel correction (SC) on a block design experiment with auditory, visual, and audio-visual stimuli, by removing non-neuronal signals from fNIRS long channels. We tested three analysis pipelines with and without SC correction. fNIRS signal was deconvoluted using Generalized Linear Model (GLM) in which 1) orthogonalized SC signal 2) a single SC signal, and 3) without SC signal was added as regressor of non-interest.

Sixteen participants (24-34 years old) were recruited. A passive sensory stimulation in visual (V), auditory (A) and audio-visual (AV) was proposed using dynamic (visual gif) ecological stimuli such as moving car or a ringing phone. Stimuli were presented for 10s duration in successive blocks containing each of the three conditions. A silent jitter interval of 5-10s was added between each block. Optodes were located bilaterally over the temporal auditory cortex and the occipital visual areas.

Results: Without the SC analysis no clear hemodynamic response can be extracted in the temporal auditory cortex in the A condition while the response signal quality increases using the single and orthogonalized SC analysis in most of the temporal optodes. In the occipital area, using SC analysis allows to extract clear visual hemodynamic response. Further we have been able to reveal from the hemodynamic response a clear deactivation with the audio stimulation. Lastly the comparison of A, V and AV responses provides significant differences only when single SC or orthogonalized SC analysis where applied.
Conclusions: This study highlights the critical role of SC in fNIRS in removing systemic signal, with orthogonalized SC being the most effective. When applying such SC noise reduction in signal, fNIRS brain imaging is a pertinent technology to observed neuromarkers in cochlear implanted children (in prep) as fNIRS is not contaminated by head movement and electrical artifacts.
These findings illustrate an interaction between auditory and visual processing, with deactivation observed in the occipital cortex during auditory stimulation, reflecting cross-modal interactions to allocate optimal processing in the modality-specific cortical areas. Such processes seem to be prominent in the visual areas and much less present in the temporal auditory areas. Moreover, the absence of strong deactivation in auditory areas highlights the importance of studying regional differences in sensory processing. On the methodological aspect, SC correction should be applied for a more efficient reduction of systemic noise.

SU19. Auditory Object Formation Across Acoustic and Electric Hearing in Cochlear Implants With Electroacoustic Stimulation

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Category: Auditory Cortex and Thalamus: Human Studies
Background: Auditory object detection relies on the interplay of acoustic feature encoding and cognitive mechanisms of regularity recognition. We previously found that listeners with acoustic hearing exhibit superior performance and stronger cortical activities than those with cochlear implants (CI) while detecting spectral and temporal alignment of multiple tone pips in a tone cloud, which indicated the advantage of acoustic hearing in auditory object formation. However, it was not clear whether CI users with both acoustic and electric stimulation could take advantage of their residual acoustic hearing in detecting auditory objects that spread across frequency ranges of their acoustic and electric hearing. Here, we aimed to answer this question through a within-subjects design experiment of auditory object formation across low-, high-, and

combined frequency ranges in bimodal CI listeners with functional low-frequency acoustic hearing.
Methods: We recruited 34 CI subjects with a better-ear unaided hearing threshold of 60 dB or lower at 500 Hz, to ensure all our subjects have functional low-frequency acoustic hearing. The stochastic figure-ground paradigm was implemented, where the stimulus is either composed of tone pips randomized across time and frequency, "Ground" condition, or consisting of several tone pips that become spectrotemporally coherent halfway through the stimulus, "Figure" condition. Crucially, the "Figure" condition was either presented in the acoustic hearing range (i.e., below 500 Hz; Fig-A), electric hearing range (i.e., above 1000 Hz; Fig-E), or in the broadband range (i.e., below 500 Hz and above 1000 Hz; Fig-B). Notably, the number of tone pips that become coherent across time and frequency dimensions, a manipulation called the 'coherence level,' was set to three for the Fig-A and Fig-E conditions and six for the Fig-B condition. Participants were required to indicate whether they heard the "Figure" while their electroencephalography (EEG) was recorded.
Results: Behaviorally, the detection of the "Figure" was most accurate in the Fig-B condition. Cortical evoked responses to detecting the "Figure" were stronger for the Fig-B condition at frontocentral electrodes. Event-related spectral perturbations revealed a strong alpha-band (8-13 Hz) response that significantly differed when the "Figure" was presented across the Fig-A, Fig-B, and Fig-E conditions; the Fig-B condition showed the strongest alpha power desynchronization. Alpha desynchronization appeared exclusively in all "Figure" conditions at about 500 ms following the presentation of the coherent tone pips. This activity may reflect top-down cognitive processes related to task-related attention.
Conclusions: These results indicate that bimodal CI listeners can integrate acoustic and electric hearing for auditory object formation across different hearing modalities. Additionally, the EEG findings suggest that this integration process recruits not just bottom-up but also top-down cortical activities, indicating the complexity of acoustic and electric integration in bimodal hearing.

SU20. Transformation of Object Representation Along the Cortical Auditory Pathways

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Category: Auditory Cortex and Thalamus: Human Studies
Background: Auditory cortex on the superior temporal plane and lateral convexity are key areas in the initial stages of cortical auditory processing. More complex perceptual representations are envisioned to occur in auditory-related cortex along the ventral and dorsal processing streams, extending into prefrontal and sensorimotor cortices. To characterize the flow of information across these stages of the auditory hierarchy that ultimately leads to sensory-driven behavioral events, we examined neural activity using intracranial electroencephalography (iEEG) during semantic categorization tasks.
Methods: Participants were 42 neurosurgical patients undergoing iEEG monitoring for medically intractable epilepsy. Stimuli were monosyllabic words, with participants pressing a button in response to words belonging to a target category. We examined iEEG data by

analyzing high gamma (70-150 Hz) power and RMS amplitude of broadband local field potentials (LFPs). Two 300 ms windows were examined: (1) 50-350 ms after stimulus onset and (2) 300-0 ms before motor response. Three patterns were identified: (1) larger activity when measured with respect to stimulus onset ("stimulus-locked"), (2) larger activity when measured with respect to motor response ("response-locked"), and (3) similar magnitude with respect to these events ("sustained").
Results: Across over 6000 recording sites, stimulus-locked activity was the predominant pattern, followed by sustained. High gamma activity within the superior temporal cortex was almost exclusively stimulus-locked with the exception of rostral areas (planum polare, anterior insula) where the sustained pattern was more common. Areas along the dorsal and ventral processing streams with predominantly stimulus-locked activity included supramarginal gyrus and the upper bank of the superior temporal sulcus. Of note, stimulus-locked activity was the most common pattern in sensorimotor cortex, whereas response-locked activity was rarely encountered there. The response-locked pattern was observed in the anterior-ventral temporal and dorsolateral prefrontal cortex and was more common in the left hemisphere.
LFPs and high gamma can be considered proxies of synaptic potentials and neuronal firing, respectively. Multiple areas were characterized by a dissociation between LFP- and high gamma-derived activity patterns. Anterior-ventral temporal cortex featured stimulus-locked LFP and response-locked high gamma activity, suggesting a role in transformation of sensory inputs into perceptual entities in this task. Activity in dorsolateral prefrontal and orbitofrontal cortex was typically response-locked for both LFP and high-gamma, suggesting perceptual-level processing related to subsequent behavioral responses.

Conclusions: Results reveal progressive stages of cortical auditory processing wherein sensory stimulus-driven activity is ultimately transformed into objects driving subsequent behavior. Additional studies requiring more complex semantic processing will evaluate whether these findings can be generalized or if profiles dynamically change as a function of the task.

SU21. Disrupting Perceptual Anchoring to Pure-Tone Sequences in Human Listeners

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Category: Auditory Cortex and Thalamus: Human Studies
Background: Perceptual anchoring, a process akin to statistical learning, occurs rapidly and without conscious awareness and is integral to our ability to successfully navigate a noisy world. In this study, we investigated anchoring abilities in typical hearing and reading participants by implementing a unique anchoring paradigm (Agus et al., 2014) using rapid pure-tone sequences (Barascud et al., 2016). Following this, we attempted to disrupt anchoring ability by applying rapid transcranial magnetic stimulation (rTMS) to frontal neural regions - an area implicated in the processing of and integration of structured tone sequence (Abla and Okanoya, 2008).
Methods: Pure-tone sequences consisted of 50 ms tone-pips that were arranged according to two segments. RAND segments were generated as tones of random frequencies, and REG segments were generated as in RAND but were then iterated to create a regularly repeating pattern.

Sequences were presented across three conditions: REPfixed (identical sequences repeated across trials), REPnovel (identical sequences not repeated across trials), and NonREPnovel (nonidentical sequences not repeated across trials).
Results: It was observed a significantly higher sensitivity to REPfixed relative to REPnovel across all participants. The disruption of frontal regions using rTMS did not significantly impact overall performance in our participants, but did alter how participants completed the task.
Conclusions: The disruption of frontal regions using rTMS did not significantly impact overall performance in our participants, but did alter how participants completed the task.

SU22. Towards a Generalized Deep Neural Network Model of Human EEG Responses to Sounds

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Category: Auditory Cortex and Thalamus: Human Studies
Background: All experiments using evoked potentials fit a model that relates input stimulus features to the output electrophysiological signal. In the simplest case, the features are stimulus onsets which are used to find the average responses. In more recent work, continuous stimulus features are extracted by applying some nonlinearity to the audio, and the response is computed by a linear transformation relating the nonlinear stimulus feature(s) to the recorded signal. In either case, the results are determined by both the stimuli that are presented and how they're represented in the feature space, both of which depend on choices made by the researchers. Here, we take steps towards training a generalized model of the human auditory system's response to sounds as measured at the scalp. The end goal is a model which takes raw stimulus audio as the input, learns from combined data recorded across electrode locations, subjects, and experiments, and runs faster than real-time. In this work, we address several challenges faced in training such a model on real EEG recordings, independent of any specific model architecture.

Methods: To train a model across subjects and electrodes for a given set of stimuli, we constructed an encoder model that computes a latent representation, with each subject- and electrode-specific signal computed as a linear projection of that representation, consistent with prior work. A greater challenge was incorporating data from multiple experiments with differing input stimuli, subjects, and electrode configurations. For this we gave each experiment its own final linear projection layer and used multi-task learning, in which the losses were computed for each experiment batch and summed before backpropagation. The issue of subject- and electrode-specific stimulus artifact was solved by adding a purely linear convolution of the input stimulus to the output that is learned during training but omitted during inference. Lastly, to address the problem of extreme additive noise in the output EEG signal—which resulted in poor predictions that were largely driven by the average value of the EEG signal—we added a penalty for low-variance predictions to the loss.
Results: We tested the effect of our proposed solutions to each of the challenges described above. By implementing them, we were able to create a model that learned from many experiments' data to predict individual subject responses.

Conclusions: We have developed methods to overcome several key challenges to training a model on real EEG data, providing a path for better models of the auditory system which may be used to develop and test new stimulus and analysis paradigms. This framework allows data from many subjects listening to many distinct sounds to be used, which both increases training set diversity and addresses practical challenges of trying to collect very large datasets from individual subjects.

SU23. Sensory Inhibition in Central Auditory Processing Disorder

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Category: Auditory Cortex and Thalamus: Human Studies

Background: Central auditory processing disorder (CAPD) is characterized by 'listening difficulties' emerging from the central auditory nervous system in individuals with normal hearing (ASHA, 2005). CAPD is included in the International Classification of Diseases (ICD-10), although its definition remains controversial due to its shared characteristics with other disorders (de Wit et al., 2018). With this in mind, there is a need to clarify the definition of CAPD and to identify neural biomarkers for this disorder. Speech perception-in-noise (SPiN) is defined as the ability to attend to a speech signal while suppressing unwanted background noise, and this ability is reduced in individuals with CAPD (Chermak and Musiek, 1992). One mechanism contributing to SPiN deficits is a decrease in thalamo-cortical sensory gating, which is defined as pre-attentive inhibition of non-novel or irrelevant stimuli (Javitt and Freedman, 2015). However, sensory gating deficits have yet to be linked with CAPD or subsets of CAPD such as SPiN deficits. This study aims to evaluate sensory gating in individuals with and without CAPD. In addition, we aim to correlate sensory gating with CAPD severity and subtypes and to estimate the neural generators of gating-related networks for CAPD.

Methods: This study will include preliminary data in five individuals with CAPD compared to control data published by Campbell et al. (2023). Audiologic evaluations will be administered, and individuals with normal hearing thresholds and present distortion product otoacoustic emissions (DPOAEs) will be included. SCAN-3 and Feather Squadron behavioral test batteries will be utilized for the diagnosis of CAPD. Participants will undergo a sensory gating paradigm via EEG. Cortical auditory evoked potentials (CAEPs) will be recorded, and differences in the amplitude of CAEPs from consecutive click stimuli (S1 and S2) will be calculated as a measure of sensory gating using MATLAB (The MathWorks, Inc.) with the EEGLAB toolbox (Delorme and Makeig, 2004).

Results: Preliminary studies have shown that individuals with SPiN deficits exhibit reduced sensory gating, with minimal differences in the amplitude of CAEPs elicited from S1 and S2 (Campbell et al., 2020). Based on this, we hypothesize pilot data will show reduced sensory inhibition significantly correlating with CAPD measures.

Conclusions: Given that SPiN deficits are associated with reduced sensory gating and also associated with CAPD, individuals with CAPD may have reduced sensory gating. Although data is currently being collected to test this hypothesis, this study can clarify the underlying neural components of CAPD, including CAPD subtypes, and inform future studies aimed at targeted interventions.

SU24. Perceptual Categorization of and Adaptation to Human Voice and Musical instruments: A Passive-Listening Study

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Category: Auditory Cortex and Thalamus: Human Studies

Background: The human voice is a highly socially relevant auditory stimulus, which has been shown to have a special status both perceptually and neurally. In addition, perceptual studies have revealed adaptation effects in the behavioral categorization of sounds as either human voice or musical instruments. However, less is known about perceptual categorization and context effects in voice and non-voice processing when the stimuli are unattended.
Methods: Electroencephalography (EEG) was recorded through non-invasive electrodes on the scalp, as the participants listened to the sound stimuli and concurrently watched a movie of their choice on mute. Data were collected from 26 adults with normal hearing, who each participated in all three experiments. In Experiment 1, vowel utterances (/a/, /o/, /u/, and /i/) and instrumental tones (bassoon, horn, saxophone, and viola) were presented with equal probability in a random sequence, and responses to different categories were compared using a t-sum cluster-based permutation test. In Experiment 2, an oddball paradigm was used, where either the vowel /a/ or the viola tone served as the rare deviant embedded in a random sequence of four stimuli (e.g., four different instruments) from the opposite category. Mismatch negativity (MMN) elicited by the rare category was calculated. In Experiment 3, ambiguous voice-instrument morphs were presented in either vocal or instrumental contexts, and the effect of context type on the responses to ambiguous sounds was analyzed both at a group level and on a trial-by-trial basis.
Results: In Experiment 1, significant differences between the brain responses to the voice and the instrument categories were observed at around 90-300 ms, primarily at the frontal and frontocentral electrodes. In Experiment 2, MMN was observed for rare instrumental tones (viola) embedded in a random sequence of four different vowels, but not vice versa, suggesting that categorization of voice and non-voice could require little to no attention but may be modulated by stimulus familiarity. In Experiment 3, although the averaged responses to ambiguous sounds did not differ significantly between vocal and instrument contexts, logistic regression models performed above chance in predicting the type of context (voice or instrument) from the responses to ambiguous morphs, providing some evidence for context effects on the neural representation of voice and non-voice stimuli.
Conclusions: The results suggest that neural signatures of both perceptual categorization and context effects can be observed in EEG responses to voice and non-voice stimuli under passive listening conditions. [Supported by NIH grant R01 DC012262.]

SU25. Parvalbumin and Somatostatin in the Songbird Auditory Cortex Suggest Conserved Mechanisms for Inhibition

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Category: Auditory Cortex and Thalamus: Structure & Function

Background: Vocal learning is a rare ability that requires the memorization and sensorimotor development of vocalizations. The songbird is an exemplary model for evaluating the role of auditory processing in vocal learning. In the mammalian cortex, nearly all inhibitory neurons express the neuropeptides parvalbumin (PV), somatostatin (SST), or the serotonin receptor 5HT3aR. The expression of these neuronal subtypes differs across cortical layers and contributes to auditory plasticity, selectivity, and attention. In general, PV neurons provide fast spiking, peri-somatic inhibition. SST neurons provide inhibition to dendrites and are part of a disinhibition circuit with 5HT3aR neurons expressing vasoactive intestinal peptide (VIP). Despite extensive study in mammals, the role of interneuron subtypes in auditory-vocal communication is not well known. Recent in-situ hybridization studies have matched mammalian PV, SST and VIP marker genes to avian GABA3/4, GABA2, and GABA5 genes respectively. We hypothesize that the subtypes of inhibitory neurons in auditory cortex serve distinct roles in vocal learning. Here, we examined PV and SST expression across the superficial, intermediate, deep, and secondary regions of the songbird auditory cortex.
Methods: We performed immunohistochemistry in the zebra finch (Taeniopygia gutatta) and long-tailed finch (Poephila acuticauda). Commercially available antibodies for GABA, parvalbumin and somatostatin were used in conjugation with fluorescent antibodies to label and map auditory interneurons by subtype. The percentage of neurons that expressed PV or SST was correlated with known physiology of cortical subregions. These percentages, along with spontaneous firing rates, were used to establish and differentiate putative PV-Like and SST-Like neurons. Species differences in vocal acoustics were also considered when evaluating interneuron subtype firing latencies.
Results: We found that the expression and localization of PV and SST differed across cortical regions. We also observed differences in PV expression along the mediolateral axis. Physiologically, PV-Like neurons exhibited significantly shorter firing latencies to tone bursts in both deep and secondary regions. Putative interneuron subtypes also differed in their frequency tuning curves and response to song stimuli. While both finch species expressed GABAergic neurons with PV or SST, we surprisingly observed PV+ neurons without GABA expression. These neurons may represent PV+ fast spiking excitatory neurons, previously only seen in the songbird robust arcopallial nucleus (RA).
Conclusions: The expression and localization of interneuron subtypes likely have critical and differential contributions to vocal learning and auditory selectivity towards birdsong. Future studies using adeno-associated virus (AAV) and brain clearing could evaluate interneuron microcircuits and synapse location. Mammalian studies that utilize PV or SST specific silencing should be replicated in the songbird. These studies could support the idea that vocal learning in mammals and birds has conserved mechanisms for complex, differentiated inhibition.

SU26. Spatial Analysis of Inner Ear Pathology in Mice With Audiovestibular Dysfunction Induced by Lassa Virus Infection

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Category: Inner Ear: Anatomy & Physiology

Background: Lassa Fever (LF) is a viral hemorrhagic fever caused by infection with Lassa Virus (LASV), which is endemic to West Africa. Approximately 25% of subjects infected with LASV develop sudden hearing loss, and up to 50% of the patients develop vestibular symptoms after clearing the acute disease phase. This study investigated spatial pathology in the inner ear by using tissue clearing and 3D reconstruction of the temporal bone from a mouse model of LF.
Methods: Female Stat1 knockout mice at 13-week-old were infected with LASV. The mice were observed for up to 35 days post-infection (dpi) unless they had to be euthanized due to distress. The mice were checked daily for behavior suggestive of vestibular dysfunction, such as head bobbing and circling. Temporal bones were fixed in 10% formalin for GREATER THAN 1 week, decalcified, and then processed for tissue clearing by Sca/e S protocol (Hama et al. 2015. Nat Neurosci 18(10):1518-1529), labeling, imaging using ZEISS Z.1 Lightsheet microscope, and 3D reconstruction with Arivis Vision4D software.
Results: The LASV-infected mice showed imbalance behaviors starting at 24 dpi. No changes were observed in the cochlear and vestibular hair cells of the mice infected with LASV. In the acute phase of infection (7-8 dpi), LASV was distributed mostly within blood vessels in both cochlear and vestibular organs. In the convalescent phase (24-35 dpi), LASV was leaked out from blood vessels, distributing into the stroma underlying the cochlear and vestibular sensory epithelium and within cochlear and vestibular ganglion cells.
Conclusions: The results suggest that LF-induced audiovestibular dysfunction is not caused by direct damage to the inner ear hair cells. The spread of LASV via blood vessels to sensory organs and ganglion cells is likely leading to LF-induced audiovestibular dysfunction.

SU27. The TECTB-C225Y Mutation Causing Autosomal Dominant Deafness in a Nicaraguan Family Enhances Sensitivity to Noise-Induced Hearing Loss in Ageing Mice

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Category: Inner Ear: Anatomy & Physiology
Background: The tectorial membrane (TM) is an extracellular matrix that lies above the mechanosensitive hair cells in the cochlea. The shear of the TM plays a major role in displacing stereocilia and facilitating hair cell mechanotransduction. TECTB encodes a major non-collagenous protein of the TM. Here, we reveal a TECTB-C225Y substitution causing an autosomal-dominant hearing loss in a Nicaraguan family and our Tectb-C225Y mouse to model the phenotype observed in patients and to investigate mutational effects on hearing. We carried

out a longitudinal study of mouse hearing, histological assessment of the effect of TECTB-C225Y upon the structure of the TM, and noise exposure tests to determine robustness of the membrane under stress.
Methods: Exome sequencing, linkage analysis and audiometry assessments were performed in a Nicaraguan family with autosomal-dominant non-syndromic hearing loss. The hearing of TectbC225Y/C225Y, TectbC225Y/+, and wild-type mice on corrected Cdh23AHL+ background were tested by ABR and DPOAE at 5 weeks, 3, 6, 9, 12, and 18 months. Samples were collected at 2, 6, and 14 months for histology. Porosity of the TM was assessed on histological sections stained with Toluidine Blue. For noise exposure, TectbC225Y/+ and wild-type mice underwent auditory testing before noise exposure at 8 weeks or 18 months, and then at 2 days and 2 weeks after noise trauma (98 dB SPL for 8-week-old mice and 100 dB SPL for 18-month-old mice, for 2 hours).
Results: The TECTB-C225Y variant segregated in individuals with mild-to-severe sensorineural hearing loss. Auditory testing showed significantly increased thresholds in TectbC225Y/C225Y mice at all time points. Auditory thresholds in TectbC225Y/+ and wild-type mice were not significantly different at any tested time point. Histological analysis showed a significantly increased degree of porosity in TectbC225Y/C225Y TMs at all ages; TectbC225Y/+ TMs showed a significantly lesser increase in porosity which was itself slightly higher than that of wild-type at some cochlear locations. Following noise exposure at 8 weeks, no significant difference was detected in auditory-threshold recovery between TectbC225Y/+ and wild-type mice. However, there was a difference at 18 months with TectbC225Y/+ mice showing a greater temporary threshold shift and a much larger permanent threshold shift in response to noise exposure than did wild-type controls.
Conclusions: We propose TECTB as an autosomal-dominant non-syndromic hearing loss gene. Testing has confirmed that TECTB-C225Y disrupts TM structure and function. This effect appears recessive in the mouse model, contrary to human pathology. However, following noise exposure, the combination of age and noise insult reveals a difference between the robustness of the TM in TectbC225Y/+ and wild-type mice. This provides new information about the role of TECTB within the TM and new insight into its role in human hearing loss.

SU28. Comparing and Optimizing Clearing Protocols for Fluorescence Microscopy of the Intact Mouse Cochlea

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Category: Inner Ear: Anatomy & Physiology
Background: Assessing the number of hair cells and spiral ganglion neurons in animal cochleae is a common task required to characterize pathophysiological conditions and therapeutic effects. Until now, hair cell counts have mostly been obtained by dissecting the sensory epithelium, which requires sophisticated dissection skills and bears the risk of rupturing hair cell rows, in particular for the basal parts of the organ of Corti. The number of spiral ganglion neurons is typically estimated by counting cells in cross-sections. A more comprehensive and less error-prone method would be to chemically clear the intact inner ear and image the translucent tissue using fluorescence light-sheet microscopy. However, none of the studies to date have

systematically compared different protocols. Moreover, since these protocols take 2-4 weeks to complete, they are currently not routinely used.
Methods: We compared five different clearing methods, three organic solvent based, two aqueous based. The two best protocols were optimized to allow tissue clearing and immunostaining of a mouse cochlea in as little as five days. Imaging was performed with a LaVision Ultramicroscope II Light Sheet Microscope (Milteny Biotech).
Results: We found clearing ability, tissue preservation and fluorescence immunostaining to be superior after iDISCO/cDISCO and ethyl cinnamate (ECi) clearing procedures. In contrast, PEGASOS and ScaleS rendered the tissue less transparent, and the FRUIT procedure was unable to clear the cochlear tissue at all. We optimized the two best methods to perform cochlear clearing in an unprecedented speed of five days for mouse cochleae, and in an inexpensive and minimally toxic way. Both fast protocols, fDISCO and fECi, cleared the tissues equally effective as the longer protocols while preserving both morphology and fluorescence immunolabelling.
Conclusions: Our newly developed rapid tissue clearing methods allow reproducible 3D imaging of the immunostained cochlea with light sheet microcopy and automatic counting of different cell types such as inner and outer hair cells by cell segmentation.

SU29. Dynamic Optical Coherence Tomography as a Tool to Identify Cellular Structures and Assess Changes in the Mouse Organ of Corti

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Category: Inner Ear: Anatomy & Physiology
Background: Optical coherence tomography (OCT) is a non-invasive microscopy technique for cross-sectional imaging that has become widespread in the research community for studying the inner ear. The restricted contrast in optical scattering of various cell types, as well as the resolution of the microscopes typically used, has been a limitation to identifying structures at the cellular level. Here, we apply dynamic OCT, a technique capable of monitoring motion by analyzing OCT signal intensity over time to enhance contrast of structures within the organ of Corti (ooC).
Methods: We used dynamic OCT to measure the frequency profile within the intracochlear tissues of freshly euthanized CBA/CaJ mice. After surgically exposing the round window (RW), CBA/CaJ mice were euthanized. Dynamic OCT data of the ooC were acquired through the RW within an hour of death. We used a 4x objective (NA 0.13) and collected B scans across the ooC repeatedly at a rate of 130 Hz. A Fourier analysis was performed on the image intensity of each pixel over the time of collection. We then split and averaged the spectrum into three different frequency bands - low (0-5 Hz), middle (5-15 Hz), and high (15-65 Hz). The power spectrum within each frequency band was represented by color - red (low), green (middle), and blue (high), and an RGB image was created from the data.
Results: Preliminary data came from a total of 3 mice (2 female, 1 male). Dynamic OCT visually improved the ability to distinguish critical structures within the ooC. These included the outer hair cells, inner hair cells, and Deiter's cells. The otic capsule bone and the osseous spiral lamina had more relative power spectrum in the low frequency band compared to the other

regions. In comparison, both the outer and inner hair cells had more relative power spectrum within both the high and middle frequency bands. Deiter's cells revealed greater variation, alternating from values similar to OHC and IHC in the mid and high value frequency band to much lower values.
Conclusions: Dynamic optical coherence tomography improves the optical contrast of critical cells and structures within the organ of Corti. Based on our preliminary data, hair cells and Deiter's cells have more movement within them at higher frequencies, likely related to their role in recycling potassium. Thus, dynamic OCT may serve as a tool for assessing cellular changes in response to trauma or ototoxic insult.

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SU30. Chd7 and Sox2 Regulate Mammalian Vestibular Sensory Epithelium Development and Tonotopic Organization of the Cochlea

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Category: Inner Ear: Anatomy & Physiology
Background: Hereditary hearing loss and balance disorders are important clinical problems that have become easier to diagnose but have not greatly benefitted from advances in molecular therapies. An important step in designing therapies for hereditary hearing loss and balance disorders is understanding their underlying mechanisms. Chd7 encodes the ATP-dependent chromatin remodeler CHD7, mutated in CHARGE syndrome. Sox2 encodes Sex determining Region Y-box 2 family transcription factor SOX2. Our previous study showed that expression of Chd7 and Sox2 is tightly regulated during inner ear development, and perturbation of the balance between these two genes leads to complex inner ear malformations, including shortened cochlea and malformed semicircular canals. In this study, we explored possible roles of Chd7 and Sox2 in tonotopic organization of mouse cochlea and vestibular sensory epithelium development.
Methods: Chd7 germline and conditional mutant mice (Chd7Gt/+; Chd7flox/flox), Sox2 inducible Cre knockin (Sox2CreER/+), and Pax2-Cre transgenic mice were used to study Chd7 and Sox2 in cochlear and vestibular development. E14.5 embryos were processed for in-situ analysis. For tonotopic organization study of cochlea, regional cochlear markers alpha-2-macroglobulin (A2M) in the base, and follistatin (Fst) and enfrin B2 (Efnb2) in the apex, were used. The vestibular organs of postnatal day 0 (P0) pups were dissected and stained with neurofilament (NF) specific antibodies and phalloid in whole-mount assays and analyzed by confocal imaging.
Results: We found that A2M was expressed in the basal cochlea whereas Fst and Efnb2 were highly expressed in the apical cochlea in E14.5 wild type embryos. In E14.5 double heterozygous (Chd7Gt/+;Sox2CreER/+) embryos, Fst and Efnb2 were also highly expressed in the apical cochlea, similar to wild type littermate controls. In contrast, expression of A2M was greatly downregulated or absent in the basal cochleae of Chd7Gt/+;Sox2CreER/+ mutant embryos. The utricle and saccule from Chd7 conditional knock out mice (Pax2-Cre; Chd7flox/flox) were smaller than those from Chd7flox/flox controls, and hair cells in the

vestibular sensory epithelium were devoid of neurofilament innervation or phalloidin-labeled stereocilia.
Conclusions: These results demonstrate that the basal cochlea in Chd7Gt/+;Sox2CreER/+ double heterozygous mutant mice is truncated, while the apical cochlea is preserved, suggesting that Chd7 and Sox2 cooperate to regulate the tonotopic organization of the mammalian cochlea. The underlying mechanism for the cochlear truncation and underdevelopment of the utricular and macular sensory epithelia might involve retinoic acid signaling defects, and this is currently under investigation. These results will inform the design of novel targets for molecular therapies to treat hearing and balance disorders associated with CHARGE syndrome.

SU31. Differential Effects of Tmie Deletion on Canal and Otolith Function in Mice

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Category: Inner Ear: Anatomy & Physiology
Background: The transmembrane inner ear (Tmie) gene encodes a protein that is part of the mechanoelectrical transduction (MET) channel complex in hair cells (Zhao et al., 2014). While Tmie's role in auditory hair cell function has been well-studied, its role in vestibular system remains to be elucidated. This study aims to investigate how Tmie deletion affects the canal and otolith vestibular function in Tmie-/- mice by measuring the rotational and translational vestibulo-ocular reflexes (VORs) and vestibular afferent spontaneous discharge and responses to head rotation and translation.
Methods: Male and female wild-type (WT), Tmie+/-, and Tmie-/- mice (3-5 months old) were used in the study. Horizontal eye movement responses to head rotation (rVOR) and translation (tVOR) were recorded to assess the canal and otolith function, respectively. The animals were subjected to sinusoidal head rotation (0.2-4Hz) and translation (0.2-2Hz) while their eye movements were recorded using an ISCAN video-based eye tracking system. Gains and phases of the rVOR and tVOR were calculated by performing a fast Fourier transform (FFT) on the de-saccaded eye velocity and head velocity signals. The canal and otolith function were further assessed through single unit recordings of vestibular afferent spontaneous activity and responses to head rotation and translation.
Results: Tmie+/- mice exhibited similar tVOR and rVOR responses to WT mice. Tmie-/- mice, however, displayed distinct canal and otolith dysfunctions. While Tmie-/- mice exhibited minimal rVOR responses, they retained reduced tVOR responses. 92 vestibular afferents were recorded from 4 Tmie-/- mice. Preliminary analysis showed that a substantial number of units responded to head translation (n=36), but only a few responded to head rotation (n=9). Both regular and irregular otolith units were recorded in Tmie-/- mice, but no regular canal unit was encountered. Vestibular afferents recorded in Tmie-/- mice exhibited reduced spontaneous discharge rates compared to WT controls.

Conclusions: Tmie is critical for the development of inner ear hair cell functions. It plays distinct roles in the semicircular canals, otolith organs, and cochlea.
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SU32. Can You Hear Without FIRE: The Impact of Microglia Loss on Cochlear Function

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Category: Inner Ear: Anatomy & Physiology
Background: The immune system is known to critically regulate the central nervous system during development, homeostasis, as well as during injury and repair. More recently, work has been undertaken to characterize the role of immune cells in the cochlea. Macrophages are the resident innate immune cells of the cochlea. Several studies on mice have reported that macrophages mediate drug uptake via the stria vascularis, promote synaptic repair after noise exposure, and participate in tissue remodeling after cochlear implantation. In other organs macrophages have shown remarkable heterogeneity. Recent work has uncovered several molecularly distinct macrophage subtypes within the cochlea, the specific functions of which remain unknown.
Methods: To examine the function of one of these subtypes – cochlear microglia, FIRE mice (Csf1rΔFIRE/ΔFIRE), a mouse model that is entirely microglia-deficient but retains other macrophage subtypes, along with Csfr1+/+ control mice were used. We assessed cochlear histology via immunostaining at postnatal days 5, 14, 6 weeks and 6 months of age, as well as cochlear function via auditory brainstem responses and distortion product otoacoustic emissions at 6 weeks and 6 months of age.
Results: Mice lacking microglia, as confirmed by the absence of Tmem119/Iba1 double positive cells in the cochlea, had the same number and organization of inner and outer hair cells in all three cochlear turns. Similarly, these mice also had a comparable number of inner hair cell synaptic complexes, with no difference in the number of orphan synapses either pre- or post-synaptically, as aged-matched control mice at all the time points examined. Furthermore, no gross differences in hearing thresholds or distortion products were observed at any frequency measured (4-32 kHz).
Conclusions: Taken together, these findings suggest that microglia are dispensable for cochlear development and maturation. Their role under pathological conditions requires further examination.

SU33. Cochlear Expression of Orexin Signaling Molecules and Their Role in Hearing

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Category: Inner Ear: Anatomy & Physiology

Background: Since we previously showed that the hypothalamic peptide CRF and its receptors are expressed in the cochlea, we were interested in assessing whether other hypothalamic homeostatic regulatory signaling systems might be expressed in the cochlea. Orexin (Ox) neurons localized to the hypothalamus project widely throughout the CNS [Song 2023] and are involved in sleep/wakefulness and glucose homeostasis. As abnormal Ox signaling via Ox receptor 2 (OxR2) is linked to sleep/wake disturbances (narcolepsy), Ox signaling has been targeted for interventions against insomnia. The Ox receptor antagonist Suvorexant (Belsomra®) represents one such drug. Use of Ox receptor antagonist drugs for insomnia, targets all tissues/organs expressing OxR's, illustrating their biological and clinical relevance. Here, we report on the expression of Ox signaling partners within the cochlea and the impact loss of Ox pre-prohormone and OrexinR1 has on hearing and cochlear organization following moderate intensity noise exposure.

Methods: Adult pre-pro-Ox (Hcrt gene) and OxR1 wild type and knockout mice were used to assess effects of loss of function on susceptibility to noise-induced hearing loss. Baseline auditory brainstem responses (ABRs) were obtained. Wild type and knockout mice were separated into two groups: 1) noise exposure (97 dB SPL, 8-16 kHz for 2 hours) and 2) no noise. ABRs were measured up to 10 days after noise exposure and threshold shifts determined. Data was aggregated and graphed (Prism). Animals were perfused 5 days – 6 weeks post noise exposure (or age-matched without noise exposure). Temporal bones were isolated, cryoprotected, and cryostat sectioned. Histochemistry (H and E) and immunohistochemistry were performed; antibodies used were Orexin A, Orexin B, Orexin R1, Orexin R2 and Aquaporin-1 [Santa Cruz BIotechnology]. Sections were DAPI counterstained and confocal imaged (Zeiss LSM 880).

Results: The Ox pre-prohormone, mature OxA and OxB peptides, and the Ox Receptors 1 and 2 (OxR1 and OxR2) are localized within the cochlea using immunohistochemistry. Neither pre-pro-Ox KO nor OxR1 KO mice recover ABR thresholds following noise exposure levels that induce classic, fully recoverable TTS in wild type mice at 7 days and 10 days, respectively. Further histological examination of OxR1 KO mice shows diminished staining intensity in the spiral ligament (hematoxylin and eosin). Aquaporin-1 protein localization is upregulated in OXR1 knockout animals and noise challenge alters pre-pro-orexin protein localization in the spiral ligament in the putative type 3 fibrocyte region.

Conclusions: Deficiency in Ox signaling may lead to greater incidence of permanent threshold shifts following exposures to even moderate intensity noise that induce only TTS in WT mice. Whether NIHL is exacerbated by Ox receptor antagonist drug use remains to be fully determined. However, given that most people using such drugs are also older and likely also suffering from age-related hearing loss, this question takes on greater significance. Supported by R21DC020326

SU34. Molecular Targeting of Alzheimer's Disease-Associated Proteins in the Spiral Ganglion and Cochlear Neural Components by Dysferlin Protein-Protein Interactions

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Category: Inner Ear: Anatomy & Physiology

Background: Protein-protein interactions presumed to underly deficits due to Alzheimer's disease (AD) in the cochlea require consistent protein co-localizations accompanying molecular targeting. A prerequisite first step is characterization and localization of Alzheimer's disease-related proteins in the wildtype animal which could be targeted in mutations occurring in AD. Without protein targets in the wildtype cochlea, AD may only alter cochlear function indirectly via neural implementation. While the expression of AD-linked proteins in the cochlea has not been studied in detail, the exception is investigation of BACE1 (β-secretase), the enzyme responsible for cleavage of amyloid precursor protein (APP) in AD. BACE1 has been determined to be required for hearing in wildtype compared to BACE1-/- mice [1]. Positive immunosignal for BACE1 was observed in both the boutons of efferent olivocochlear fibers and post-synaptic afferent spiral ganglion fibers, suggesting neural sites of action [1]. Tau itself was previously identified in nerve fibers under the inner and outer hair cells of the organ of Corti and osseous spiral lamina by immunoelectron microscopy [2].

Methods: In the present investigation, confocal microscopy with 0.6-1 μm optical slice z-stacks was used to differentiate immunofluorescence emanating from direct/indirect binding partners of wildtype Alzheimer's disease proteins conducted with a Zeiss LSM 780 instrument at 63× magnification of mid-modiolar 4-5 μm cochlea sections of the adult rat. Immunoreactivity was initially determined with 3,3′-diaminobenzidine (DAB) serving as chromogen (Bio-Genex, San Ramon, CA). Yeast two-hybrid analysis identified direct protein interactions of rat CNGA3 with PSENEN, presenilin enhancer gamma secretase subunit, a component of the PSEN1 protein complex modified in Alzheimer's disease.

Results: Evidence of separate groups of protein clusters/co-localizations were replicated in the spiral ganglion (SG) and nerve fibers approaching the habenula perforata. BACE1 co-localized with dysferlin in nerve fibers approaching the habenula perforata and with FKBP8 in mitochondria of type I spiral ganglion cell bodies. Tau co-localized with FKBP8. PSEN1, in contrast, co-localized with ryanodine receptors on the cell membrane of type I cell bodies within the SG, with evidence of internalization of both proteins approaching the mitochondria layer.

Conclusions: Two types of protein clusters related to AD are present in type I spiral ganglion cell bodies and afferent nerve fibers that would enable AD mutation-linked hearing deficits. The first, which would have input from dysferlin and dysferlin protein-protein interaction pathways, are mitochondrial targets anatomically defined by localization of the mitochondria outer membrane marker protein, FKBP8, along with AD proteins BACE1 and tau. A second protein complex on the cell body membrane includes the ryanodine receptors that would regulate Ca2+ and AD protein PSEN1.

[1] Dierich M et al. J Neurosci 39:9013-9027, 2019. PMID 31527119.

[2] Slepecky NB, Ulfendahl M. Hear Res 57:201-215, 1992. PMID 1733913.

SU35. 3D Characterization of Arteries in the Sheep Cochlea Using Contrast-Enhanced Micro-Computerized Tomography

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Category: Inner Ear: Anatomy & Physiology

Background: The cochlea is highly vascularized to meet its high metabolic demand. The main blood supply to the cochlea comes from the labyrinthine artery. Arterioles branched from the common cochlear artery travel through the central modiolar region of the cochlea to supply the microvasculature in different compartments. While the capillary network in the lateral wall has been extensively studied, the vasculature in other regions are less studied due to the technical challenges associated with imaging the vessels encapsulated in the bony portion of the cochlea, particularly in large animals. The aim of this project is to characterize the vasculature in the cochlea of the sheep, an emerging large animal model, to make a comparison with human cochlea.

Methods: Temporal bones from 6-week-old and 3-4-year-old female adult New Zealand Romney ewes were extracted within 1.5 hours post-mortem. Temporal bones were flushed and perfused with pre-warmed BriteVu through the carotid arteries and immediately chilled on ice and fixed in 4% paraformaldehyde. After decalcification in 8% EDTA for 8-12 weeks, tissues were microdissected and visualized by micro-computerized tomography (micro CT) using the Bruker Skyscan 1272 (Bruker, Kontich, Belgium). Reconstructed using InstaRecon CBR premium (Urbana, IL, USA) plus Bruker Nrecon and visualized in 3D using Bruker CTVox. quantifications and segmentations were performed using 3D slicer. In parallel, tissues were labelled with tomato-lectin, phalloidin or anti-CD34 antibodies, cleared with the PEGASOS method and imaged using Leica FV1000 (Leica).

Results: In 3D, common cochlear arteries, spiral modiolar arteries and radiating arterioles were visualized. External and internal radiating arterioles were very curvy within the bone; a feature that was prominent in the basal turn of the cochlea, however, became less evident towards the apex (n = 3). The outer diameter of spiral modiolar arteries of the sheep cochlea ranged between 37-48um in diameter, while the radial artery diameter ranged from 18-25um, and capillary size down to ~7um. Focusing on the round window, a capillary network was observed on both the middle ear and inner ear surface of the round window membrane; these capillaries did not penetrate through the round window membrane.

Conclusions: The step-wise increment in the sizes of vasculature and their anatomy in the sheep cochlea may mean that sheep could be a useful animal model for experimentation to understand the vascular flow in the human cochlea.

SU36. Micro-Ct and Histological Analysis of Sheep Cochlea and Tympanic Membrane

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Category: Inner Ear: Anatomy & Physiology

Background: Large animal models play an important role in translational research, such as drug-delivery, pharmacokinetic, or disease model research. Literatures show that the anatomy of sheep's outer, middle, and inner ear are similar to those of humans, which presents a promising area for research. However, some of the histology and anatomy in the sheep tympanic membrane and cochlea haven't been characterized yet. Therefore, this study aims to establish an anatomical and histological baseline in sheep cochlea and tympanic membrane.
Methods: Cochleae and the external auditory canal (EAC) were freshly dissected from 4-6-year-old female New Zealand (NZ) Romney sheep and fixed with 4% paraformaldehyde. After decalcified by 8% ethylenediaminetetraacetic acid, the cochleae were analysed by serial cryosection or whole mount preparation followed by haematoxylin and eosin staining, peripherin-1 immunofluorescence labelling, or phalloidin labelling. Fixed sheep cochleae and tympanic membranes were scanned by micro-computerized tomography, followed by the segmentation and 3D analysis with 3D slicer.
Results: The size of the NZ Romney sheep cochlea was 7.2±0.6 mm in length, 5.0±0.3 mm in width, and 3.2±0.8 mm in height (n = 3). The sheep cochlea had 2.17±0.11 turns, with a length of 7.2±0.6 mm and a total volume of 64.8±3.9 µl (n = 4). The inner hair cell and outer hair cell densities were 91.0±6.4 and 338.6±23.1 cells per mm of the basilar membrane, respectively (n = 3). The total spiral ganglion neurons (SGN) cell count was 18,867±3,201 cells per cochlea, with a density of 600.2±90.7 cells/mm² (n = 4). Of the SGN population, 5.3%±0.4% were positively labelled by anti-peripherin-1 antibody, a marker for type II SGNs (n = 3). The sheep EAC had a vertical diameter of 6.4±0.8 mm and a horizontal diameter of 4.2±0.3 mm (n = 3). The sheep pars tensa was approximately 30–100 µm thick with a surface area of 45.1±4.4 mm², while the pars flaccida was approximately 200–400 µm thick with a surface area of 25.7±2.7 mm² (n = 3).
Conclusions: Overall, the NZ Romney sheep cochlea is approximately 70% of the size of the human cochlea with a comparable shape and has a similar SGN and cochlear hair cell density to that of humans. In contrast, the sheep EAC diameter is about 40% smaller than that of humans, with a relatively thinner pars tensa and a larger pars flaccida. These differences may need to be considered when adapting the intratympanic injection procedure for sheep.

SU37. Molecular Profiles Defining Adult Cochlear and Vestibular Hair Cells

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Category: Inner Ear: Anatomy & Physiology
Background: The cochlear and vestibular sensory epithelia contain four different hair cell (HC) types for detecting sound and motion. Although the basic function of HCs is similar, there are substantial differences in morphology and function among them. The difference includes variations in ion channel composition and synaptic structure as well as vulnerabilities to ototoxic drugs, genetic deficits and aging. Furthermore, the hair bundle of vestibular HCs contains both stereocilia and kinocilia while kinocilia in the hair bundle of cochlear HCs disappear during HC

maturation. Kinocilia in HCs are necessary for planar cell polarity, stereocilia orientation, and signal sensing during development. The molecular mechanism underlying differences between adult cochlear and vestibular HCs as well as between type I and type II HCs is not clear. It is also unknown about the nature and property of kinocilia in adult vestibular HCs.
Methods: We used single-cell RNA-sequencing to examine gene expression of 1,522 HCs collected from adult mouse cochlear and vestibular sensory epithelia. Gene expression was validated by single molecule fluorescent in situ hybridization. Immunostaining and high-resolution confocal microscopy were used to examine protein localization in stereocilia and kinocilia.
Results: We identified approximately 1,000 genes which likely constitute the core genes for basic properties of HCs as mechanosensitive receptor cells. Comparison among different types of HCs also identified new marker genes as well as common and unique genes mediating mechanotransduction, ion channels and pre- and post-synaptic mechanisms in different HC populations. Gene enrichment analysis showed that vestibular HCs have differential expression of genes related to kinocilia and cilium motility including those related to the organization of axonemal repeat, the foundation for motile cilia. Immunostaining and high-resolution confocal imaging detected the expression of motile cilia regulator FOXJ1 in vestibular HCs and several key proteins for motile cilia in vestibular kinocilia, including the 96-nm rulers CCDC39 and CCDC40, and force generating protein DNAH5.
Conclusions: Our analysis provides new insight into molecular mechanisms defining mechanosensitive HCs and underlying morphological and functional differences between cochlear and vestibular HCs. Expression of motile cilia related genes/proteins in kinocilia of vestibular HCs implicates novel kinocilia-driven mechanism in enhancing mechanosensitivity in all HCs with kinocilia.

SU38. Hair Cell Proteins in Stereocilia Embedded in the Tectorial Membrane: Dysferlin, Annexin A2, FKBP8, Bcl2, and RyR3, Forming a Molecular Pathway to Regulate Ca2+

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Category: Inner Ear: Anatomy & Physiology
Background: Dysferlin direct protein-protein interactions (PPI) have been elucidated with surface plasmon resonance (SPR) and predicted to underlie membrane repair in mechanotransducing myofibrils of striated muscle [1]. In mechanotransducing inner ear hair cells, dysferlin is detected in the stereocilia and their inserts embedded in the tectorial membrane (TM). We have ascertained with SPR direct, tight positively-Ca2+-dependent interaction of dysferlin with anti-apoptotic protein FKBP8 [1]. FKBP8 was previously identified by Labay and Griffith [2] as directly interacting with the hair cell mechanosensory ion-channel component, TMC1. Dysferlin carboxy terminus would compete with TMC1 carboxy terminus for binding to the carboxy terminus of FKBP8, consistent with co-localization of dysferlin with TMC1 observed in myofibrils [1].
Methods: Confocal microscopy immunofluorescence Z-stack analysis was carried out with a Zeiss LSM 780 instrument at 63× magnification and 0.6–1.0 µm optical slices (Zen 2.1

software), immunolocalizing dysferlin PPI proteins for mid-modiolar 4-5 µm cochlear sections. Immunoreactivity was initially determined with the chromogen 3,3′-diaminobenzidine. Yeast two-hybrid analysis identified direct protein interactions of CNGA3 with ryanodine receptors (RyRs). Western analysis was carried out on proteins expressed in dysferlinopathy.
Results: Observation of expression of FKBP8 in the cochlear tectorial membrane at P6 in rodents by Zak et al. [3] has been extended to determination of FKBP8 expression in IHC and OHC stereocilia TM insertions at P28 with confocal Z-stack immunofluorescence. Evidence was obtained of co-localization of members of a dysferlin/annexin A2/FKBP8/Bcl2/RyR PPI pathway in hair cell stereocilia, with concomitant appearance of the stereociliary insertions in the tectorial membrane. When overexpressed, FKBP8 evokes an elevation of anti-apoptotic Bcl2, inhibition of ryanodine receptor activity and consequent reduction in Ca2+ release. Bcl2 couples to all three ryanodine receptors initially detected with a pan antibody. A specific RyR3 antibody localized RyR3 to the tip of the TM in close association with a third-row OHC stereociliary Bcl2-positive insertion "hanging" from the TM, also the site of dysferlin, annexin A2, FKBP8, RyR accumulation. RyR3 was also found in the IHC and OHC stereociliary arrays.
Conclusions: A dysferlin/annexin A2/FKBP8/Bcl2/RyR3 interaction pathway is expressed in inner ear hair cells and their stereociliary TM inserts, possibly contributing to regulation of TM Ca2+ and consequently MET currents and otoacoustic emissions [4] controlling hearing sensitivity [5].
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SU39. Non-Invasive Imaging of the Inner Ear in Animal Models

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Category: Inner Ear: Cochlear Mechanics
Background: Scala media, containing endolymphatic fluid, has high potassium and low sodium concentrations compared to its surroundings (scala tympani and scala vestibuli, which have high sodium concentrations). The role of this inner ear ionic homeostasis equilibrium in various cochlear pathologies remains unclear clinically, as invasive methods used in animal models are not feasible for clinical measurements in patients. Therefore, we performed initial investigations of non-invasive, clinically relevant methods to study the status of the cochlear endolymph system. We employed advanced imaging techniques, including SPECT/PT/CT, and MRI, to visualize the inner ear in CBA/CaJ mice and guinea pigs, and assess their possibilities for clinical applications.
Methods: Potassium Imaging: Single photon emission computerized tomography (SPECT) was used to image the inner ear of young adult CBA/CaJ mice. A potassium analogous radio tracer –

Thallium-201 (201Tl), was intravenously injected into tail veins; and the brain, cochlea, and whole body were scanned using a micro PET/SPECT/CT system. CT imaging was carried out for anatomical referencing.
Sodium Imaging: Positron emission tomography (PET) was used to image the inner ear of young adult CBA/CaJ mice. A sodium analogous radiotracer – Sodium-22 (22Na), was injected into tail veins using a catheter. Like before, CT imaging was done for anatomical references, and the brain and cochlea were scanned. Further, the Na-MRI technique was used to image sodium in the inner ear of guinea pigs, and a standard MRI was performed for anatomical referencing.

In all the experiments, animals' body temperature was maintained at 37 oC, and physiological metrics were monitored continuously to ensure animal health during the imaging experiments.

Results: Initially, detectable radiation 201Tl signals were observed from phantom experiments; the signal increased with the phantom volume. A SPECT 201Tl signal was observed in the CBA/CaJ mice cochlea, confirming that 201Tl can enter the mammalian cochlea. Similarly, Na-22 radiation (PET imaging) signals were also observed from the cochlea of CBA/CaJ mice. The region of interest (ROI) was drawn with the help of CT structural images, and subsequently, cochlear signals were measured. Equal absorption of radiotracers was observed for both right and left cochleae.
Similarly, Na signals were observed in the guinea pig's cochlea in Na MRI experiments. Proton MRI was used for anatomical referencing, and cochlear regions of interest (ROI) were identified. As expected, both right and left cochleae had equal signals.

Conclusions: Our study shows that non-invasive molecular/nuclear and functional MRI imaging techniques can be used to measure key physiological properties of the inner ear effectively.
This work is supported by the following grants: NIH-NIDCD 1R21DC020091, American Otological Society (AOS) – Research Grant, USF strategic investment pool grant, and Missouri University of Science and Technology Start-up.

SU40. Electrical and Mechanical Basis for Prestin's Role in Mammalian Hearing Adaptation to Loud Sound

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Category: Inner Ear: Cochlear Mechanics
Background: The mammalian auditory system's remarkable ability to process a vast spectrum of sound intensities hinges on the interplay between prestin-mediated amplification and mechanoelectrical transduction (MET) channel adaptation. This complex mechanism allows for precise sound processing across varying intensities, forming the foundation for our investigation into cochlear adaptation, particularly during exposure to loud sounds. Over 400 million people worldwide suffer from hearing loss, with more than a billion young individuals at increasing risk from unsafe sound levels, underscoring the importance of understanding these adaptive mechanisms.

Methods: Methods:
We employed a novel approach combining extracellular recordings of MET channel receptor potentials with AI-enhanced high-speed confocal imaging in acute guinea pig temporal bone preparations. Measurements were conducted in the low-frequency region of the cochlea. Our protocol included baseline, mild stimulation (70 dB SPL), loud stimulation (105 dB SPL), and recovery phases, using 160 Hz tone bursts. This method enabled simultaneous capture of hair cell electrical responses and mechanical changes in outer hair cells (OHCs) and surrounding structures, providing insights into cochlear adaptation dynamics across different sound intensities.

Results: Our study revealed a fast electrical adaptation mechanism with a time constant of 66 microseconds in control conditions, which increased 30-fold to 2 ms upon prestin activity reduction with salicylate. We also identified a slow electrical adaptation process with time constants of 11.2 ms and 325.8 ms for fast and slow components, respectively. Mechanical analysis demonstrated that OHCs exhibited distinctive adaptive behaviors in their displacements and surface area changes, operating on the same timescale as slow electrical adaptation. Notably, we observed a fundamental shift in OHC mechanics between loud and mild stimulation, with axial and radial displacements showing a negative correlation ($r = -0.21$) under loud sounds and a strong positive correlation ($r = 0.64$) during mild sounds. Salicylate severely disrupted both electrical and mechanical OHC-specific responses, while EGTA and dihydrostreptomycin, which reduce MET channel Ca2+ and K+ entry respectively, exerted only mild effects. Non-sensory structures displayed minimal adaptation, underscoring the specialized role of OHCs in cochlear mechanics.
Conclusions: Our findings provide compelling evidence for prestin function's pivotal role in both electrical and mechanical adaptation to sounds, supporting its function in adjusting cochlear operating points across different sound intensities. The observed inversion of OHC mechanical responses between loud and mild stimulation may represent a protective mechanism during intense auditory input. These insights open new avenues for developing targeted interventions to prevent noise-induced hearing loss, addressing a growing global health concern affecting over 1 billion at-risk individuals worldwide. Further research into these adaptive mechanisms could lead to innovative therapies for hearing disorders and improved strategies for preserving auditory function in challenging acoustic environments.

SU41. Two-Dimensional Organ of Corti Motion in the Mouse Apex

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Category: Inner Ear: Cochlear Mechanics
Background: Despite tremendous progress in uncovering cochlear micromechanics with the introduction of optical coherence tomography (OCT), two- and three-dimensional organ of Corti motions are still not well understood. In addition, recent work in gerbil (Cho and Puria, 2022) showed that the reticular lamina (RL) moved more than the basilar membrane (BM) and that the motion varied between rows of outer hair cells. Passive mechanics measurements in gerbil (Zhou et al., 2022) showed similar transverse motion between the BM and RL and increased radial

motion in the RL. Previous work in the mouse apex in vivo (Lee et al., 2016) suggested that radial tuning was present in both the BM and RL and was of a similar magnitude in the two directions. However, their equipment did not provide the resolution to resolve motion along the different rows of hair cells. We aimed to explore radial and transverse motions of the BM and RL in the mouse apex at the three rows of outer hair cells in vivo.
Methods: We used our 905 nm center wavelength, single beam Thorlabs OCT system mounted on a six-axis robot arm to capture two-dimensional organ of Corti motion measurements at a higher resolution than previous experiments. We achieved planar rotations using a tool reference frame aligned to that of our OCT B-scan for measurements at the same longitudinal place. To quantify our measurement angles, we implemented the orientation program described in Frost et al., 2022 and Frost et al., 2023. Vibration measurements in mice were obtained using methods similar to those described in Cho and Puria (2022, Scientific Reports). Tone sweeps were presented through the ear canal, and vibrations of the basilar membrane and organ of Corti structures were measured using VibOCT and SyncAv—LabView-based programs built in-house.
Results: Our preliminary results suggested that the RL of the first row of hair cells moved similarly in the radial and transverse directions and that this motion was greater than that of the BM. Furthermore, high-level measurements showed a decrease in motion from the first to third row of hair cells. The BM did also show radial tuning, and this motion was less than in the transverse direction.
Conclusions: Combining our single-beam, high-resolution OCT system and six-axis robot arm allowed us to capture motion along different rows of outer hair cells. This is an important step in untangling the intricate motions within the organ of Corti and support future development towards three-dimensional motion measurements. [Supported by the Amelia Peabody Charitable Fund and NIDCD R01DC07910, F31DC021079, and T32 DC000038.]

SU42. Sex Differences in TRPA1-Mediated Changes to the Operating Point of the Organ of Corti After Noise Exposure

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Category: Inner Ear: Cochlear Mechanics
Background: Transient receptor potential ankyrin 1 (TRPA1) channels detect tissue damage throughout the body. TRPA1 channels are highly expressed in nociceptive neurons and in the Hensen's cells of the organ of Corti. Our laboratory recently found that TRPA1 plays a role in the noise-induced temporary threshold shift through a mechanism likely involving the cochlear supporting cells (Velez-Ortega et al., Nat Commun, 2023). Specifically, TRPA1 activation in Hensen's cells results in prolonged calcium responses that propagate across the organ of Corti and cause long-lasting tissue displacements. We hypothesize that these TRPA1-mediated tissue displacements affect the stiffness and/or geometry of the organ of Corti thus modifying its operating point. Here, we used auditory brainstem responses (ABR) to evaluate TRPA1-dependent changes in cochlear microphonics (CM) in the same mice before and after noise exposure.

Methods: We used TRPA1-deficient mice (Kwan et al., Neuron, 2006) and wild-type littermates, as well as mechanotransduction-deficient CIB2 F91S mutant mice (Giese et al., Nat Commun, 2017), all maintained in a C57Bl/6 background. Ratios of roughly 50:50 male:female mice were utilized for all experiments. ABR to click and tone-burst stimuli were recorded in anesthetized mice, before and after the exposure to broadband noise at 100 dB SPL for 30 minutes. We subtracted the 0° and 180° phase ABR recordings to extract CM data. With this approach we obtained CM recordings in live mice that were significantly larger than those recorded in post-mortem mice or live mice lacking hair cell mechanotransduction (CIB2 F91S).
Results: Our results show significant differences in the amplitude of the summating potential (SP) in click-evoked ABR between TRPA1-deficient mice and wild-type littermates. However, five days after noise exposure, when mice from both genotypes still exhibited elevated hearing thresholds, the SP differences were no longer observed. In addition, mice showed a direct current (DC) shift in the CM elicited by an 8 kHz tone burst as the sound intensity increased, which was delayed in TRPA1-deficient mice. We also observed an overall reduction in CM amplitudes in the TRPA1-deficient mice which was not seen in wild-type littermates. Interestingly, this reduction was driven by the female TRPA1-deficient mice. In addition, we observed a larger amplitude of the ABR wave I in response to high intensity stimuli (GREATER THAN 90 dB SPL) exclusively in the female TRPA1-deficient mice.
Conclusions: TRPA1 activation after noise exposure changes the operating point of the organ of Corti, which could serve as a protective mechanism against noise-induced hearing loss. Additionally, we discovered a previously unknown sex difference in TRPA1-mediated regulation of hearing sensitivity.

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SU43. Analysis of Phase and Displacement Consistency in Optical Coherence Tomography Vibrometry Measurements From the Guinea Pig Cochlear Apex and a Piezo Driven Model

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Category: Inner Ear: Cochlear Mechanics
Background: Signal processing approaches inevitably influence interpretation of optical coherence tomography vibrometry (OCTV) data derived from the inner ear. Multiple approaches exist, and each has its own pros and cons. We recently used a region of interest, signal to noise ratio (SNR) criterion and median averaging approach to provide robust signal quality from our in vivo OCTV data.
Methods: In order to validate our approach, we have investigated the distribution of the phase and displacement of pixels corresponding to apical organ of Corti in the guinea pig and using a piezo mounted phantom model with similar reflectivity to tissue.
Displacement and phase data obtained from three locations within the cochlear apex of anesthetized animals, and previously published (Burwood, G., Hakizimana, P., Nuttall, A.L. and

Fridberger, A., 2022. Best frequencies and temporal delays are similar across the low-frequency regions of the guinea pig cochlea. Science Advances, 8(38), p.eabq2773.), and that obtained from the phantom, was analyzed with different regions of interest, and using a statistical approach. The distributions of responding pixels, their optical and mechanical signal properties, and the effects of our analysis technique upon outliers was appraised.

Results: Displacement histograms indicate that the region of interest approach produces a normal distribution in behavior. Radial scanning position had a minimal effect on phase. Concerning phase, we noted a small percentage of pixels satisfying our SNR criterion were outliers, creating a bimodal distribution. This outlier population increased with displacement, indicating that the noise is one of optical rather than mechanical origin. Our median averaging approach for data that satisfied our mechanical SNR criterion effectively minimized the contribution of outliers. We noted that pixels with lower optical signal level, close to strongly reflective pixels, were more likely to contribute to phase noise akin to the findings of Lin et al. 2017 (Lin, N.C., Hendon, C.P. and Olson, E.S., 2017. Signal competition in optical coherence tomography and its relevance for cochlear vibrometry. The Journal of the Acoustical Society of America, 141(1), pp.395-405.).
Finally, we double checked our analysis of propagation time using a column and row-wise examination, rather than a whole organ of Corti region of interest approach. We found that the degree of propagation difference changed dependent on where in the organ of Corti the row or column was derived, but that overall our findings of group delay coherence or evidence of dominant reverse propagation were confirmed.

Conclusions: In conclusion, our signal processing approach used prior is sufficient to describe displacement accurately, minimize an optically sourced phase noise, and capture the unusual mechanical qualities of the cochlear apex.

SU44. Vibration Patterns of Outer Hair Cell-Deiters' Cell Complex

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Category: Inner Ear: Cochlear Mechanics
Background: The complex vibrating pattern of the organ of Corti holds the key to understanding how OHCs modulate hearing sensation because the pattern varies depending on the level of outer hair cell (OHC) action. Recent experimental observations of tomographic vibrometry have offered opportunities to analyze the complex vibrating patterns. The OHC-Deiters' cell complex (ODC) vibrations characterize sensitive and insensitive cochlea well. We measured ODC vibrations from isolated cochleae using optical coherence tomography at a resolution fine enough to distinguish individual cells. We treated the OHC and the Deiters cell as the member elements of a complex and analyzed its 2D vibration patterns in the radial section.
Methods: Cochleae were acutely excised from young Mongolian gerbils (15-30 days old, both sexes). After being reduced to a single turn centered around a target location (10 kHz CF), the excised cochlea was placed in a custom-designed chamber. Acoustic pressures and alternating currents were applied to the tissue at different frequencies to evoke the passive and the active

vibrations, respectively. The resulting vibrations were measured at two orientation angles using an optical coherence tomography system. Forty to sixty M-scans were acquired across the span of the organ of Corti (2-5 μm between M-scans).
Results: The ODC had distinct vibration patterns when it was subjected to acoustic or electrical stimulations. The ODC vibrated like a rigid body when stimulated by acoustic pressures. Meanwhile, the motion due to electric stimulation revealed interesting relative motions between two sub-components (the OHC and the Deiters cell). When subjected to acoustic pressures, the ODC underwent mostly translational motion and minimal bending deformation. When the compound was subjected to electrical stimulation, the ODC bent mostly at the Deiters cell root. Most bending deformation took place in the lower end of the Deiters cell. While there was minimal translational motion at the Deiters cell root (on the basilar membrane side), there was greater motion in the upper part of ODC (on the reticular lamina side). The vibration patterns of the ODC changed as the frequency of electrical stimulation increased.
Conclusions: The Deiters cells lock and unlock the organ of Corti frame depending on two stimulating modes. When acoustic pressures vibrate the organ of Corti, the ODC deforms minimally. On the other hand, when vibrated by OHC motility, the ODC is deformed in its upper part due to the deflection of the Deiters cell base.

SU45. Meniere's Disease: The Mechanism of Low-Frequency Hearing Loss Due to Bony Labyrinth Cracks

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Category: Inner Ear: Cochlear Mechanics
Background: Meniere's disease is an inner ear disorder characterized by recurrent episodes of vertigo, accompanied by auditory symptoms such as hearing loss, tinnitus, and a feeling of fullness in the ear, with endolymphatic hydrops recognized as its underlying pathology. The hearing loss associated with Meniere's disease initially presents as low-frequency hearing loss, which is said to expand to affect the entire frequency range as the symptoms progress.
Methods: The authors propose a cochlear fluid dynamics model that rigorously addresses the acoustic phenomena of the cochlea by taking into account the compressibility of the lymphatic fluid. This model has been used to elucidate auditory mechanisms and hypothesize the causes of various auditory disorders. Based on the authors' experience with cochlear simulations, it has been confirmed that low-frequency hearing loss can occur when: (1) a fistula forms in the lateral wall of the scala vestibuli, allowing internal lymphatic fluid to come into contact with external air; (2) bubbles are present at a midpoint in the scala vestibuli, preventing sound waves from propagating to the apical side; and (3) a hole opens in the basilar membrane connecting the scala vestibuli and scala tympani. However, none of these phenomena sufficiently explain Meniere's disease, and the authors believe that the underlying mechanisms must be distinct.
It has long been believed that the bony labyrinth, composed of particularly hard bone, is incapable of fractures or cracks. However, the authors have found specimens with cracks in the thin bone separating the scala vestibuli of the lower turn from the scala tympani of the upper turn. These cracks are extremely small, making them difficult to detect even with high-resolution

CT scans. The authors modeled these cracks in their proposed cochlear fluid dynamics model and simulated.

Results: The simulation results indicated that a crack measuring 0.1 mm in width and 5 mm in length could cause significant low-frequency hearing loss in the bony labyrinth. Furthermore, as this crack enlarges, the hearing loss can extend from low frequencies to the entire frequency range.
Conclusions: Since the bone forming the bony labyrinth is said not to develop granulation tissue, cracks would not fully heal; this means that only the mucosa is likely to repair. In such a case, the symptoms of vertigo might subside because lymphatic fluid does not move through the crack. However, once an external force is applied to the cochlea with reduced stiffness, a crack may reappear in the bony labyrinth. Additionally, as the crack further expands, low-frequency hearing loss could extend to a broader range. This could explain the temporal progression of Meniere's disease. The authors hope for collaboration from medical research groups in validating the authors' hypothesis of Meniere's disease.

SU46. Outer Hair Cells Suppress and Boost the Organ of Corti Vibrations

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Category: Inner Ear: Cochlear Mechanics
Background: Outer hair cells (OHCs) modulate the organ of Corti (OoC) vibrations so that responses to soft sounds are enhanced selectively near the best frequency (BF). In neural responses, the hearing threshold increases near the BF and decreases in the tail region after suppressing active OHCs. Recent observations seem to enlarge the difference between neural and mechanical tuning. For instance, OHCs boost OoC vibrations non-selectively. While fluid dynamics in the scala have been extensively studied, the interaction between active OHCs and their supporting structures in the OoC is drawing increasing attention. Moreover, the interaction between the OoC and the Corti fluid (the extracellular fluid in the OoC) has hardly been investigated. Through model simulations, we demonstrate how active OHCs interact with elastically through the OoC frame and viscously through the Corti fluid.
Methods: The Virtual Cochlea, a computational model of cochlear mechanotransduction, incorporated 3D OoC micromechanics, scalae fluid dynamics, and the kinetics of hair cell mechanotransduction and motility. The model incorporated the 3D OoC geometry of the gerbil cochlea. New to this study, the model includes the Corti fluid dynamics, solved simultaneously with the Virtual Cochlea. The Corti fluid boundary motion determined the cross-sectional area changes of the Corti fluid space. For validation, simulated OoC vibrations were compared to 2D vibrations measured from excised gerbil cochleae, especially at the OHC-Deiters cell joint (ODJ).
Results: Viscous coupling through the Corti fluid resulted in three distinct frequency tuning patterns. First, the basilar membrane vibrations were amplified near the BF. Second, the ODJ motion was amplified over the entire frequency range. Third, the reticular lamina motion was amplified near the BF but suppressed toward low frequencies. These different frequency responses disappeared as the Corti fluid dynamics were turned off. To further investigate the suppression by active OHCs, the effect of locally inhibited OHCs was simulated. Inactivating

OHCs at the BF location reduced amplification. Similar OHC inactivation at a location half an octave below the BF increased vibrations over the entire frequency range. Our results suggest that OHCs modulate (amplify and suppress) OoC vibrations depending on frequency.
Conclusions: Our computational study demonstrates that active OHCs modulate cochlear vibrations to achieve better frequency tuning. Active pressure transmission through viscous Corti fluid is required to explain the suppression by OHCs. Our observation of the reticular lamina frequency response is in line with the tail region hypersensitivity observed in neural tuning responses after suppressing or damaging OHCs (Liberman and Dodds, 1984; Henry, Kale, Heinz, 2016).

SU47. Measurements in Vivo Show That Active Outer-Hair-Cell Motion Exceeds and Can Drive Basilar-Membrane Motion up to Frequencies of 40 Khz

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Category: Inner Ear: Cochlear Mechanics
Background: Cochlear responses to sound are thought to be amplified by outer hair cells (OHCs) expanding and contracting lengthwise in response to audio-frequency changes in their transmembrane potential. However, how OHCs produce this cochlear amplification is not understood. For individual OHCs in micro-chambers, the injected-current to measured-displacement characteristics are low pass with corner frequencies of a few kHz. In vivo, OHC corner frequencies were estimated to be ~3 kHz. Since cochlear motion is amplified for tones at many tens of kHz, the low OHC corner frequency has appeared to be a problem for high-frequency amplification to be produced by OHCs.
Methods: We explored the relationship between OHC motion and basilar-membrane (BM) motion using high-resolution optical-coherence-tomography (OCT) with results in seven gerbils at the 38-45 kHz best-frequency (BF) region. We measured transverse organ-of-Corti (OoC) motions at the OHC top near the reticular lamina (RL), at the OHC bottom near the OHC-Deiters-Cell junction (ODJ), and at the BM. From these in-vivo measurements we determined the transverse motions of individual OHCs. Data from all three locations were available on all gerbils only for the third OHC row (Cho and Puria, 2022), which provided the results described here.
Results: Cochlear motion changed from being greatest at the ODJ for frequencies an octave and more below BF, to being greatest at the RL for frequencies near BF. The motion magnitudes near BF were 2 to 20 times higher at the RL than at the ODJ. From the lowest frequencies up to ~40 kHz, the phase differences between the motions at the tops and the bottoms of the OHCs were close to ½-cycle. RL motion was ahead of ODJ motion by 0.4-0.5 cycle throughout most of the frequency region; at 30 and 40 kHz the phase difference was 0.5 +/- 0.1 cycle in most gerbils. All gerbils had OHC length changes that were greater than BM motion (usually by a factor of 2 or more) from low frequencies up to their BFs of 38-46 kHz. The OHC length changes were accommodated at low frequencies, by a decrease in Deiters-cell length with little change in overall OoC height, but at frequencies near BF produced a net cyclic increase in OoC height.

Conclusions: Our measurements show that, in vivo, OHCs expand and contract with amplitudes greater than BM motion up to 40-50 kHz, frequencies many times higher than the OHC corner frequencies measured in a dish or in vivo. Thus, a low OHC corner frequency does not prevent OHCs from moving significantly at frequencies up to tens of kHz so that OHCs can add energy to overcome cochlear damping and provide cochlear amplification. [Work supported by R01 DC07910 from the NIDCD of NIH (to SP).]

SU48. Harmonic Distortion Products Within the Cochlear Partition in the Basal Turn of Sensitive Gerbil Cochleae

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Category: Inner Ear: Cochlear Mechanics
Background: Auditory harmonic distortion has been demonstrated psychoacoustically in humans and electrophysiologically in experimental animals. It has been shown in vivo that the harmonics in the reticular lamina vibrations are significantly larger and have broader spectra and shorter latencies than those in the basilar membrane vibration. These observations imply that the mechanic harmonics are generated by motile outer hair cells over a broad cochlear region. To reveal the distribution of the mechanical harmonics within the cochlear partition, we measured the magnitude and phase of the second and third harmonic distortion products from the cross-section of the cochlear partition in the basal turn of the gerbil cochlea.
Methods: Young Mongolian gerbils of either sex with normal hearing were used in this preliminary study. The bulla on the left side was opened through a ventrolateral surgical approach. After the stapedial artery was removed from the bony surface of the cochlea, an opening was made in the lateral wall of the scala tympani of the basal turn. When the cochlear partition was positioned approximately in the horizontal plane, the magnitude and phase patterns of the vibrations were measured at stimulus frequency f0 and harmonic frequencies 2f0 and 3f0 from the cross-section of the cochlear partition using a scanning heterodyne low-coherence interferometer.
Results: The harmonic distortion products were detected at the intermediate and high sound pressure levels. At the best frequency, the reticular lamina in the outer hair cell region vibrates significantly more than the basilar membrane and other parts of the cochlear partition. Except for a sharp phase transition between the third-row outer hair cells and Hensen's cells, the vibration phase is constant across the cochlear partition. While the largest 2f0 is at the reticular lamina, the maximal 3f0 is located at the basilar membrane. At low stimulus frequencies, the f0, 2f0, and 3f0 all occur at the basal ends of the outer hair cells and in the Deters' cell region.
Conclusions: The present results indicate that the generation location of the harmonic distortion depends on the stimulus frequency and that the dynamic mechanical properties of the organ of Corti vary with the location within the cochlear partition in the sensitive living cochleae.

SU49. Translating a Computational Model of the Human Auditory Periphery to Gerbil and Mouse for Comparative Auditory Research

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Category: Inner Ear: Cochlear Mechanics

Background: Realistic computational models of the peripheral auditory system can assist researchers in better understanding how sensorineural hearing loss (SNHL) affects sound coding. Here we adapted a one-dimensional human non-linear cochlear transmission-line model [Verhulst et al., Hearing Research, 360, 55-75 (2018)] to mouse and gerbil models, aiming to facilitate cross-species research on SNHL.

Methods: We modified key model parameters such as cochlear length and height based on species-specific anatomical data. The characteristic frequency range of each model was adjusted to correspond to the tonotopic map of each animal. The pole trajectories of the local basilar-membrane (BM) admittance were calibrated to reproduce realistic cochlear tuning and compressive behavior. The models have been assessed by comparing BM and auditory-nerve (AN) output along with simulated distortion-product otoacoustic emissions (DPOAE) to in-vivo recordings from mice and gerbils. For the inner hair cell (IHC) drive in the gerbil model both BM velocity, as is used in the original human model, and BM desplacement were tested.

Results: The models closely mirror the observed data trends in BM-velocity compression and capture measured AN frequency tuning characteristics accurately. Whereas mouse AN thresholds require no further refinement, gerbil AN thresholds are too high when the BM velocity is applied as the drive for the IHCs. When BM displacement is applied as IHC drive, the AN thresholds in the lower frequency range are lowered and resemble the in vivo measured gerbil thresholds more closely. Finally, the DPOAE's produced by the human and mouse model show realistic values within the matching hearing range. The gerbil model on the other hand shows unrealistically high peaks and dips.

Conclusions: The developed models represent a new tool that can guide experimental research in mice and gerbils. Furthermore, extending the transmission-line model from humans to common laboratory animals allows for more thorough testing of the model, particularly in the case of SNHL, as more direct pathophysiological data is available for these species. The IHC drive in the gerbil model is better modeled as BM displacement as opposed BM velocity, resulting in improved AN thresholds. Our research shows that realistic features of the animal models can be captured by translating the human model to research animals.

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SU50. Effect of Subclinical Cochlear Damage on Brainstem Encoding of Amplitude Envelope

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Category: Brainstem: Structure & Function

Background: Natural sounds, speech, and music contain amplitude modulations (AM). Accurate encoding of AM information is essential for understanding speech. The impact of auditory damage on AM neural encoding is not fully understood. Animal studies suggest that noise-induced sensorineural hearing loss enhances temporal precision and amplitude envelope encoding. However, human studies on the relationship between auditory damage and amplitude envelope coding have yielded inconsistent results. Few studies in the context of cochlear synaptopathy have reported reduced envelope following responses in individuals with suspected synaptopathy, while others have found no difference in neural encoding. Interestingly, others have reported enhanced neural envelope encoding in hearing-impaired listeners, consistent with psychophysical studies of AM detection. Subclinical cochlear damage, a potentially major variable, was not adequately controlled for in human studies investigating neural encoding of AM. This study aimed to investigate the influence of subclinical hearing loss on brainstem encoding of sustained amplitude envelope, as measured by envelope-following responses. We used extended high-frequency hearing loss as a model for subclinical cochlear damage, as listeners with this condition have subtle cochlear deficits despite clinically normal audiograms.
Methods: Envelope-following responses were recorded from 30 young adults with normal audiograms using a two-channel recording system. Stimuli were sinusoidally amplitude modulated and presented monaurally at 80 dB SPL through an ER2A transducer, with a carrier frequency of 4000 Hz and a modulation frequency of 80 Hz. Modulation depths of 40%, 63%, and 100% were used.
Results: The initial analysis focused on examining the relationship between envelope following response magnitude, signal-to-noise ratio, and phase coherence measures. Detailed results and discussion will be presented in the context of early cochlear damage and functional implications for speech understanding in noisy environments.
Conclusions: The findings have significant implications for understanding the neural mechanisms underlying speech perception difficulties in individuals with clinically normal hearing.

SU51. Optical Coherence Tomography Imaging of the Posterior and Lateral Semicircular Canal in Mice

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Category: Inner Ear: Membranes & Fluids

Background: Endolymphatic hydrops (ELH) is associated with symptoms of fluctuating hearing loss, tinnitus, and vertigo, characteristic of Meniere's disease. The presence of cochlear ELH with distention of Reissner's membrane in mice after noise exposure has been characterized with Optical Coherence Tomography (OCT). In vivo OCT imaging of the membranous labyrinth in the horizontal and posterior semicircular canals (SCC) has not yet been described. We hypothesize that ELH present in the cochlea should also appear in the vestibular system. We

therefore used OCT to determine whether ELH present in the cochlea after noise exposure is also present in the horizontal and posterior SCC.
Methods: Wild type CBA/CaJ mice 6-11 weeks old were anesthetized with ketamine and xylazine anesthetic without and after 8-16 kHz noise exposure at 100dB for 6 hours . The left cochlea and horizontal and posterior SCC were surgically exposed. OCT was used to image the cochlea, visualize Reissner's membrane position, and establish whether ELH was present. The horizontal and posterior SCC were also imaged with OCT. Finally, we used a higher resolution optical coherence microscopy (OCM) system with a 4x objective to image the SCC in an attempt to improve the image quality. With this OCM system, we could also perform dynamic OCM imaging, which improves optical contrast by assessing for variations in pixel intensity over time. All animal experiments were approved by the USC Institutional Animal Care and Use Committee.
Results: The membranous labyrinth in the horizontal and posterior SCC could be identified using OCT. However, the mouse SCCs are small, and we found that the membranous labyrinth could be better resolved with OCM. Dynamic OCM imaging improved the ability to distinguish the membranous labyrinth even further. We found two spaces. One was large and in the center of the SCC. The other was a thin rim around the periphery. Gold nanoparticle injection via the posterior SCC was found to perfuse the perilymphatic space, confirming that the large central fluid chamber was endolymph. We then used ImageJ to measure the cross-sectional area of the two chambers and calculated the endolymph to perilymph (E/P) ratio. The E/P ratio was consistent between the lateral (2.9 +/- 0.4) and posterior (3.1 +/- 0.6) SCCs in control mice (n=4). Noise exposure resulted in ELH with distention of Reissner's membrane in the cochlea, but no significant difference in the E/P ratio in the lateral (3.6 +/- 0.8) and posterior (3.3 +/- 0.5) SCCs (n=4) as compared to control mice (p GREATER THAN 0.05, unpaired t-test).
Conclusions: OCT techniques resolve the membranous labyrinth and distinguish between the endolymphatic and perilymphatic spaces of the mouse horizontal and posterior SCCs. However, the large endolymphatic compartment relative to the small perilymphatic compartment makes it challenging to detect ELH in the mouse SCCs.

SU52. KCNQ2/3 Potassium Channel Activator Mitigates Noise-Trauma-Induced Hypersensitivity to Sounds in Mice

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Category: Inner Ear: Damage and Protection of Hair Cells
Background: Exposure to loud noise is the leading cause of highly debilitating hearing disorders, such as tinnitus and hypersensitivity to sound. Tinnitus and sound hypersensitivity often coexist. Our previous work has established that downregulation of KCNQ2/3 potassium channels in the Dorsal Cochlear Nucleus (DCN) after noise trauma contributes to the development of tinnitus in mice. Importantly, transient delivery of a highly selective and potent KCNQ2/3 channel activator, RL-81, after noise trauma, mitigates the development of tinnitus in mice. Because tinnitus and hypersensitivity to sounds often coexist and may share similar

underlying plasticity mechanisms in the auditory pathway, here, we tested whether transient delivery of RL-81 after noise-exposure could impact noise-trauma-induced hypersensitivity to sounds in mice. Next, because the increased sound-evoked activity of the auditory cortex (AC) is suggested to contribute to the development of hypersensitivity to sounds after noise exposure, we tested whether transient delivery of RL-81 after noise exposure reduces the increased sound-evoked activity of AC associated with hypersensitivity to sounds.
Methods: To assess hypersensitivity to sounds in mice after noise exposure (unilateral, 116 dB, 8-16 kHz, 1 hr), we employed an operant Go/No-Go behavioral assay. To measure the sound-evoked activity of AC after noise exposure, we employed in vivo wide-field calcium imaging in awake mice.
Results: Utilizing our operant Go/No-Go behavioral assay, we confirmed that application of salicylate or noise exposure causes hypersensitivity to sound in mice. Using our behavioral assay, we found that transient treatment with RL-81 after noise exposure mitigates the development of hypersensitivity to sounds in mice. Also, we found that RL-81 treatment after noise exposure mitigates the reduced reaction time to sounds associated with hypersensitivity to sounds. Finally, we found that RL-81 treatment after noise exposure reduced the increased sound-evoked responses of AC associated with hypersensitivity to sounds.
Conclusions: These results suggest that KCNQ2/3 channel plasticity likely contributes to the development of hypersensitivity to sounds, and highlights RL-81 as a promising clinical candidate for treating tinnitus and hypersensitivity to sounds.

SU53. Susceptibility to Noise Trauma in Deaf OTOF Knockout Mice

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Category: Inner Ear: Damage and Protection of Hair Cells
Background: Congenital deafness due to mutations in the OTOF gene (DFNB9) is due to a defect of vesicle release at inner hair cell ribbon synapses. Otoacoustic emissions, a measure of cochlear amplification, can be normal in newborn children but typically get lost within months to years after birth. However, we only observed a moderate acceleration of age-related outer hair cell degeneration in Otof knockout mice (Stalmann et al., Front. Cell Neurosci 2021). We hypothesized that the absence of protective reflexes (stapedial reflexes, olivocochlear reflexes) makes deaf ears more susceptible to noise damage.
Methods: We applied lower- (15 min 103 dB) and higher-intensity (2 h 120 dB) acute noise trauma as well as a novel chronic noise trauma paradigm (80-85 dB, 8h/night, 5 days/week, 3 months) to Otof knockout and wildtype mice. Auditory function was assessed by recordings of auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE). The degeneration of inner and outer hair cells and of synapses were assessed by immunohistochemistry and confocal microscopy.
Results: Acute lower-intensity noise trauma led to a slightly more pronounced decrease of DPOAE in Otof knockout mice than in wildtype littermates but no difference in hair cell counts. Following higher-intensity noise trauma, DPOAE and basal turn outer hair cells were lost in both genotypes. Chronic noise trauma resulted in more severe loss of inner and outer hair cells in Otof knockout mice than in wildtype controls.

Inner hair cell ribbon synapse numbers were reduced in wildtype mice following all three types of noise trauma. Ribbon synapse numbers were reduced in all Otof knockout mice, but noise trauma inced only a small further decrease.

Conclusions: Our findings are consistent with an increased susceptibility of Otof knockout outer hair cells to noise trauma, likely due to the absence of protective reflexes. Consistent with an excitotoxic mechanism of noise-induced synaptopathy, the remaining inner hair cell ribbon synapses in Otof knockout mice were resistant to noise trauma.

SU54. Repurposing Pravastatin and Metformin for the Treatment and Prevention of Noise-Induced Hidden Hearing Loss in a Mouse Model

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Category: Inner Ear: Damage and Protection of Hair Cells
Background: Noise-induced hidden hearing loss (NIHHL) is a subtle form of hearing damage where auditory thresholds recover but synapses between inner hair cells and afferent nerve fibers are permanently damaged. Currently, there are no FDA-approved therapeutics for the treatment or reversal of NIHHL. Using a noise-response molecular atlas and the DrugCentral database, our lab identified statins and metformin as potential NIHHL therapeutics. Both drugs inhibit HMG-CoA reductase, an enzyme in the mevalonic acid pathway, which is linked to hearing protection. Our lab showed that this enzyme is upregulated in key auditory cells after noise-induced trauma. This study aims to evaluate whether statins and metformin can protect against NIHHL.
Methods: Seven-week-old B6CBAF1/J male and female mice were used in this study. Pravastatin (25 mg/kg/day) and metformin (200 mg/kg/day), alone and in combination, were dissolved in drinking water, with treatment starting at 8 weeks of age. Baseline ABR and DPOAE thresholds were recorded at 9 weeks. At 10 weeks, mice were exposed to noise (97 dB SPL, 8-16 kHz, 2 hours) to induce NIHHL without permanent threshold shifts. ABR and DPOAE were measured at 24 hours, 1 week, and 6 weeks post-exposure to assess hearing. Cochlear tissue was collected at each time point for histological analysis, focusing on outer hair cell (OHC) loss and inner hair cell (IHC) synapse loss using the pre- and post-synaptic markers, CtBP2 and GluR2, respectively.
Results: Consistent with a HHL-inducing exposure, auditory thresholds returned to baseline in all groups 1-week post-exposure. The combination treatment of pravastatin and metformin reduced ABR threshold shifts 24 hours post-noise exposure at 16, 24, and 32 kHz in male mice. A similar protective trend was observed in female mice across all measured frequencies, but this was not statistically significant. DPOAE analysis revealed a statistically significant reduction in threshold shifts at 16, 24, and 32 kHz in female mice treated with either pravastatin or metformin compared to placebo mice, 24 hours post-noise exposure. ABR wave-I amplitude analysis indicated larger amplitudes in male mice treated with the combination of pravastatin and metformin one-week post-exposure compared to the placebo group, though no significant differences were found, likely due to the limited sample size. Histology analysis is ongoing, and we will present the outcomes related to synapse loss during the ARO conference.

Conclusions: These findings suggest that the combination of metformin and pravastatin may improve ABR threshold shifts 24 hours after noise exposure in male mice. However, further analysis is required to assess changes in ABR wave-I amplitude (indicative of synaptopathy), as current data are limited to a small sample size. Based on preliminary results, the combination of pravastatin and metformin holds potential for pre-clinical studies on hearing preservation in males.

SU55. Not Fishy at All: Hair Cell Damage and Protection in the Zebrafish Model System

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Category: Inner Ear: Damage and Protection of Hair Cells
Background: Zebrafish are a popular model for many critical areas of auditory research, including studies of inner ear development, genetics of hearing loss, hair cell damage and protection, and hair cell regeneration. Like other fishes, zebrafish have two hair cell-bearing sensory systems: an inner ear and an external system called the lateral line. The external location of lateral line hair cells makes zebrafish an ideal model for damage and protection studies, offering the ease of visualization comparable to an in vitro preparation and the intact physiology of an in vivo system. Our lab uses both larval and adult zebrafish to understand the cellular mechanisms of hair cell damage and develop potential otoprotective therapies. Our work compares cellular mechanisms across different damage types – ototoxin exposure, acoustic trauma, and age-related hearing loss – to better understand common cellular damage pathways vs. those distinct to specific toxic insults. Here, we present research from three of those intersectional studies using different damage environments: 1) chemical ototoxicity from COVID-19 drug therapies in clinical trials, 2) synaptic damage associated with neurotransmitter modulation, which may serve as a proxy for noise over-exposure, and 3) auditory dysfunction in aging zebrafish.
Methods: The first two studies use fluorescent reagents to label hair cells and pre- and post-synaptic elements in the larval lateral line, followed by microscopy to assess hair cell morphology and cell survival. The third study uses fluorescence microscopy to examine morphological changes in the lateral line and inner ear of young vs. aged adult zebrafish.
Results: We demonstrate that lateral line hair cells are susceptible to damage from several putative COVID-19 therapies, such as mefloquine and prazosin, with comparable hair cell loss to a known ototoxin, gentamicin. We further show that synaptic damage can be modulated through multiple mechanisms. Finally, we show that despite robust regenerative capacity, zebrafish still lose hair cells as they age, likely due to decreased supporting cell proliferation.
Conclusions: Our studies underscore the potential of zebrafish as an excellent model for understanding hair cell damage and provide a valuable platform for developing preventative therapies targeted to specific toxic insults. Our future studies will determine ototoxin-specific signatures to identify targets for therapeutic intervention.

SU56. Prevention of Acute Mouse Model of Aminoglycoside-Induced Outer Hair Cell Loss by Targeting Calcium/Calmodulin Kinases

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Category: Inner Ear: Damage and Protection of Hair Cells

Background: Calcium (Ca2+) overload in outer hair cells (OHCs) is a well-established contributor to the pathogenesis of aminoglycoside-induced hearing loss. Increases in Ca2+ concentration activate Ca2+/calmodulin (Ca/CaM) complex proteins including Ca/CaM-dependent protein kinase kinases (CaMKKs) and Ca/CaM-dependent protein kinase, type II (CaMK-II). Both CaMKKβ and CaMK-II are serine/threonine protein kinases and are the predominant isoforms in brain tissues. CaMKKβ serves as an upstream activator of AMP-dependent protein kinase α subunit (AMPKα). Recently, we reported that traumatic noise exposure activates CaMKKβ in OHCs, leading to synapse disruption and OHC loss. In this study, we investigated the role of CaMKKβ and CaMK-II in the inner ear using acute models of aminoglycoside-induced hearing loss both in vivo and in vitro. We aim to develop a novel strategy to mitigate aminoglycoside-induced hearing loss by targeting CaMKKβ or CaMK-II signaling.

Methods: We have modified an acute mouse model of aminoglycoside-induced hearing loss using a single subcutaneous dose of kanamycin (KM) at 800 mg/kg followed by an intraperitoneal injection of 200 mg/kg furosemide (FU) to 6-week-old CBA/J mice. Immunohistochemistry was performed to detect CaMKKβ expression. Small interfering RNA targeting CaMKKβ (siCaMKKβ) was administered to the inner ear via the posterior semicircular canal (PSC) to silence its expression. Hearing function was assessed by auditory brainstem response (ABR) and distortion product otoacoustic emissions (DPOAE). Myosin VIIa immunolabeling and DAB staining were employed to evaluate hair cell loss. Cadaverin and FM-143 dye were utilized to measure the permeability of the stria vascularis and hair cells, respectively. In vitro, cochlear explant culture medium was treated with gentamicin with or without co-treatment of KN93, a specific CaMK-II inhibitor. Damage to explanted hair cells was measured by immunolabeling for apoptosis-inducing factor (AIF).

Results: Treatment with KM followed by FU to adult CBA/J mice induced massive OHC loss and hearing loss, accompanied by activation of CaMKKβ. OHC loss appeared as early as 6–8 h after KM and FU treatment. Application of siRNA via the PSC effectively silenced CaMKKβ expression in sensory hair cells and protected from KM-FU-induced OHC loss. Importantly, silencing CaMKKβ did not affect the permeability of the stria vascularis or hair cells. Furthermore, AIF is activated in sensory hair cells during gentamicin treatment in explant culture. Application of KM93 protected against gentamicin-induced hair cell loss.

Conclusions: Calcium/calmodulin signaling is activated in an acute aminoglycoside-induced hearing loss in mice. Targeting CaMKKβ or CaMKII offers a promising strategy to prevent aminoglycoside-induced hair cell loss and hearing loss.

SU57. Treatment With FK506 Attenuates Noise-Induced Hearing Loss via Mediation of Endoplasmic Reticulum Stress

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Category: Inner Ear: Damage and Protection of Hair Cells

Background: Noise-induced hair cell loss is a common cause of acquired hearing loss, referred to as noise-induced hearing loss (NIHL). Endoplasmic reticulum (ER) stress plays an important role in the development of cochlear hair cell apoptosis and loss of hair cells. Accumulation of unfolded and misfolded proteins alters ER function. Heat shock protein 90 (Hsp90) is a cytoplasmic molecular chaperone that regulates protein folding and degradation of ER-associated misfolded proteins.
FK506 is an FDA-approved immunosuppressant that acts as a potent inhibitor of calcineurin function. Beyond its immunosuppressive effects, FK506 mediates neurotrophic and neuroprotective effects via several mechanisms. Previously, we reported that treatment with FK506 attenuates noise-induced hair cell loss and NIHL in adult CBA/J mice, but the downstream mechanisms by which FK506 attenuates NIHL remain unknown. Here we show that treatment with FK506 attenuates NIHL in adult FVB/NJ mice and such protection is associated with inhibition of noise-induced ER stress.

Methods: Eight-week-old-old FVB/NJ mice were exposure to noise. Auditory function was measured by auditory brainstem responses (ABRs) and distortion product of otoacoustic emissions (DPOAEs). Western blots using cochlear homogenates and immunolabeling of cochlear surface preparations were performed to detect Hsp90 and GRP94 levels and localization of Hsp90 and Grp78 in in sensory hair cells by confocal images. Myosin VIIa labeling and DAB staining were employed to evaluate hair cell loss.
Results: We have characterized noise-induced permanent threshold shifts and hair cell loss in adult FVB/NJ mice in both sexes and confirmed that treatment with FK506 significantly attenuates noise-induced hair cell loss and hearing loss. Furthermore, we found that noise exposure significantly decreases Hsp90 and increases ER stress markers, such as Grp78. Treatment with FK506 increased Hsp90 in the inner ear and effectively prevented noise-diminished Hsp90 and noise-increased Grp78 levels in inner ear tissues and sensory hair cells.
Conclusions: Taken together, these results indicate that treatment with FK-506 attenuates noise-induced OHC loss and hearing loss through increasing Hsp90 levels and inhibiting ER stress.

SU58. Sex-Dependent Galectin-3 Response to Noise-Induced Cochlear Stress in Mice

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Category: Inner Ear: Damage and Protection of Hair Cells

Background: Galectin-3 (Gal-3) is a β-galactoside-binding protein involved in various important biological processes, including immune responses, apoptosis, and cell adhesion. Our previous study revealed that Gal-3 deficiency leads to sex-dependent hearing loss, predominantly affecting female mice (Zhang, Hearing Research). However, the underlying mechanism remains

unclear. This study explored whether Gal-3 exhibits a differential response to cochlear stress in male and female mice.
Methods: Three-month-old CBA mice, both male and female, were divided into two groups: noise-exposed and control. The noise group was subjected to broad-band noise exposure at 120 dB SPL for 2 hours. Female mice in the noise group were exposed to the noise during the estrus stage of their estrous cycle, as determined by vaginal cytology. Auditory function was assessed using auditory brainstem response (ABR) testing. Cochlear tissues were collected for hair cell quantification, Gal-3 mRNA, and protein expression analysis. Cochlear immune cells were also evaluated.
Results: Noise-induced ABR threshold elevation was more significant in males than females, especially at higher frequencies. The level of hair cell loss between males and females was not significantly different (485.8 ± 240.6 vs. 480.1 ± 119.3), with females displaying greater variability. Gal-3 mRNA expression was similar between control males and females. Following noise exposure, Gal-3 mRNA levels increased significantly in both sexes, with males showing a larger increase than females (fold change 3.13 ± 1.18 vs. 1.90 ± 0.50, $p = 0.00025$). Immunostaining of Gal-3 showed strong expression in Hensen's cells and resident immune cells in the lateral wall of the cochlea under normal conditions. After noise exposure, Gal-3 expression increased in both Hensen's cells and macrophages, with distinct sex-specific differences. Males exhibited higher Gal-3 expression in Hensen's cells, while females had higher Gal-3 expression in macrophages.
Conclusions: This study identified sex-specific differences in Gal-3 expression changes in the cochlea following noise exposure. Males showed elevated Gal-3 expression in Hensen's cells, whereas females had increased expression in macrophages. These findings suggest that Gal-3 may play a sex-dependent role in the cochlear response to noise-induced stress, which could contribute to sex differences in noise-induced hearing loss. Gal-3 represents a potential target for future therapeutic interventions.

SU59. Cisplatin Induced Hearing Loss Through Multi-Cycle Single Low Dose Intravenous Injection

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Category: Inner Ear: Damage and Protection of Hair Cells
Background: Cisplatin is used as a potent anti-tumor drug accounting for use in nearly 40% of cancer treatments, but an unfortunate side effect of this treatment is severe hearing loss that can be debilitating for patients. Previous cisplatin-induced hearing loss (CIHL) animal models simulating clinical settings involve multiple cycles of 4-day low doses of cisplatin treatment through intraperitoneal injections. Significant threshold shifts was induced, but at the cost of the health of the animals. Our new method proposes using low dose intravenous (IV) injection through tail vein once per defined cycle, every 2 weeks in our case. Honokiol, a pleiotropic polyphenol which has been shown to protect hearing, was also used in the form of a liposome to prevent against CIHL.
Methods: One week prior to the beginning of the experiments, FVB mice were placed on a high fat diet and continued on it for the duration of the experiments. After measuring the baseline

auditory brainstem response (ABR), they went through three 14-day cycles of treatment. In each cycle, animals were treated with liposomal honokiol and cisplatin on day 1, followed by a 13-day recovery period. The dose for cisplatin was 4 mg/kg, and the dose for honokiol was 10 or 20 mg/kg. Saline and blank liposome were used as controls. Both honokiol and cisplatin were delivered through IV injection, with honokiol been given 1 hour before cisplatin. Intensive caring was provided during the experiments, including twice daily hydration support and high caloric supplementation. Six treatment groups were included in the study: 1) Control; 2) Honokiol 10; 3) Honokiol 20; 4) Cisplatin only; 5) Honokiol 10 + Cisplatin; and 6) Honokiol 20 + Cisplatin, with honokiol dose indicated in the group names. ABRs were measured on Day 14, 28, and 42 during the treatment.
Results: ABR threshold elevation was observed at frequencies 36 kHz (±19 dB) and 42 kHz (±16 dB) in Cisplatin only treatment groups (p LESS THAN 0.0001) at day 42. Honokiol treatment significantly reduced this cisplatin induced hearing loss, although it is still shown at 36 kHz (p LESS THAN 0.01). Mice who received only 10 mg/kg of honokiol had less severe threshold shifts as compared to those treated with 20 mg/kg. No significant weight loss was observed in any of the treatment groups.
Conclusions: We established an IV injection model for CIHL, which is more reliable and clinically relevant, ruling out the influence of deteriorated health on the hearing of the animals. Honokiol also prevented the higher frequency threshold shifts as compared to cisplatin treatment alone.

SU60. Foxo3 Mechanisms in Noise Induced Hearing Loss

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Category: Inner Ear: Damage and Protection of Hair Cells
Background: Noise exposure is a leading cause of adult-onset hearing loss. In the periphery, traumatic noise can kill outer hair cells, injure cellular structures, and raise hearing thresholds. Previously, we have found that Foxo3-ko/ko mice, which do not express functional FOXO3 protein, are significantly more sensitive to noise exposure compared to Foxo3-wt/wt mice of the same strain. In particular, outer hair cells in Foxo3-ko/ko mice appear to be primed to die in response to noise exposure. They undergo a form of caspase-independent apoptosis called parthanatos within four hours of the onset of a mild noise exposure. Here we investigate the gene expression profile of outer hair cells in the Foxo3-ko/ko mouse using RPL22(HA) technology, also called RiboTag.
Methods: A mouse line was created that harbored both the inducible CRE recombinase under control of the Slc26a5 (Prestin) promoter and the HA-tagged RPL22 protein, which is incorporated into translating polyribosomes. These mice were bred to the Foxo3-ko/ko line to obtain mice with one allele of Slc26a5-iCRE, one or two alleles of RPL22(HA), and either two wild-type or two knockout alleles of Foxo3. Mice were injected with tamoxifen to enable expression of RPL22(HA) in outer hair cells. After euthanasia, inner ears were processed to enrich for organ of corti cells. Immunoprecipitation was used to enrich for HA+ ribosomes from both Foxo3-wt/wt and Foxo3-ko/ko tissues, which were compared to their respective input

samples. RNA samples were reverse-transcribed into cDNA and sequenced. Results were analyzed using R Studio and cross-referenced to cell-type atlases of the inner ear, available on the gEAR website.
Results: Outer hair cells readily expressed HA-tag upon injection of tamoxifen. Notably, Foxo3-ko/ko mice that also harbored Slc26a5-iCRE and RPL22(HA) were not present in Mendelian ratios, suggesting a toxic genetic interaction. In analyzing sequence data, we focus our attention on changes in expression of three categories of genes: proteins that interact with actin, proteins that regulate excitotoxicity, and members of the proteostasis network. Validation of identified Foxo3-dependent outer hair cell genes is ongoing.
Conclusions: RiboTag is a powerful genetic tool for identifying shifts in gene expression in rare cells such as outer hair cells, especially for cells that are too fragile to isolate. Downstream targets of FOXO3 are crucial for outer hair cell resilience and survival, and may represent future targets for interventions to protect hearing from noise.

SU61. Lysosomal Roles in Delayed Hair Cell Death After Aminoglycoside Uptake

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Category: Inner Ear: Damage and Protection of Hair Cells
Background: Mechanosensory hair cells in the inner ear are complex and highly specialized cells that fulfill a pivotal role in hearing and balance. Hair cells are susceptible to ototoxic damage from therapeutic agents, like the widely used aminoglycoside (AG) family of antibiotics. Despite their known ototoxic effects, AGs, like neomycin and gentamicin, are still considered a standard treatment for patients. Our group has extensively explored the multiple adverse effects of AG on hair cells of the zebrafish lateral line, including the distinct hair cell death patterns that different AGs generate. During uptake, neomycin accumulates mostly in the cytoplasm of hair cells generating acute death of the hair cell. Other aminoglycosides, like gentamicin or G418, accumulate mostly in lysosomes within hair cells, and they generate delayed hair cell death. These observations led us to hypothesize that vesicular compartmentalization and lysosomal function could be influencing a delayed hair cell death. Our ultimate goal is to understand the mechanisms and lysosomal signals that participate in delayed hair cell toxicity.
Methods: In order to understand how lysosomal function is able to influence hair cell death, we perturbed normal lysosomal function. By using lysosomotropic drugs, protease inhibitors, and multiple calcium channel agonists we dissected specific pathways that might participate in the delayed hair cell death processes. These effects will be assessed by performing dose response curves, as well as live imaging and automated image analysis.
Results: Our results support the previous observation that most G418 accumulation happens in late endosomes/lysosomes, labeled by Rab7. Lysosomal protease activity inhibition doesn't affect hair cell death outcome. On the other hand, osmotic stress generated by GPN in the lysosomal compartment is able to protect hair cells against G418, but not against neomycin. TPC2-A1-N, a Two Pore Channel 2 (TPC2) calcium agonist, is also able to protect against G418, but not against neomycin. This protection is only present when TPC2-A1-N is added before G418, suggesting that there are other mechanisms necessary for protection. Moreover, longer

incubations in this agonist don't change the outcome of AG toxicity. Interestingly, other drugs that alter lysosomal calcium dynamics do not show any protective effects.
Conclusions: By perturbing the lysosomal compartment with GPN we can protect hair cells against G418 toxicity, but not neomycin. Moreover, specific lysosomal calcium release pathways are linked to hair cell protection whereas others are not. Overall, our data suggests that lysosomes are a key factor on the delayed, but not acute, hair cell death process in hair cells, and calcium could play an important role during protection.

SU62. Characterization of Auditory Function and Histological Changes in a Novel Blast-Tube Model

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Category: Inner Ear: Damage and Protection of Hair Cells
Background: Hearing loss is the most common service-connected disability among U.S. veterans. A risk that increases the chances of hearing loss in United States military personnel, is exposure to blast. Some of the primary blast injuries result from the over-pressurization wave which can cause multi-organ damage. Of particular interest is tympanic membrane rupture and concussions. Blast injury can result in disruption of stereociliary bundles in the outer hair cells, and reduction in synaptic ribbons of the inner hair cells. Together, this can lead to sensorineural hearing dysfunction. It is imperative to develop preclinical model to characterize further the acute and long-term auditory function post blast and implore potential therapeutics that can reduce or intervene in sensorineural hearing dysfunction. In study we aim to characterize hearing function, as well as central and peripheral morphological changes in animals exposed to a novel blast paradigm developed by our group. This will help identify potential therapeutic targets that that may circumvent blast-induced auditory dysfunction.
Methods: We used male Brown Norway rats (14 – 16 weeks, n =4) exposed to a novel blast-tube paradigm under anesthesia (ketamine/xylazine). Naive animals (n = 4) underwent anesthesia and were in the vicinity of where the blast occurs. In both groups, Auditory Brainstem Responses (ABR) were measured prior to blast exposure and at days 7, 14, and 28 after trauma (click, 2, 4, 8, 16, 24, and 32 kHz). At day 28 post-blast, cochlea and brain tissue were collected to perform histological studies to determine multi-organ damage effects from blast overpressure.
Results: We observed no instances of blast-induced mortality in the cohort studied. Additionally, the novel trauma paradigm didn't cause disruption of the tympanic membrane unlike prior devices used. Measurements performed as early as day 7 reveal that blast exposure caused significant shifts in ABR thresholds at all frequencies tested (Click: 48.8±24.6, 2kHz: 56.3±17, 4kHz: 36.2±10.3, 8kHz: 40.3±17.3, 16kHz: 55±18.8, 24kHz: 46.2±17.5, 32kHz: 57.5±13.2). Increased auditory threshold were observed up to day 28, suggesting permanent functional damage. By day 28, we also observed higher variability in responses between subjects, indicating potential individual differences in long-term outcomes.

Conclusions: The blast-tube paradigm successfully induced auditory functional damage. Additional studies are underway to increase the sample size, collect outcomes following repeated blast exposures, and assess long-term peripheral and central changes post-blast.

SU63. Therapeutic Potential of Dexamethasone Palmitate Lipid Nanoparticles in Protecting Against Aminoglycoside Ototoxicity

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Category: Inner Ear: Damage and Protection of Hair Cells

Background: Glucocorticoids are the first-line therapy for idiopathic sudden sensorineural hearing loss (I-SSNHL), with intratympanic steroid injection (ITSI) used in patients with incomplete recovery from hearing loss 2 to 6 weeks after symptom onset. However, effective drug delivery to the inner ear remains a significant challenge, leading to recent efforts to explore new drug delivery technologies.

Dexamethasone palmitate (DP), a dexamethasone prodrug, is encapsulated in solid lipid nanoparticles composed of palmitic acid. Due to its hydrophobic properties, which differ from the hydrophilic nature of conventionally used dexamethasone sodium phosphate (DSP), DP's lipid nanoparticles are expected to enhance drug delivery efficacy when administered intratympanically. Before clinical application, ex vivo evaluations were conducted to assess DP's effectiveness against ototoxic drugs.

Methods: Ex vivo experiments with mouse cochleae were conducted. Kanamycin, an aminoglycoside (AG) antibiotic, was used to induce ototoxicity. The effects of DP were compared with those of DSP.

In the ex vivo experiment, explanted mouse cochleae were treated with DP and DSP 24 or 0 hour prior to the treatment of AG, and incubated for 24 hours. The number of surviving inner hair cells (IHCs) and outer hair cells (OHCs) was then counted from three cochlear regions: apex, middle, and basal turns.

Results: In the ex vivo model, 0.7mM of AG induced ototoxicity to cochleae. While there was no significant difference in IHC survival between DP- and DSP-treated cochleae, DP-treated cochleae exhibited a higher number of surviving OHCs, particularly when administered 24 hours prior to AG exposure (53 OHCs/100μm for DP vs. 35 OHCs/100μm for DSP in the apex). When treated simultaneously with AG, DP-treated cochlea also showed a higher number of surviving OHCs in the middle turn compared to DSP-treated cochlea (18 OHCs/100μm for DP vs. 8 OHCs/100 μm for DSP in the middle turn).

Conclusions: The exact mechanism of AG-induced ototoxicity remains unclear. However, it is considered that signaling pathway activated via reactive oxygen stress induces pro-apoptotic cascades, which leads to programmed cell death.

It is well known that DP can protect ototoxicity-induced HC death, and proposed protective mechanism of DSP involves the inhibition of mitochondrial apoptotic pathway and prevention of the tumor necrosis factor-a, leading to anti-apoptotic and anti-inflammatory effect.

In our ex vivo experiment, DP demonstrated equal or superior protective effects against AG-induced ototoxicity compared to DSP. The hydrophobic nature of DP may have contributed to these differences.

To find out the mechanisms that induced these difference, further in vitro study to evaluate the mechanisms of drug-of-action is needed to be followed. Furthermore, in vivo experiments are necessary to validate the efficacy of DP's to improve drug delivery to the inner ear, potentially replacing DSP in ITSI treatment for patients with conditions such as I-SSNHL.

SU64. A Multicenter Investigation of Risk Factors Associated with Aminoglycoside-Induced Hearing Loss in Patients With Cystic Fibrosis

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Category: Inner Ear: Damage and Protection of Hair Cells

Background: Patients with cystic fibrosis (PwCF) frequently receive intravenous aminoglycoside (IV-AG) antibiotics to manage chronic pulmonary exacerbations. However, few studies address the risk factors associated with ototoxicity-related hearing or balance loss in this population. We examined the associations of sensorineural hearing loss (SNHL) with clinical factors in PwCF treated with IV-AGs.

Methods: This investigation was conducted as part of a larger project focused on genetics and hearing. Six CF centers participated with a total cohort including 710 patients (352 females), mean age (17.5 years; SD=10.5; range 5-72 years). Audiometry (up to 16,000 Hz) and 226 Hz tympanometry were prospectively obtained, and electronic medical chart review was conducted for each PwCF. SNHL was defined as any threshold GREATER THAN 20 dB HL for test frequencies ranging from 250-16,000 Hz in either ear. Patient and clinical risk factors, including age at audiogram, cumulative (lifelong) IV-AG dosing and presence/absence of cystic fibrosis-related diabetes (CFRD) were evaluated in preliminary models. PwCF were categorized into four age-groups (LESS THAN 12, 12–18, 19–24, and GREATER THAN 24 years) and divided into quartiles based on cumulative IV-AG dose exposure, with Q1 representing the lowest, and Q4 the highest, dosing quartile. Associations between SNHL (yes/no) with independent variables (age at audiogram, CFRD status, and cumulative IV-AG dosing) were tested using logistic regression.

Results: 506 PwCF (1,001 ears) contributed audiometric data in the sensitive extended high frequency range (EHF) from 9,000-16,000 Hz. Of these, 394 PwCF (788 ears) had complete datasets with cumulative IV-AG dosing and CFRD status for analyses. 139 of 394 PwCF (35%) had confirmed CFRD. 34 of 64 (53%) PwCF in the highest dosing quartile had SNHL, compared to 35 of 137 (26%) in the lowest dosing quartile. Age at audiogram (p LESS THAN 0.001), cumulative IV-AG dosing (p LESS THAN 0.002), but not CFRD (p= 0.65) were associated with presence of SNHL. The highest quartile of AG exposure had 3.74 times higher odds of

developing SNHL than Q1, while each additional year of age increased the risk of SNHL by 14% (both p LESS THAN 0.001). Further analyses will be conducted with these variables to control for multicollinearity, including % predicted FEV1, on hearing for the low-to-high frequency hearing range and for cumulative IV-AG dosing quartiles.
Conclusions: These data support our previous single center observation on the correlation between higher cumulative IV-AG exposure and risk of EHF SNHL. These data will also serve as a basis for performing a genome-wide association study that may identify genetic markers that alter the risk of aminoglycoside-induced ototoxicity, as the association of high IV-AG dosing and SNHL was not uniform.

SU65. The Role of XIRP2 in Stereocilia F-Actin Repair

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Category: Inner Ear: Damage and Protection of Hair Cells
Background: Hearing depends on the proper function of auditory hair cells, which contain specialized hair bundles that deflect in response to sound, activating mechanically-gated ion channels necessary for mechanotransduction. However, because mammalian auditory hair cells do not regenerate, these cells are vulnerable to damage from loud noise and can accumulate mechanical damage over time, leading to progressive hearing loss. Understanding endogenous mechanisms of damage repair is critical for identifying potential avenues for treating hearing loss. Our lab found that damage to the filamentous actin (F-actin) cores of stereocilia in mice induced by loud noise exposure exhibited repair within one week, indicating an endogenous repair mechanism. This study focuses on elucidating the role of XIRP2, a protein enriched in hair cell stereocilia that accumulates at sites of F-actin damage, in mediating this repair process. The lab has shown that Xirp2 knockout mice exhibit increased stereocilia damage post-noise exposure and progressive hearing loss. We hypothesize that XIRP2 detects and responds to F-actin damage, playing a crucial role in preventing permanent hair cell degeneration after mechanical insults.
Methods: To explore the role of XIRP2 in F-actin repair, we have employed an in vitro model of actin damage to identify which domain of XIRP2 is responsible for its recruitment to F-actin lesions and to investigate a possible autoinhibition mechanism. We used a laser photoablation system that locally induces strain sites in the stress fibers of fibroblast cells transfected with Halo-XIRP2 or various truncated versions of XIRP2 fused to a Halo tag. Recruitment indices of each XIRP2 subfragment were compared to determine their mechanosensitivity. Additionally, we generated mice with endogenous XIRP2 tagged with GFP, utilizing a split GFP approach. This allows us to live image the movement of XIRP2 in stereocilia within hair cell explants. Lastly, we developed a proximity labeling approach to identify XIRP2-interacting proteins after F-actin damage in vivo. We did this by knocking a TurboID into the Xirp2 locus, enabling biotinylation of proximal proteins that can be pulled down for downstream mass spectrometry (MS) analysis.
Results: Preliminary findings suggest that XIRP2 is recruited to F-actin strain sites in a force-dependent manner. We identified that the C-terminal domain of XIRP2 is responsible for responding to F-actin damage, although this behavior appears to be masked in the full-length

protein. Additionally, I have confirmed successful genetic knock-in of the biotin ligase in the TurboID-Xirp2 mouse model, wild type-like XIRP2 expression and localization, and successful biotinylation of stereocilia proteins. This indicates efficient and specific biotinylation of XIRP2-proximal proteins in the hair bundle, priming us to conduct MS analysis.
Conclusions: XIRP2's recruitment to damaged F-actin is dependent on its C-terminal domain. Further experiments using a proximity labeling approach will provide deeper insights into XIRP2's repair mechanisms within the hair bundle.

SU66. OPEN BOARD

SU67. Discrepancies in Serum Composition via Different Blood Collection Routes

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Category: Inner Ear: Damage and Protection of Hair Cells
Background: The outer hair cells (OHCs) act as amplifiers, undergoing somatic electromotility to achieve high sensitivity and precise frequency selectivity in our hearing. Prestin is the molecular basis for OHC electromotility. OHCs are also one of the most vulnerable components in the cochlea and are typically the first to be damaged by various assaults. Since prestin is the most abundant protein in the plasma membrane of OHCs, it has been investigated by numerous labs as a potential serological biomarker for OHC damage. We have examined the specificity of prestin-ELISA kits using prestin-KO mice as a negative control. Our data shows that commercial ELISA kits cannot specifically identify prestin in the bloodstream (Zheng et al. 2024). Optical density (OD) levels, which are supposed to correspond to concentrations of prestin-ELISA, are similar among WT mice and prestin-KO mice regardless of whether these mice have OHC loss. Interestingly, we noticed differences in OD records after we began to use the Retro-orbital blood collection technique in the lab when previously all blood was collected only via decapitation. To avoid the potential influence of anesthetization reagents, age, sex on the composition of serums, we performed the following experiment to quantify the difference among different blood collection rounds.
Methods: WT and prestin -KO mice received one of the following subcutaneous injections (1) 8 g/kg of HPβCD, (2) 0.9% NaCl. Blood was first collected serially at 4 hours post injection and then decapitated, then another group at 1 day post injection and then decapitated. Mice were first anesthetized, and then proparacaine was applied to the eye from which blood would be collected. Retro-orbital blood was collected via the use of capillary tubes containing hematocrit. While the mouse was still under deep anesthesia, verified via paw pinch reflex, it was decapitated quickly, and blood was collected free flowing from the body cavity into a tube. With these 2 methods, hemolysis was minimized as it could interfere with ELISA assay readings.
Results: Regardless of the treatment group or mice models, almost all samples obtained via retro-orbital collection had significantly lower Prestin serum concentration compared to the blood collected through decapitation (p LESS THAN 0.0001). OD values of serum samples collected from decapitation ranged from 2 to 5 times the OD readings of those collected from retro-orbital, and are well beyond the detectable range defined by Prestin-ELISA kit.

Conclusions: Although OD readings from prestin-ELISA do not reflect the prestin levels in the bloodstream, the difference between two different blood collection routes are unexpected. It raised the question whether blood composition is similar among different vessels. What is precisely being measured in Prestin-ELISA needs further investigation and verification. (Work supported by the Knowles Leadership Fund and NIH R56DC020542 to JZ and NIH R01DC019434-01 to XT).

SU68. Investigating the Role of the Lipid Scramblase XKR8 in Inner Ear Function

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Category: Inner Ear: Damage and Protection of Hair Cells
Background: Hearing loss is a complex condition influenced by genetic mutations, ototoxic drug exposure, and noise. Among the genetic factors, the Xkr8 gene has emerged as a key player in hereditary auditory neuropathy, a specific type of hearing loss. Xkr8 encodes a caspase-activated lipid scramblase critical for apoptotic cell clearance and developmental axon pruning. A dominant variant of Xkr8 (c.710G GREATER THAN A, p.W237X) has been linked to late-onset hearing loss in both humans and mice, highlighting its vital role in inner ear physiology.
Methods: We generated two CRISPR-Cas9 knock-out mouse models: one with a 600 bp deletion in exon 1 (E1 strain) and another with an 800 bp deletion in exon 3 (E3 strain). To validate the knock-out strains and confirm Xkr8 expression in the cochlea, we performed reverse transcription quantitative PCR (RT-qPCR) on young adult tissue from Xkr8+/+, Xkr8+/- and Xkr8-/- littermate mice. XKR8 localization in the inner ear was confirmed via RNA in-situ hybridization (RNA ISH) and immunofluorescence (IF). Auditory brainstem responses and distortion product otoacoustic emissions were tested in the knock-out mice. To better understand the auditory phenotype differences between the Xkr8W237X/W237X and Xkr8-/- mice, we analyzed the expression, localization, and functionality of the XKR8 wild type and W237X mutant in mammalian cell lines. We validated two commercially available and one customed anti-XKR8 antibody in mammalian cell lines and our knock-outs.
Results: Auditory brainstem responses and distortion product otoacoustic emissions in these knock-out mice were normal up to 20 weeks, suggesting the presence of compensatory mechanisms or a gain-of-function effect associated with the p.W237X mutation. We looked at the expression of other Xkr genes to assess the presence of potential compensatory mechanisms in Xkr8-/- mice. Preliminary IF results revealed XKR8 localization in stereocilia, the surface of Deiters' cells, and spiral ganglion neurons in young adult mice. The expression of the Xkr8 gene and its protein predominantly in spiral ganglion neurons suggests a significant role for XKR8 in inner ear development and neural homeostasis, particularly in axon pruning.
Conclusions: This study enhances our understanding of the role of membrane regulatory proteins like XKR8 in hearing loss and lays the groundwork for future diagnostic and therapeutic innovations. Further work is needed to elucidate the implications of XKR8 in auditory function,

thereby illuminating the complex genetic landscape of hearing loss, with a specific focus on XKR genes.

SU69. Exploring Synergistic Effects of Dexamethasone With Alpha Lipoic Acid and Diltiazem in Noise-Induced Hearing Loss Models

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Category: Inner Ear: Damage and Protection of Hair Cells
Background: Dexamethasone (DEX) is the most widely used drug for treating inner ear diseases, and intratympanic (IT) injection allows for higher efficiency and reduced systemic side effects. However, there is a need to explore other potential drugs that could complement DEX. This study aimed to compare the efficacy of drugs reported to be effective when administered via IT and to investigate whether combining the most effective drug with DEX would produce a synergistic effect.
Methods: Four drugs reported in the literature to have protective or restorative effects on the inner ear with IT injection (DEX 5mg/ml, Alpha lipoic acid (ALA) 0.75mg/10ul, Diltiazem (DIL) 1mg/ml, N-acetylcysteine (NAC) 20mg/ml) were administered consecutively for four days. Inner ear penetration of the drugs was confirmed by uHPLC, and tympanic membrane (TM) inflammation was examined using oto-endoscopy. Drug efficacy was evaluated in a noise-induced hearing loss mouse model by measuring ABR thresholds and comparing the morphology of the organ of Corti (OC) using H and E staining. The most effective drugs were selected, and their mechanisms of action were compared with DEX by analyzing the expression of inflammasome markers (NRLP3 and caspase-1) and inflammatory markers (TNF-α and NF-kB) using immunofluorescence and western blot. To determine if the selected drugs had synergistic effects when combined with DEX, ABR thresholds and OC morphology were assessed after co-administration of DEX and the selected drug via IT injection.
Results: uHPLC confirmed that all drugs effectively penetrated the inner ear. Among the four drugs, NAC showed no effect on both ABR thresholds and OC morphology, while the DEX, ALA, and DIL groups demonstrated significant recovery effects. Therefore, ALA and DIL were selected for comparison with DEX regarding their mechanisms of action. There were no significant differences between groups for inflammasome markers NRLP3 and caspase-1; however, the inflammatory marker NF-kB showed suppressed expression in both the ALA and DIL groups. Finally, to assess potential synergy, ALA and DIL were each combined with DEX and administered intratympanically for four consecutive days. While no significant restoration in ABR threshold was observed compared to the DEX-only group, the combination of DEX and DIL led to the most effective recovery of organ of Corti morphology.
Conclusions: In this study, it was confirmed that IT-ALA and IT-DIL injections in the noise-induced hearing loss animal model may achieve inner ear restoration through mechanisms different from DEX. Therefore, it was expected that the combination of DEX with DIL would show a higher synergistic effect than with ALA. However, the combined injections appeared to cause increased inflammation of the tympanic membrane, which resulted in no significant improvement in hearing recovery. Thus, further research is needed to explore methods of

minimizing the inflammatory response during combined administration to maximize therapeutic efficacy.

SU70. The Therapeutic Potential of Natural Compounds Against Noise-Induced Hearing Loss

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Category: Inner Ear: Damage and Protection of Hair Cells

Background: Hearing loss is a major health concern in our society, affecting more than 300 million people worldwide. Furthermore, according to the World Health Organization, unaddressed hearing loss costs the global economy US$980 billion annually due to health sector costs and costs of educational support, resulting in a reduction in productivity and quality of life. Despite these significant social and economic ramifications, the US Food and Drug Administration (FDA) has only recently approved the first therapy for the treatment of cisplatin ototoxicity in pediatric cancer patients3. Thus, it is imperative that we develop effective therapies to improve the quality of life of millions of people and at the same time reduce this financial burden.

Previous work from our laboratory demonstrated the therapeutic potential of piperlongumine (PG) – a natural alkaloid derived from the Indian long pepper (Piper longum) – against aminoglycoside-induced hearing loss. Given this, we decided to assess whether PG can also protect against noise-induced hearing loss (NIHL).

Methods: CBA/CaJ mice (7- to 8-week-old) received PG treatment before noise exposure or after noise. Noise stimulus levels were 97dB SPL (temporary threshold shifts) and 112dB SPL (permanent threshold shifts). Hearing tests (auditory brainstem responses [ABRs] and distortion product otoacoustic emissions [DPOAEs]) were performed before and after noise exposure. Twenty-one days after noise, the inner ears were micro dissected and processed for confocal analysis.

Results: Preliminary results suggest PG can protect from noise-induced synaptopathy when given immediately after the noise exposure. Likewise, PG has the potential to act as a prophylactic compound since pre-treatment also resulted in protection of the hearing function. We are currently finalizing experiments in animals exposed to 112dB SPL.

Conclusions: The present work demonstrates the beneficial effect of a natural product against noise-induced hearing loss and set the bases for future studies aiming to address PG's molecular target(s) as well as its potential as an otoprotectant for other forms of acquired hearing loss.

SU71. Lack of Integrin Alpha-8 Function Increases Hair Cell Sensitivity to Ototoxic Damage

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Category: Inner Ear: Damage and Protection of Hair Cells
Background: Previous work from our laboratory identified a functional interaction between integrin alpha 8 (Itga8) and the Usher protein, protocadherin-15 (Pcdh15) in zebrafish. Because integrins are involved in the regulation of Wnt/β-catenin pathway and since we have evidence that Itga8 regulates the transcriptional co-activator, Yap, we decided to assess the effect of Itga8 dysfunction in zebrafish hair cells. We hypothesize that lack of Itga8 will result in an increase in susceptibility to ototoxic insult
Methods: 5-7dpf zebrafish (wild type and Itga8 mutants) were incubated with kanamycin or tobramycin to induced hair cell loss. Neuromast hair cells were immunostained for otoferlin and quantified under a fluorescent microscope. To address whether there Itga8 affects hair cell survival, we incubated the fish with the ototoxin and performed TUNEL or EdU labeling at different time points. Animals were fixed, and processed for confocal microscope
Results: Dose-response experiments identified the EC50 for kanamycin (400uM) and tobramycin (10ug/mL) in wild type zebrafish. These conditions were applied to the Itga8 mutants. There was an increase in ototoxin sensitivity in the Itga8 mutants compared to wild type animals. Moreover, we found that absence of Itga8 results in an increase in hair cell death and a decrease in hair cell proliferation
Conclusions: We demonstrated a key role for Itga8 in hair cell function and survival. Future studies aim to address the link between this integrin and Yap signaling pathway

SU72. The Role of NPTN in Stereocilia of Cochlear Hair Cells

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Category: Inner Ear: Damage and Protection of Hair Cells
Background: Neuroplastin (NPTN), an adhesion molecule that belongs to the immunoglobulin superfamily, plays important roles in the brain and inner ear sensory cells. In the inner ear, Nptn-/- mice show profound deafness from early postnatal age. According to previous reports, two hypotheses, a reduction in the connection between stereocilia and the tectorial membrane (TM) and a decrease in plasma membrane Ca2+ ATPase 2 (Atp2b2, PMCA2) which maintains Ca2+ homeostasis are expected to cause hearing loss in Nptn-/- mice. However, the mechanisms underlying how the absence of Nptn causes hearing impairment remain elusive. Therefore, we decided to investigate the function of NPTN in the cochlea and the molecular mechanism of hearing loss in Nptn-deficient mice.
Methods: The Nptn or Atp2b2-deficient mice in this study were generated using CRISPR-Cas9. We tested the hearing function using ABR (Auditory Brainstem Response) and DPOAE (distortion product otoacoustic emission). Also, we observed protein localization in stereocilia using immunofluorescence. Hair bundle morphology and TM imprint were observed using scanning electron microscopy.
Results: Nptn-/- mice exhibit significantly elevated auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE) thresholds as early as postnatal day 15 (P15). Despite the profound hearing impairment at P15, no obvious differences were observed in the TM-attachment crowns (crown-like structures surrounding the tips of the tallest stereocilia,

forming attachment links) or TM imprints. Consistently with previous reports, we found that immunofluorescence signals of PMCA2 disappear from OHC stereocilia at P15 in Nptn-/- mice. To investigate the phenotypic effects of NPTN deficiency on stereocilia, we performed scanning electron microscopy (SEM) and observed stereociliary fusions and reduced tip links in the OHC stereocilia bundles of Nptn-/- mice. These two stereociliary defects were also found in Atp2b2-deficient mice. Notably, the fusion was alleviated with the partial restoration of PMCA2 in Nptn-delta mice (genetically modified mice lacking the extracellular domain but with a fully preserved interaction site with PMCA2), suggesting that the observed defects are caused by the loss of PMCA2.
Conclusions: These results indicate that NPTN is important for maintaining normal stereociliary spacing and tip links, suggesting that PMCA2 mediates the cochlear functions of NPTN.

SU73. Lipidomics Identifies Beta-Oxidation as a Key Process in Noise-Induced Hearing Loss

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Category: Inner Ear: Damage and Protection of Hair Cells
Background: Hearing loss is a major global health concern, affecting more than 20% of the world's population. Noise contributes significantly to this burden; ¼ of hearing loss globally is caused by noise overexposure, and more than 15% of U.S. teenagers show evidence of noise induced hearing loss (NIHL) on audiological testing. To identify noise-induced metabolic changes, our group previously compared the inner ear metabolome of noise-exposed and control mice by measuring aqueous metabolites. To further explore the metabolic changes induced by noise, we now investigate the lipidome.
Methods: Awake mice were subjected to noise for 2 hours (98-100dB SPL@8-16 kHz), or to a sham exposure. Immediately afterward, otic capsules were dissected, flash frozen, mechanically lysed and subjected to untargeted lipidomics profiling. To narrow down which lipids are influenced by hair cell activity and which changes occur in the tissue, we used mice that were transcardially perfused after the exposure, as well as mice in which hair cells were ablated (Pou4f3-DTR).
Results: Polyunsaturated Free Fatty Acids (PUFAs) were the only lipid group changed by noise in hearing but not in deaf mice. Moreover, this lipid change occurred regardless of perfusion status, suggesting that PUFAs are metabolized in the inner ear itself. To characterize these lipid changes in greater detail, we performed targeted lipidomics of Free Fatty Acids (FFAs) and acylcarnitines (CAR) on a second cohort of hearing mice. PUFAs decreased after noise exposure, demonstrating consistency between the methods. Furthermore, CARs increased while carnitine levels decreased. This metabolic profile indicates that noise increases beta-oxidation, suggesting an increase in cellular energy demands under noise.
To determine if the lipid changes depend on the intensity of the noise, we used a 2-hour, 112dB SPL (8-16kHz) exposure. Remarkably, in this case, there were no changes in FFAs or CARs, suggesting that at louder noise levels, there is either a lack of fatty acid consumption, or increased production in addition to consumption. To distinguish between these possibilities, we

treated mice with Etomoxir, a drug that prevents beta-oxidation through permanent inhibition of CPT-1A. Both noise levels increased fatty acids, but the increase was larger at 112dB. This indicates that beta-oxidation increases under noise conditions, with greater consumption and production occurring at louder noise levels. Finally, we found that blocking beta-oxidation with Etomoxir does not affect hearing function under normal housing conditions but reduces the extent of hearing loss induced by a 2-hour, 112dB SPL (8-16kHz) exposure.

Conclusions: These results implicate beta-oxidation as an important energy source for the inner ear. However, its inhibition paradoxically reduces the threshold shifts caused by loud noise. Our findings provide insights into cochlear energy metabolism and suggest that its modulation could be targeted to reduce noise-induced hearing loss.

SU74. Protective Effect of an FDA- Approved Drug via Middle Ear Delivery Against Noise Exposure in Swine Model

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Category: Inner Ear: Damage and Protection of Hair Cells

Background: Hearing loss ranks among the top five global disability, affecting over 1.5 billion people worldwide, and noise-induced hearing loss (NIHL) disproportionally affects military service members and veterans. Noise exposure (NE) can damage the inner ear hair cells, the synapses, and the auditory neurons, resulting in permanent hearing impairment. Despite the severity and prevalence, there are no FDA-approved drugs that prevent or treat NIHL. Identification of such drugs will be significant for patients with NIHL. Our research in hair cell regeneration identified Notch1 as a key factor in reprogramming the inner ear. Significantly, Notch activation, genetically or by small molecule Valproic acid (VPA), potently protects mice against NIHL. We initiated a study into the role of this FDA drug in protecting against NIHL in a large animal swine model.

Methods: Anesthetized Micro-Yucatan pigs (12-14 kg, aged 3-4 months) were placed within a sound booth and subject to 120 dB white noise exposure (NE) for 2 hours over two consecutive days. One day before NE, 0.5 mL of VPA (100 mg/mL) was injected into one ear via tympanic membrane, with vehicle (saline) injected into the contralateral ear as control. Auditory brainstem responses (ABR) were measured pre-NE and at various intervals post-NE. Inner ears were collected 28 days post-NE for characterization, including immunohistochemistry with markers for hair cells (MYO7A) and synapses (CtBP2) and quantification.

Results: Compared to the baseline, NE significantly increased ABR thresholds from 35-40 dB pre-NE to 55-80 dB post-NE across a frequency range of 4-16 kHz and reduced ABR wave amplitudes. Three days post-NE, pig ears treated with VPA exhibited a reduction of 17.9 dB at 4 kHz (p LESS THAN 0.05), 17.1 dB at 8 kHz (p LESS THAN 0.05), and 16.8 dB at 16 kHz compared to the saline-injected controls. By day 7 post-NE, VPA-treated ears showed reductions of 20 dB at 4 kHz (p LESS THAN 0.05), 13 dB at 8 kHz, and 10 dB at 16 kHz. At 28 days post-NE, all ears demonstrated partial hearing recovery, with the VPA treated group showing

reductions of 8 dB at 4 kHz, 13 dB at 8 kHz (p LESS THAN .05) and 14 dB at 16 kHz (p LESS THAN 0.05). Analysis revealed NE damaged the inner ear by an increase in the loss of basal outer hair cells, significantly reduced MYO7A labeling in basal-mid turn inner hair cells, and the loss of CtBP2 labeling across the entire cochlea. VPA treatment rescued the inner ears in all the parameters.
Conclusions: Our findings strongly support that VPA pretreatment mitigates the acute effects of NE on ABR thresholds and preserves cochlear function. VPA mediates the rescue effect by maintaining homeostasis of the inner ear cells. The data is consistent with the results from the mouse studies, making VPA a strong candidate in a clinical study against NIHL.

SU75. An Antioxidative Treatment to Prevent Further Noise-Induced Hearing Loss in Hearing Impaired Mongolian Gerbils

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Category: Inner Ear: Damage and Protection of Hair Cells
Background: Hearing loss affects nearly half a billion people worldwide, with the leading causes in adults being recreational and occupational noise exposure. In these cases, noise exposure increases the reactive oxygen species known as superoxides. High levels of superoxides cause oxidative damage to the inner and outer hair cells of the cochlea eventually leading to apoptosis and hearing loss.
Pharmacological approaches using antioxidative treatments reduce oxidative damage; however, route of administration and variability in absorption impedes efficacy of the therapy. One antioxidant, superoxide dismutase (SOD), is a protein that is naturally expressed in many animal species. SOD reduces superoxide levels by catalyzing them into hydrogen peroxide.

Therefore, we have developed a hair-cell targeted AAV to express the transgene for SOD at levels that compensate for noise induced increases in superoxide production.

In our previous study we demonstrated that prophylactic use of this gene therapy in an animal model of noise induced hearing loss significantly reduced noise induced hearing loss.

Here we have asked whether adult animals that are already affected by varying levels of hearing loss, will see neuroprotection from further exposures to damaging levels of noise when the AAV therapy is administered after the induction of moderate hearing loss.

Methods: Adult (P86) Mongolian gerbils (Meriones Unguiculatus) received baseline auditory brainstem response recordings and DPOAEs followed by five days of noise exposure (110 dB/2 hours) to induce moderate hearing loss.
After four weeks of recovery the gerbils were then given intra cisterna magna injections of either

AAV without the transgene or our AAV gene therapy containing the SOD1 (intracellular), SOD2 (mitochondrial), or SOD3 (extracellular) transgene.

After three weeks of transgene expression, animals were then exposed to an additional round of noise exposure (110 dB/2 hours). Auditory thresholds were taken weekly throughout noise exposures and statistically compared to baseline thresholds.

Results: Compared to controls SOD transgene expression offered significant protection from initial hearing loss (prophylactic) and moderate protection from further hearing loss (treatment).
Conclusions: These data suggest that increasing the bioavailability of SOD in individuals that already suffer from hearing loss can reduce the progression of additional hearing loss over time.

SU76. Mitigation of Cochlear Damage With Liraglutide Treatment in Chinchillas Exposed to Repeated Blasts

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Category: Inner Ear: Damage and Protection of Hair Cells
Background: Blast-induced hearing loss is a prevalent issue among Service members. Previous studies have reported that liraglutide, a glucagon-like peptide-1 (GLP-1) receptor agonist, can mitigate blast-induced hearing damage in chinchillas. However, the detailed pathological understanding of cochlear dysfunction in relation to liraglutide treatment remains unclear. This study aims to investigate the cochlear damage in chinchillas with liraglutide treatment under repeated high-intensity blast exposures and provide histological evidence to understand the hearing function changes.
Methods: Young adult chinchillas, with and without earplugs, were subjected to three blasts at a peak level of 15-25 psi (103-172 kPa) on days 1 and 4, respectively. Liraglutide was administered to the treatment groups at a dose of 0.25 mg/kg daily, starting two days before (pre-blast treatment) or after (post-blast treatment) blasts on day 1, for a total of seven days. The blast control group received an equivalent volume of saline. Auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs) were measured on Day 1 pre- and post-blast, Day 4 post-blast, and on Days 7, 14, and 28. Cochleae were harvested at the end of experiment on days 14 or 28 for histological study, either dissected for whole-mount immunostaining with myosin VIIa to assess hair cell integrity, or sectioned to slide preparation for spiral ganglion neurons (SGNs) staining with toluidine blue. Both hair cells and SGNs were quantified and analyzed.
Results: Images of outer and inner hair cells and SGNs were collected. For earplugged chinchillas with liraglutide pre-blast treatment, the SGN density was higher compared to the blast control group, corresponding to hearing function restoration by 28 days. For open ear chinchillas, mild damage of hair cells was caused by the repeated blasts. The post-blast treatment group showed less hair cells damage compared to the blast control group.
Conclusions: Liraglutide administration mitigates blast-induced cochlear damage, preserving hair cells and spiral ganglion neurons and therefore promoting hearing function restoration. Further research is needed to explore liraglutide's full impact on cochlear pathology and its potential as a therapeutic intervention for blast-induced hearing loss.

SU77. Donut Mitochondria: A 'Hot and Ready' Mitochondrial Repair/Defense Mechanism?

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Category: Inner Ear: Damage and Protection of Hair Cells

Background: Cisplatin is a chemotherapy drug used to successfully treat a wide variety of cancers. However, it is known to have severe side effects, including permanent hearing loss. Cisplatin damages and kills hair cells–the sensory receptors for hearing. High doses of cisplatin are shown to kill hair cells through apoptosis. These doses have been explored to understand the immediate hearing loss patients encounter. A more clinically troubling question is the phenomenon of delayed/progressive hearing loss onset in patients years post treatment. This clinical presentation drove us to investigate sub-lethal doses of cisplatin in sensory hair cells to define its pathology. Previous studies have shown cisplatin localization and accumulation in the mitochondria is an early and causative event of hair cell death (Lee 2024). This along with other data informed us to further investigate the effect of sub-lethal doses of cisplatin on hair cell mitochondria.

Methods: We used a transgenic zebrafish line tg(myo6b:mitoGCaMP) which expresses a genetically encoded calcium indicator in hair cells. Larvae were exposed to drug treatment at 6 days old. Treatment conditions were established using logarithmic concentrations to establish a dose dependent response curve. The cisplatin lesion protocol implemented a 2-hour exposure to a cisplatin solution and subsequent rinses and recovery in Embryo Media. Larvae were allowed time to recover depending on the condition. Larve were fed with rotifers daily with 50% media changes. Post recovery larvae were sedated on ice, then fixed using a paraformaldehyde solution.

Results: We are currently characterizing and quantifying cisplatin-induced mitochondrial changes using super resolution confocal imaging and 3D analysis. Our data indicate cisplatin is altering mitochondrial morphology in surviving hair cells, even at sublethal doses that produce no hair cell loss. From this data we see a trending decrease in the number of mitochondria from no recovery to 48-hour recovery. An interesting finding from preliminary data is the presence of donut, or toroidal, mitochondria post cisplatin treatment. With increasing cisplatin dosage, donuts appear to be more numerous and persist even post 48-hour recovery.

Conclusions: Our observations indicate loss of neuromast hair cell mitochondria and changes in mitochondrial morphology in response to sublethal doses of cisplatin. These changes persist in neuromast hair cells even 48 hours post cisplatin exposure. We posit that donut formation in response to cellular stress is a mitochondrial repair or defense mechanism which may permit hair cell survival. Ongoing studies will determine the functional impact of these morphological changes using functional imaging of hair cells, electrophysiology, and behavioral assays.

SU78. Otoprotective Effect of MnTBAP in Cisplatin-Induced Hearing Loss

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Category: Inner Ear: Damage and Protection of Hair Cells

Background: Cisplatin (cis-diammineplatinum (II) dichloride), a third-generation platinum-containing coordination complex widely used in the treatment of various solid tumor cancers, causes ototoxicity, which is a progressive and irreversible dose-limiting side effect. Cisplatin treatment leads to the generation of reactive oxygen species and reactive nitrogen species in the inner ear, triggering the production of peroxynitrite and 3-nitrotyrosine, which eventually leads to cochlear apoptosis resulting in hearing loss. Selective targeting of cochlear nitrative stress with metalloporphyrins appears to be an attractive strategy for preventing cisplatin-induced hearing loss because, unlike broad-spectrum antioxidants, selective inhibition of nitrative stress did not interfere with the anti-cancer activity of cisplatin. Here, we test the efficacy of a commercially available metalloporphyrin in preventing cisplatin-induced hearing loss.
Methods: Male and female six-week-old CBA/J mice were treated with a short-term cisplatin treatment regimen (3 mg/kg, ip daily for 5 days). MnTBAP (Mn (III)tetrakis (4-benzoic acid) porphyrin Chloride), a metalloporphyrin, which in its pure form selectively scavenges peroxynitrite, was used to inhibit cisplatin-induced cochlear nitrative stress. The mice were cotreated with 10 mg/kg of MnTBAP (ip) daily for 8 days. Auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE) were recorded before and after the treatment to assess hearing loss.
Results: Cisplatin treatment resulted in a 10-15% loss of body weight and induced an 8-12 dB shift in ABR thresholds across multiple frequencies (p LESS THAN 0.05; n=6). It also affected the activity of outer hair cells by significantly decreasing the DPOAE amplitudes (16 kHz; p LESS THAN 0.05; n=5-6). However, MnTBAP cotreatment prevented the cisplatin-induced weight loss, the hearing threshold shifts, and the decrease in the DPOAE amplitudes (p LESS THAN 0.05; n=5-6).
Conclusions: This study demonstrated that even short-term cisplatin treatment causes significant weight loss in mice and results in hearing loss. More importantly, the results indicated that MnTBAP cotreatment prevents cisplatin-induced weight loss and offers protection against cisplatin-induced hearing loss. Together, these findings suggest that targeting nitrative stress is a plausible approach for preventing cisplatin-induced ototoxicity and MnTBAP is a promising interventional drug for preventing cisplatin-induced hearing loss.

SU79. Characterization of a Progressive Early Onset Hearing Loss in SIRT3 Knock-Out Mice

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Category: Inner Ear: Damage and Protection of Hair Cells
Background: Sirtuins are a highly conserved family of NAD+ dependent histone deacetylases, consisting of seven members (SIRT1-7) in mammals. SIRT3 is found in the mitochondria where it regulates oxidative phosphorylation. The major proteins which interact with SIRT3 are SOD2, FOXO3A, and IDH2. Together, they contribute to antioxidant and redox signaling and suppress reactive oxygen species. Despite its critical roles in various cellular process, complete knockout of SIRT3 (SIRT3-/-) only leads to mild phenotypes in the animals. SIRT3 has been associated with age-related, noise-induced, and drug-induced hearing loss using animal models. However, the molecular mechanism of SIRT3 in hearing protection requires more research.

Methods: A constitutive SIRT3 knock-out mouse model (129-Sirt3tm1.1Fwa/J) was obtained from Jackson Laboratory. The hearing of the three genotypes (+/+, +/-, and -/-) was assessed by auditory brainstem response measured 6-, 8-, and 12-week-postnatal. At the endpoint, the mice were euthanized, and their cochleae were collected. Cochlear whole mount segments in frequency range of 9-36 kHz were dissected after decalcification and they were stained with antibodies targeting SIRT3, SIRT5, and pre- and post-synaptic components. In addition, fresh cochlear whole mounts were collected from four animals each for SIRT3+/+ and SIRT3-/- genotypes for RT-qPCR. Sixteen gene targets were selected from existing literatures which had investigated SIRT3 previously.
Results: At week 6, SIRT3+/+ (n = 33), SIRT3+/- (n=39), and SIRT3-/- (n = 29) mice did not show significant difference in ABR thresholds at all frequencies measured. However, male SIRT3-/- mice showed significantly higher (51.16 dB ±5.05, SD) median ABR thresholds than females (35.06 dB ±1.65, SD) at 36 kHz (p LESS THAN 0.01). Similar patterns were observed at week 8 and 12, and a progressive ABR threshold elevation was observed in male SIRT3-/- mice. The progressive early onset hearing loss was not observed in SIRT3+/+ and SIRT3+/- groups. Immunostaining showed that SIRT3 was expressed in the hair cells of all other animals except SIRT3-/- mice. RT-qPCR showed that SIRT5 was upregulated ~ 1.74 fold (2^-(ΔΔCt)) in male SIRT3-/- mice compared to male SIRT3+/+ mice. In females, SIRT3-/- mice showed downregulation of GPX4 with ~ 0.38 fold difference compared to SIRT3+/+.
Conclusions: We report an early onset hearing loss in male SIRT3-/- mice, which initiated by as early as 6 weeks and progressed until up to 12 weeks, but not in female SIRT3-/- mice. The hearing impairment was not observed in SIRT3+/+ and SIRT3+/- mice. These results indicate that SIRT3 deficiency is more punishing in the hearing of the males than that of the females. SIRT3 deficiency may drive a compensatory antioxidant mechanism through overexpression of SIRT5, although insufficient for rescuing the hearing of the males. In females, SIRT3 deficiency may have caused downregulation of GPX4, a key antioxidant enzyme, but their hearing was unaffected.

SU80. Effects of BDNF-TrkB Activation on Blast-Induced Hearing Loss in a Mouse Model

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Category: Inner Ear: Damage and Protection of Neurons & Synapses
Background: Blast exposure is known to cause substantial long-term damage to both the peripheral and central auditory systems, affecting not only the outer and middle ears but also leading to hearing loss, tinnitus, and central auditory processing disorders. In this study, we investigated the effects of unilateral blast exposure on auditory functions using a blast-injured mouse model, generated by a highly compressed air pressure system. Additionally, we explored the potential therapeutic effects of 7,8-dihydroxyflavone (7,8-DHF), a small molecule that mimics the activity of brain-derived neurotrophic factor (BDNF) by selectively activating the TrkB receptor. Given the critical role of BDNF-TrkB signaling in neuronal survival and

plasticity, we hypothesized that treatment with 7,8-DHF could facilitate hearing recovery following blast-induced damage.
Methods: To mimic blast-induced auditory damage, we developed a highly compressed air-induced hearing loss model in adult C57BL/6J mice (WT) and BDNF mutant mice (heterozygous, BDNF+/–) using repetitive unilateral exposures. Air pressure ranging from 40 to 70 psi and 80 to 90 dB at the ejection port, with varying diameters, was produced using a paintball gun. ABRs in both ears were obtained from sham, blast-injured, and treated mice at different time points during the recovery period, both before and after blast injury (at 3 hours, 1 day, 1, 4, and 8 weeks). 7,8-DHF (10 mg/kg) was intra-peritoneally injected into mice at 3 hours after blast exposure. During the recovery period, we evaluated cell populations and structural changes in cochlear hair cells, spiral ganglion neurons, and auditory brainstem nuclei.
Results: A single blast exposure resulted in minimal hearing threshold elevation, with only a temporary effect observed at 1-day post-exposure. In contrast, dual continuous blast sessions caused significant threshold shifts within 24 hours, followed by near-complete recovery within 2 weeks at a pressure of 38 psi. Exposure to blasts at 60–70 psi led to hearing thresholds rising to approximately 80 dB within 3 hours, gradually decreasing to 63.2 dB in the ipsilateral ear. The contralateral ear also experienced elevated thresholds (~80 dB) within 1-week post-exposure but showed significant recovery by 4 and 8 weeks. Interestingly, BDNF-deficient mice exhibited severe and irreversible hearing damage after blast exposure, with both ipsilateral and contralateral hearing loss, indicating that endogenous BDNF plays a critical role in determining the extent of hearing loss caused by high-pressure air exposure. Post-treatment with 7,8-DHF, administered 3 hours after blast exposure, led to hearing improvements in both WT and BDNF heterozygous mice, showing faster reductions in ABR thresholds during the recovery period.
Conclusions: The high-pressure air delivery system provides an effective and comparable model for studying blast-induced hearing loss. The TrkB agonist, 7,8-DHF, demonstrates significant potential as a therapeutic candidate for mitigating blast-induced damage in the auditory system.

SU81. Human Umbilical Cord Perivascular Cells Reverse Noise-Induced Damage in the Brain and Cochlea in a Rat Model of Noise-Induced Hearing Loss

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Category: Inner Ear: Damage and Protection of Neurons & Synapses
Background: Sensorineural hearing loss (SNHL), a common sensory impairment in humans, results from damaged hair cells or spiral ganglion neurons. One common cause of SNHL is noise exposure, which leads to noise-induced hearing loss (NIHL). NIHL is a significant health problem in the present era and often remains unrecognized and undertreated. The inner ear was thought to be an immune-privileged organ. However, recent studies have shown that proinflammatory mediators contribute to the NIHL. Despite the growing knowledge, there is no specific treatment for SNHL. Recent advancements in regenerative medicine have opened potential cell-based therapies for SNHL. Our group has characterized first-trimester (FTM) human umbilical cord perivascular cells (HUCPVC), a rich and potent source of mesenchymal

stromal stem cells. We hypothesized that noise injury induces inflammation and apoptosis in the brain and cochlea and that FTM HUCPVC treatment can prevent that noise-induced inflammation in NIHL rat model.
Methods: Long-Evans rats were randomly divided into control and noise-exposed groups. The noise-exposed group rats were exposed to white noise (110 dB, 8-16 kHz) for 2 hrs and administered with 1 million FTM-HUCPVC or vehicle (HBSS) via tail vein injection 72 hours after the noise exposure. Cochlea and brain tissues were collected 24 hours, 5 days, 2 weeks, and 4 weeks after noise exposure. The effect of noise exposure and the role of FTM-HUCPVC treatment on the inflammatory proteins and transcripts, hair cell damage and spiral ganglion neuron damage in the brain and cochlea were assessed using Western blot, qPCR, and confocal microscopy.
Results: Our study revealed several significant findings. Firstly, we observed no adverse effects of systemic delivery of FTM-HUCPVC on the overall health of the rats. Secondly, we found that noise exposure led to a significant increase in the expression of inflammatory genes in the brain (CRP ~6-fold, p LESS THAN 0.005; Tnf-α ~2 fold, p LESS THAN 0.05; CCl2 ~2 fold, p LESS THAN 0.005) and cochlea (Tnf-α ~2 fold, p LESS THAN 0.05; CRP ~5-fold, p LESS THAN 0.005) and a decrease in anti-inflammatory IL10 gene expression in the brain (~4.5-fold, p LESS THAN 0.005). Tnf-α, NfKb and cleaved-caspase 3 protein levels were also increased significantly in the brain tissue (~3.5, ~1.5, and ~2 fold respectively) after noise exposure. Additionally, we noted a significant decrease in BDNF gene expression in the brain (~3.5-fold, p LESS THAN 0.0005) and the cochlea (~7-fold, P LESS THAN 0.05) and visible degeneration of inner ear synapses after noise exposure. Importantly, all these changes were significantly reversed in the HUCPVC-treated group.
Conclusions: The findings from our study provide compelling evidence that noise exposure triggers inflammatory damage in the brain and inner ear. Importantly, we demonstrate that FTM HUCPVC effectively reverse these damages by preventing noise-induced inflammation. This work holds significant promise for clinical applications, offering an effective and practical approach to treating noise-induced damage in the inner ear and brain.

SU82. Characterization of Spiral Ganglion Glial Cells Following Selective Ablation of Spiral Ganglion Neurons in Neonatal Mice

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Category: Inner Ear: Damage and Protection of Neurons & Synapses
Background: Glial cells initiate neuronal repair by dedifferentiating and proliferating in response to peripheral nerve injury. Sox-2 is a transcription factor that plays a key role in inner ear growth, development, and regeneration. During postnatal development, Sox-2 expression in the spiral ganglion glial cells (SGGC) undergo dynamic changes before the onset of hearing. In adult cochlea, Sox-2 is upregulated and Sox-2 positive SGGCs proliferate in response to spiral ganglion neuron (SGN) injury. However, the role of Sox-2 positive SGGCs in response to acute SGN degeneration in the neonatal cochlea is unclear. Therefore, in this study we sought to characterize the histological changes of Sox-2 positive SGGCs following selective SGN ablation

in the neonatal mice. We further examined the expression of transcription factor c-Jun to assess the reparative capacity of neonatal SGGCs.
Methods: We delivered AAV2/Retro-FLEx-DTA-mCherry driven by neuronal hSYN promoter (AAV-hSYN-DTA) in neonatal (P1-2) Parvalbumin-Cre (PVCre) or PLP-eGFP+/PV-Cre+/- (PLP/PVCre) and C57 strain. Vectors were microinjected via the posterior semicircular canal at the original titer (1.2 x 10^13 GC/mL) and the 1:1 dilution (6 x 10^12 GC/mL) with artificial perilymph. Neonatal mice were euthanized at 1, 2 ,3, 5, 7, and 14 days post-injection. We performed immunohistochemistry with cryosection tissue staining with TUNEL, anti-beta-tubulin III, sox-2, c-Jun followed by western blot analysis for sox-2 and c-jun expression protein levels.
Results: In the AAV-hSYN-DTA injected and non-injected contralateral control cochleae, Sox-2 positive SGGCs increase during P1-4 and then become stable after P7 while the SGN population remains intact. The PVCre and PLP/PVCre neonatal mice injected with AAV-hSYN-DTA demonstrated selective cre-dependent ablation of SGN without directly damaging adjacent Sox2/PLP positive SGGCs or hair cells. We observed an average of 30 and 60% degeneration in the basal turns of the cochlea at 3 and 7 days, respectively. Dilution of the vector titer did not affect the degree of AAV-hSYN-DTA induced neuronal degeneration. At later time points we observed a gradual decline of Sox-2 positive SGGC population in the AAV-hSYN-DTA injected mice by P7 which remained stable at P14. In the surviving glial cells, we observed an increase in c-Jun and Sox-2 double positive cells, suggesting that SGGCs undergo repair in the neonatal mice.
Conclusions: Our preliminary results suggest that SGGCs in the neonatal cochlea can dedifferentiate into reparative phenotypes. The surviving Sox-2 positive SGGCs upregulate transcription factor c-Jun, suggesting the key reparative molecular pathways are functional. However, the number of Sox2-positive SGGCs does not increase in response to acute neuronal damage. We speculate that neuron-glial interaction is necessary for Sox-2 positive SGGCs to survive and proliferate. Early denervation may compromise the survival of Sox-2 positive SGGCs. Future research focusing on the molecular cues from neuron-glial interaction and factors contributing to the maturation of SGGCs may provide better insight.

SU83. Restoration of Cochlear Synapses from Noise-Induce Synaptopathy by Introcochlear Infusion of TrkC Agonist Antibody in Mice.

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Category: Inner Ear: Damage and Protection of Neurons & Synapses
Background: Neurotrophins, BDNF and NT-3, can promote cochlear synapse regeneration following noise-induced cochlear synaptopathy (NICS) in animal models, with NT-3 being particularly effective. However, neutrophins are sticky molecules, difficult to formulate and deliver intracochlearly. To address these limitations, we have developed agonist antibodies that display improved physical properties relative to the neurotrophins and activate the cognate neurotrophin receptors, TrkB or TrkC, directly. This study evaluates the efficacy of these Trk agonist antibodies delivered via intracochlear infusion in restoring cochlear synapses, directly compares them to NT-3 in a mouse NICS model.

Methods: NICS was caused by exposing 12-14 week-old CBA/CaJ male mice to 100 dB SPL, 8-16 kHz octave band noise for 2 hours. Auditory brainstem responses (ABR) were recorded at 8, 16, and 32 kHz before noise exposure, immediately after noise, and on postnoise days 12-14 (PND14) to assess, respectively, baseline thresholds, temporary threshold shift, and threshold recovery. For each mouse, the postnoise ABR measures were normalized to the prenoise measure in a within-subject design. TrkC agonist antibody ZEB146 (TrkC-Ab) is a fully human IgG2 derived from immunizing Xenomouse II mice. TrkB agonist antibody ZEB85 (TrkB-Ab) is a single-chain variable fragment-Fc selected from a human combinatorial antibody library. TrkC-Ab, NT3, or TrkB-Ab, dissolved in 0.1% mouse serum albumin in artificial perilymph, was infused into the left cochleae via the round window or lateral semicircular canal using a minipump/cannula, with the right ear serving as the unoperated noise-exposed control. Infusion was initiated within 3 hours postnoise and continued until PND14. Postsynaptic densities, presynaptic ribbons and inner hair cells (IHCs) were visualized using anti-PSD95, anti-Ribeye/CtBP2 and anti-myosin antibodies, respectively, in organ of Corti wholemounts at the 8, 16, and 32 kHz location. Synapse counts were quantified as the number of colocalized PSD95 and CtBP2 puncta per IHC.
Results: All mice exhibited elevated ABR thresholds at PND1. By PND14, ABR thresholds in unoperated noise-exposed control right ears returned to baseline. In operated noise-exposed left ears, ABR thresholds recovered to prenoise baseline in approximately two-thirds of the cases, with the remainder excluded from analysis. Both noise-exposed TrkC-Ab and NT3-infused ears showed similar recovery of ABR wave-I amplitude, while noise-exposed control vehicle- or TrkB-Ab -infused ears didn't show significant recovery of ABR wave-I amplitude. Consistent with this observation, synapse numbers were significantly lower in noise-exposed control vehicle- or TrkB-Ab-infused ears, while synapse numbers in noise-exposed TrkC-Ab- and NT3-infused ears were comparable to those in non-noise-exposed control ears and significantly higher than in noise-exposed control vehicle- or TrkB-Ab -infused ears.
Conclusions: These results confirmed that intracochlear delivery of TrkC-Ab postnoise effectively promotes cochlear synapse restoration, with results comparable to NT3. In contrast, TrkB-Ab did not demonstrate similar benefits.
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SU84. Translatability of the Larval Zebrafish Lateral Line Neuromast Model as a High-Throughput Screening (HTS) Method for Noise-Induced Hearing Loss Drug Discovery

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Category: Inner Ear: Damage and Protection of Neurons & Synapses
Background: Noise-induced hearing loss (NIHL) is a significant global health issue, affecting approximately 1.3 billion people and accounting for more than 30% of all hearing loss cases. It is recognized as the leading preventable cause of acquired hearing loss, primarily due to noise exposure in occupational settings. Despite its prevalence, there are currently no FDA-approved therapies to prevent or mitigate NIHL. It is well-known that glutamate excitotoxicity plays a critical role in the mechanisms underlying noise-induced hair cell and auditory nerve damage.

One of the major barriers hindering NIHL drug discovery is the lack of high-throughput screening (HTS) platform. In this work, we evaluate the chemically induced glutamate excitotoxicity hair cell damage zebrafish model that was initially proposed by Sheets (Sci Rep. 2017) and the translatability of its outcomes to rodents.
Methods: Approximately 100 small molecule compounds that were tested in NIHL rodent models have been extracted from the literature. The outcomes of these compounds have been stratified based on ABR and/or DPOAE threshold shift reduction after noise exposure. The compounds are tested in the Kainic acid (KA) and NMDA-induced glutamate excitotoxicity larval zebrafish lateral line neuromast model. The results are compared to the outcomes of these compounds in rodents. FM1-43 assay is used to determine whether MET channel blockers, which is a potential confounding factor that needs to be considered.
Results: Compared to published rodent model results, the zebrafish assay offers good predictability in identifying protective/non–protective compounds. It also provides qualitative agreements on the level of protection/mitigation against NIHL.
Conclusions: The zebrafish assay correlates reasonably well with mammalian model data and even human subject data in some cases. Our work demonstrates that the zebrafish assay is an efficient and effective HTS drug screening tool to speed up and facilitate the NIHL drug development process. The evaluation of ribbon synapse damage and protection in zebrafish as a drug screening model is underway.

SU85. Chronic Trk Receptor Inhibition Increases Noise-Induced Cochlear Synaptopathy in Adult Mice

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Category: Inner Ear: Damage and Protection of Neurons & Synapses
Background: The Trk tyrosine kinase receptors and their ligands, the neurotrophins BDNF and Ntf3, play key roles in the development and survival of vestibular and cochlear sensory neurons during the embryonic period, but their role in adulthood remains unclear. We previously demonstrated that Ntf3 regulates ribbon synapse density in the neonatal cochlea and its over-expression induces synapse regeneration after acoustic trauma in adult mice.
To determine the importance of Trk signaling in the adult inner ear, we tested if chronic inhibition of Trk receptors alters auditory function and the impact of noise using Larotrectinib/LOXO-101, a Trk inhibitor that is used as a cancer treatment.

Methods: To test the effects of Trk inhibition on hearing, adult (8w-old) CBA/CaJ mice were treated with Larotrectinib/LOXO-101 (100mg/kg/day, gavage) or vehicle (10% DMSO and 5% glucose) daily for 21 days. To test the effects of Trk inhibition on the response to noise, another set of 8w-old mice were exposed to a 100dB 8-16kHz band noise for 2hs. Larotrectinib/LOXO-101 or vehicle was delivered immediately after the exposure and continued daily for 14 days. In all cases, cochlear function (ABR and DPOAE) was measured at baseline and every 7 days. At the end of the treatments, ears were harvested for histological analysis.

Results: Chronic Trk receptor inhibition in un-exposed mice caused a mild ABR threshold shift only at 32kHz and a decrease in ABR P1 amplitudes at mid and high frequencies when compared with control mice. As previously demonstrated, noise-induced temporary ABR and DPOAE threshold shifts were seen from 16 to 32kHz. Whereas in control mice, ABR and DPOAE thresholds returned to normal 2 weeks after exposure, mice treated with LOXO-101 showed mild permanent ABR threshold shifts at 32kHz and permanently reduced ABR P1 amplitudes at high frequencies. Histological analysis is ongoing.
Conclusions: These results indicate that Trk signaling is necessary for the preservation of normal hearing in adulthood and that its loss makes the cochlea more sensitive to noise-induced damage. These observations also suggest that cancer patients treated with Trk inhibitors might be susceptible to developing hearing problems.

SU86. Roles of D-Serine in Kainate-, and Noise-Induced Cochlear Synaptopathy and Repair

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Category: Inner Ear: Damage and Protection of Neurons & Synapses
Background: NMDA receptor (NMDAR) activation in the cochlea has a multifaceted role in auditory signal processing, synaptic plasticity, neuroprotection and excitotoxicity. Traditionally, glycine was considered the main co-agonist for NMDAR activation. However, growing evidence suggests that D-serine, an endogenous amino acid, plays a more prominent role in modulating cochlear NMDAR function in the cochlea. However, the molecular mechanisms behind the pathological activation of NMDAR induced by D-serine in the cochlea are not well understood. Understanding these roles could provide insight into the mechanisms underlying hearing adaptation and the pathophysiology of auditory disorders such as hearing loss and tinnitus, where NMDAR dysfunction or dysregulation may be involved.
Methods: To investigate the role of D-serine-induced NMDAR activation in cochlear synaptopathy and repair, we utilized an in vitro kainate-induced synaptopathy model using neonatal P3 mouse cochlear explants and an in vivo model using an impulse noise (peak pressure: 146 dB SPL, 1 pulse/second, for 700 seconds) exposure. By integrating molecular biology techniques, cochlear ultrastructural analysis, in vivo electrophysiology, and acoustic startle reflex tests, we evaluated the potential involvement of D-serine in NMDAR dysfunction or dysregulation, as well as its impact on the pathophysiology of auditory disorders.
Results: Our results demonstrated that, in the presence of NMDA, D-serine exerts a protective effect against kainate-induced acute synapse loss in cultured cochlear explants, as well as against impulse noise-induced temporary threshold shifts in adult mice. This protective effect was completely abolished when cochleae were co-treated, both in vitro and in vivo, with the selective receptor antagonist, 5,7-dichloro-4-hydroxyquinoline-2-carboxylic acid (DCKA), which blocks the glycine/D-serine binding site of the NMDAR.

Conclusions: These results suggest that understanding the pathways involved in excitotoxicity is of critical importance for the future clinical treatment of many auditory neurodegenerative diseases.

SU87. Intracochlear Insertion Force and Hydrostatic Pressure Measurements During Cochlear Implantation and Coiling in 3D Printed Artificial Cochlea and Mastoid Models

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Category: Inner Ear: Damage and Protection of Neurons & Synapses
Background: Despite cochlear implants (CI) being the most successful neural prosthetic device to date, damage to residual hearing during cochlear implantation still occurs in approximately 30% of patients. Increased hearing preservation following surgery is associated with improved speech perception, pitch discrimination, musical recognition, and quality of life in CI patients. Insertion force (IF) and hydrostatic pressure (HP) are two physical mechanisms by which damage to residual hearing can occur and can be influenced by extracochlear factors such as surgical approach, insertion method, and electrode trajectory. Individual temporal bone anatomy, particularly mastoidectomy shape and posterior tympanotomy (PT) alignment, can constrain the operative approach during CI insertion and coiling. Although significant research has been conducted on developing "soft-surgery" techniques for cochlear implantation, lead coiling in the mastoid cavity following CI insertion has been largely overlooked in terms of its impact on insertion trauma. This study aims to investigate how intracochlear IF and HP measurements vary based on mastoid anatomy during CI insertion and fixation, using anatomically accurate artificial models and a high-fidelity insertion simulation setup.
Methods: Four cadaveric temporal bones underwent mastoidectomy and formation of a PT and round window approach. Micro-computed tomography scans were obtained and segmented to produce mastoidectomy and PT surface reconstructions. A custom MATLAB script was used to characterise the cochlea anatomy and align the mastoid cavities to a representative average scala tympani model. One cochlea and four interchangeable mastoidectomy models were then fabricated using high-resolution 3D printers and integrated into a custom CI insertion setup with a 6-axis force and pressure sensor, allowing for simultaneous IF and HP measurements throughout manual cochlear implantation by a fellowship trained CI surgeon.
Results: Mean mastoid cavity volume lateral to the PT was 3.93ml (standard deviation (SD) 0.57). Facial nerve-chorda tympani angle had a mean value of 35.35□ (SD 15.33). Mean PT width and length, at an angle with adequate visualisation of the RW, were 2.45mm (SD 0.38) and 8.38mm (SD 0.57), respectively. During CI insertion, IF had the highest peak in the axis of electrode advancement (x-axis) and a smaller peak in the vertical axis (z-axis). During CI coiling in the mastoidectomy cavity, IF peaked in the z-axis. HP changes were greatest during CI insertion.
Conclusions: Continuous IF and HP measurements in a high-fidelity insertion simulation have demonstrated that forces peak during different stages of CI insertion and coiling. The higher IF exerted by vertical movements (z-axis) during CI coiling increases the risk of damage to the

basilar membrane, which may result in further residual hearing loss, depending on the location of damage and the remaining frequencies of residual hearing. These findings may help to better inform surgical decision-making, particularly when securing the implant, to further optimise hearing preservation and CI performance in patients.

SU88. TRPA1 Deficiency Leads to Reduced Volume of Outer Hair Cell Ribbon Synapses After Noise Exposure

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Category: Inner Ear: Damage and Protection of Neurons & Synapses
Background: TRPA1 channels are involved in detecting pain-like signals in nociceptive neurons. They are activated directly and indirectly via several pathways present during tissue damage. TRPA1 channels are also highly expressed in the cochlear epithelium. We recently found that, after acoustic overstimulation, mice lacking TRPA1 channels exhibit a shorter temporary threshold shift (TTS) and permanent changes in the waveform of auditory brainstem responses (ABR) (Velez-Ortega, et al., Nat Commun, 2023). Thus, TRPA1 signaling appears to protect against noise-induced cochlear damage. Noise exposure is known to affect counts, volumes, and subcellular localizations of hair cell ribbon synapses. We previously evaluated ribbon counts in inner hair cells (IHC) before and after noise exposure and found no differences between TRPA1-deficient mice and wild-type controls. Here, we tested whether TRPA1-deficient mice are more susceptible to changes in outer hair cell (OHC) ribbon synapses after noise exposure.
Methods: We used C57Bl/6 TRPA1-deficient mice and wild-type controls. Anesthetized young (4-week-old) mice were exposed to 100 dB SPL broadband noise for 30 minutes, and temporal bones were collected two weeks after noise exposure. Cochlear epithelia were immunolabeled with antibodies against CtBP2/RIBEYE, myosin VIIA. Confocal images were obtained with a Leica SP8 upright confocal microscope. Ribbon counts and positions were quantified using ImageJ software. Ribbon volumes were determined using Imaris 10.0.
Results: Our results show a similar decrease in the OHC ribbon counts two weeks after noise exposure in both wild-type and TRPA1-deficient mice. This conflicts with a previous study performed on wild-type mice where noise exposure of awake mice led to no differences in the total number of ribbons in OHC (Wood et al., JARO, 2021). Interestingly, after noise exposure, we also found that OHC ribbons are significantly smaller in TRPA1-deficient mice than in wild-type controls at multiple cochlear locations. Next, we plan to explore whether these noise-induced changes in OHC ribbons of TRPA1-deficient mice also affect their postsynaptic densities.
Conclusions: Our results indicate that TRPA1 knock-out mice show a greater decrease in ribbon volumes after noise than wild-type mice. Continued examinations of OHC ribbon counts, volumes, and positions are required to fully understand their dynamic changes and functional consequences after noise exposure and TRPA1-mediated signaling in anesthetized and awake conditions.

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SU89. Identification of Biomarkers Associated With Cochlear Synaptic Loss

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Category: Inner Ear: Damage and Protection of Neurons & Synapses

Background: Synaptic loss is an early sign of major neurodegenerative diseases, including noise-induced or age-related hearing impairment. Currently, there are no clinical diagnostic tools to detect human cochlear synaptic loss in vivo. In animal models, seminal longitudinal studies with a combination of electrophysiological and post-mortem histological methods clearly demonstrated synaptic loss between inner hair cells and spiral ganglion neurons due to noise insult or aging. However, in humans, due to a lack of effective diagnosis tools, "hidden hearing loss" is used to describe cochlear synaptopathy without changes in hearing thresholds. Since clinical diagnosis of human hidden hearing loss cannot include histological quantification, various attempts have been made to detect cochlear synaptic loss or cochlear synaptopathy based on functional measurements. So far, those efforts have led to inconclusive results. One major obstacle is a high variability of wave amplitudes from sound-evoked electrical potentials. To address this important issue, firstly, we applied machine learning to identify unique electrophysiological features specifically associated with cochlear synaptic loss from well-established mouse models of noise-induced cochlear synaptopathy. Secondly, in the same mouse models, we collected blood samples and identified blood molecular markers associated with cochlear synaptic loss. These blood molecular markers could provide an independent validation for non-invasive functional biomarkers in human diagnosis. Thus, our data provided the first set of biomarkers associated with noise-induced cochlear synaptic loss and paved the way to develop an effective clinical diagnosis of synaptic loss.

Methods: CBA/CaJ mice were used to identify biomarkers for cochlear synaptopathy. Three groups of animals were: control, 91-dB, and 96-dB SPL groups (8-16 kHz; for 2 hours). Quantitative histology was used to determine inner and outer hair cell populations and synaptic loss. Cochlear synaptic loss was found only in the 96-dB group. Based on our recent paper the curvature measurement of ABR Wave I is more sensitive for detecting CS than its amplitude measurement. Using both curvature and latency quantifications we applied machine learning approaches to identify ABR features associated with noise-induced synaptic loss. To identify blood molecular markers associated with cochlear synaptopathy, we collected blood samples and carried out transcriptomic analysis of peripheral blood monocyte cells.

Results: We identified six new ABR features associated with noise-induced synaptic loss by machine learning. In addition to these non-invasive functional features, we identified mRNA markers from peripheral blood monocyte cells, which can be used to cross-validate cochlear synaptopathy with functional markers.

Conclusions: This study combined both electrophysiological recordings and transcriptomic analysis and identified new biomarkers for noise-induced synaptic damage of the inner ear.

SU90. Gene Regulatory Elements Enable Cell-Specific Delivery of GJB2 to Treat a Mouse Model of DFNB1 Deafness

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Category: Gene Therapy

Background: DFNB1, caused by mutations in GJB2, is the most common form of hereditary hearing loss. Because null mutations do not always produce profound deafness, DFNB1 might be treatable with gene therapy. GJB2 encodes GJB2/connexin26, a gap-junction protein expressed by epithelial cells and fibrocytes, but not by hair cells. Although the GJB2 coding sequence is small enough to be packaged in a single AAV vector, published reports have not demonstrated significant functional rescue in an animal model of DFNB1.

Methods: We used mouse models of DFNB1 with mild and severe phenotypes to test gene addition therapy. The GFP or GJB2 coding sequence was packaged in AAV9-PHP.B, which robustly transduces GJB2-expressing cells, hair cells, and other cochlear cells. Expression was driven by the CBA promoter or by a GJB2-specific promoter and regulatory elements. Vectors were injected through the round window membranes of neonatal mice or juvenile nonhuman primates.

Results: In wild-type mice, vectors using the ubiquitous CBA promoter to drive GJB2 expression were often lethal and caused hearing loss in surviving mice, but not if GJB2 carried the 35delG truncating mutation. To express GJB2 in appropriate cells and prevent widespread expression in hair cells and brain, we used the human GJB2 promoter and proximal gene regulatory elements (pGREs). Vectors encoding GFP transduced only GJB2-expressing epithelial cells and fibrocytes. In Gjb2-floxed mice with Cre recombinase driven by the Sox10 promoter (Gjb2^fl/fl, Sox10-Cre), we found that the organ of Corti has normal morphology at P6 but degenerates to a flat epithelium by P30. Injection of Gjb2^fl/fl, Sox10-Cre+ mice at P1 with AAV9-PHP.B with pGREs driving expression of mmGJB2.HA (AAV-pGRE-GJB2.HA) prevented the degeneration of the organ of Corti, and injection of Gjb2^fl/fl, Sox10-Cre- control mice caused no lethality or hearing loss. Untreated Gjb2^fl/fl, Sox10-Cre+ mice showed profound hearing loss, with little or no ABR response at 120 dB. Despite rescue of cochlear morphology, AAV-pGRE-GJB2.HA produced only slight rescue of ABR responses at P30. Suspecting that the earliest cochlear pathology in mice begins before the vector can express GJB2, we treated a milder model in which Gjb2 carries the common M34T mutation (Gjb2^fl/M34T, Sox10-Cre). The progressive hearing loss observed in untreated mice was completely prevented by P1 injection of AAV-pGRE-GJB2.HA. To confirm specificity in a primate, we injected juvenile cynomolgus monkeys with AAV-pGRE-hsGJB2.HA. Anti-HA label was seen in nearly all GJB2-expressing cell types, and injections generally produced no elevation of ABR threshold.

Conclusions: We conclude that pGREs are necessary to restrict GJB2 expression to the appropriate cells and to avoid lethality. AAV-pGRE-GJB2.HA prevents degeneration in severe models, but substantially rescues the ABR only in a mild model of DFNB1. Proper localization and absence of toxicity confirmed in nonhuman primates indicate promise for treatment of DFNB1.

SU91. Restoration of Hearing in a Novel Gjb2^M34T Mouse Model with Gene Addition Therapy

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Category: Gene Therapy

Background: More than 140 genes are casually linked to hearing loss, but the most prevalent form of hereditary hearing loss results from mutations in one gene, GJB2, which encodes the gap junction protein Cx26/GJB2. GJB2 is expressed by epithelial cells of the organ of Corti and by fibrocytes elsewhere in the cochlea. GJB2 mutations occur in up to 50% of patients with hereditary hearing loss, with severity ranging from moderate to profound. In European populations, the p.M34T mutation in GJB2 is most frequent, estimated to be 1 out of every 4000 births, and results in mild-to-moderate, possibly progressive hearing loss. As hearing loss is not profound, the sensory epithelium might not degenerate in patients and p.M34T may be a good target for gene therapy.

Methods: We first generated a p.M34T mouse model by CRISPR-mediated insertion of donor DNA that encoded GJB2 with the p.M34T mutation. Homozygous mice were embryonic lethal, so we paired the p.M34T allele with a floxed allele and a Cre active in the cochlea: Gjb2^M34T/fl, Sox10-Cre. All mice carried the Cdh23-Ahl corrected allele. Viral vectors used the AAV9-PHP.B capsid and a GJB2-specific promoter to drive the expression of wild-type GJB2 with a C-terminal HA tag. Mice were injected at P1 through the round window membrane. Auditory thresholds were measured at P30, P60, P90 and P120 and compared between mutants and controls. Cochleas were extracted after P120 and prepared for histological analysis.

Results: We first evaluated a group of untreated Gjb2^M34T/fl,Sox10-Cre mice (n=16) along with both Cre- littermate controls (n=6) and C57BL/6J wild-type controls (n=5). Gjb2^M34T/fl,Sox10-Cre mice displayed a mild-moderate, broad-frequency progressive hearing loss that progressed over 120 days to ~30 dB threshold elevation and was variable among animals. Histology revealed that GJB2 is still present; however, mice displayed some hair cell loss at P120. Control animals showed wildtype hearing thresholds. Gjb2^M34T/fl,Sox10-Cre mice were injected with the vector at P1 (n=13). We observed complete rescue of hearing, with treated mice displaying wildtype thresholds at all ages. To test toxicity, we injected the vector into Cre- littermate controls (n=7) and observed no threshold elevation. At P120, antibody labeling of the HA tag showed expression in most cell types that normally express GJB2.

Conclusions: This novel mouse model of M34T-associated hearing loss recapitulates the human phenotype, with variable, mostly moderate hearing loss. Delivery of GJB2 to the appropriate cells using the GJB2 promoter completely rescued the hearing loss and showed no toxicity in controls. This vector might be adapted to treat the most common deafness mutation in European populations.

SU92. GJB2 Gene Therapy-Response of Two Pre-Clinical Mouse Models of the Most Frequent Form of Human Deafness, DFNB1

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Category: Gene Therapy

Background: More than 1.5 billion people worldwide live with hearing loss (HL). Sensorineural HL affects one new-born in 700-1000 and approximately one child or young adult in 500 before the age of 20. Approximately 70% of congenital severe to profound deafness cases are hereditary due to monogenic defects, thus potentially treatable by gene therapy (GT).
GJB2-related autosomal recessive non syndromic HL (also referred as DFNB1) is the most common genetic cause of congenital sensorineural HL in many world populations, frequently accounting up to half cases. In addition, it is also responsible for deafness occurring later on, even after 40 years of age. GJB2 encodes connexin-26 (Cx26) gap-junction channel protein that plays a key role in metabolites and ions homeostasis necessary to cochlear development and sensory hair cell function and survival. Notably, Cx26 contributes to endolymph ionic composition and endocochlear potential, the driving force of the sensory mechanotransduction. Hundreds of pathogenic GJB2 variants have been reported which outcome spans from mild to profound deafness caused by genomic variants including large deletions, loss-of-function (LOF) and missense variants.

Methods: In order to develop GT approaches for deafness caused by GjB2 defects, we developed several experimental mouse models. Among those that are models for GJB2 human deafness forms, one Gjb2del/del resulting in a biallelic Gjb2 inactivation mimics the most common form of DFNB1, and the other, Gjb2del/Hmut, a compound heterozygote, expressing a human missense pathogenic variant (Hmut), corresponds to a frequent human GJB2 genotype. To circumvent embryonic mouse lethality caused by Gjb2 inactivation, conditional knockouts were generated here using the same Cre-recombinant mice which displays a large cochlear spatial expression, i.e. in all Gjb2-positive cells.
Results: Both models showed an elevation of the hearing threshold of 110dB and 90dB on average, in Gjb2del/del and Gjb2del/Hmut mice, respectively. In both models, inner ear delivery of a GT-GJB2 recombinant adeno-associated virus (AAV) improved the hearing threshold. Histological analysis showed cochlear structure preservation in both Gjb2 defective recombinant mice. Especially, treated Gjb2del/del mice, GT-GJB2 fully impeded their dramatic hair cells and supporting cells lost.
Conclusions: Thus, GT-GJB2 improves audition in two preclinical models of human pathogenic GJB2 variants.

SU93. CRISPR/Cas9-Mediated Exon Skipping to Restore Premature Translation Termination in a DFNB4 Mouse Model

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Category: Gene Therapy

Background: Hearing loss is a common sensory disability impacting more than 1.5 billion individuals worldwide. Approximately two-thirds of congenital deafness instances result from genetic abnormalities. Pendrin, encoded by the SLC26A4 gene, is crucial for anion transmembrane transport, affecting the endolymphatic pH in the inner ear. Mutations in SLC26A4 result in DFNB4 non-syndromic hereditary hearing loss and Pendred syndrome, characterized by enlarged vestibular aqueduct (EVA) and cochlear partition anomalies. The prevalent SLC26A4 c.919-2A GREATER THAN G splice-site mutation in East Asian populations results in a loss of exon 8 and frameshifted, truncated pendrin. In this study, we developed a novel mouse model, Slc26a4△E8+E9/△E8+E9, by deleting exons 8 and 9 to restore the open reading frame (ORF) of pendrin, and to examine whether the protein expression levels and audiovestibular function were restored in comparison to prior SLC26A4 splice-site mutation mice.

Methods: A CRISPR/Cas9-mediated exon-skipping approach was employed to generate Slc26a4△E8+E9/△E8+E9 mice. Vestibular function was evaluated using swimming and open-field tests, and auditory function was quantified through auditory brainstem response (ABR) testing. Furthermore, pendrin expression and the integrity of cochlear hair cells were assessed by immunofluorescence and confocal microscopy.

Results: The developed novel mouse model harboring exon-skipped SLC26A4 transcript effectively expressed reframed pendrin in the endolymphatic sac and restored vestibular-related balance function. However, despite the modification of the pendrin protein, hearing loss persisted, demonstrated by increased ABR thresholds and the degeneration of cochlear hair cells. The enlarged endolymphatic sac and vestibular aqueduct, prevalent characteristics in DFNB4, were also not reserved.

Conclusions: The study creates mouse models with targeted deletions of SLC26A4 exons 8 and 9 to assess the occurrence of ORF restoration and its impact on pendrin expression and audiovestibular functionality. This innovative finding presents a potential path for exon-skipping therapeutics aimed at SLC26A4-related auditory impairment.

SU94. Hearing Evaluation of Exon-Deleted Mouse Models of Ush2a to Determine Dispensable Exons for Exon Skipping Therapeutic Approaches

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Category: Gene Therapy

Background: USH2A is the most common form of blindness-deafness syndrome in humans. USH2A is encoded by one of the largest genes in the human genome, with 15.6kb of coding sequence. This seriously hampers the possibility of traditional gene augmentation therapy, as it far exceeds the packaging capacity of AAVs. The strategy of exon skipping to delete the non-essential mutation harboring exons is a viable approach to restore USH2A function and rescue vision and hearing. The strategy has been used to skip exon 13 with mutations, resulting in the

production of properly localized USH2A protein Usherin. In addition to exon 13, there are 24 in-frame exons within USH2A that could potentially be skipped to produce functional USH2A mRNA. However, it is not known which of the 24 exons can be skipped without losing their biological functions, which can be potentially applied to treat USH2A patients.
Methods: We selected 6 in-frame human exons (exon 16, 18, 21, 38, 43, 45) as targets and 2 out-of-frame exons (exon 6 and 20) as controls. Dual sgRNAs targeting the flanking introns of the exon-of-interest were synthesized and co-injected with Cas9 protein into the mouse zygote to delete the targeted exons and generate the mouse models. The genotype was validated by Sanger sequencing. These mouse lines were crossed with Ush2a knockout mice (DEL5/KO, DEL15/KO, DEL17/KO, DEL19/KO, DEL20/KO, DEL37/KO, DEL42/KO and DEL44/KO), and their hearing function (auditory brainstem response) was evaluated at ages 2 and 4 months.
Results: At 2 months, significant difference in hearing threshold was observed at 32kHz. Compared to DEL5/KO and DEL19/KO mice (out-of-frame controls, 89□22 dB SPL), DEL15/KO, DEL17/KO, DEL20/KO, DEL37/KO, and DEL44/KO mice showed preserved hearing (52□9 dB SPL, p LESS THAN 0.05, Mann Whitney U test), whereas DEL42/KO showed similar poor hearing (87□19 dB SPL, p=0.17). The average ABR threshold for WT/KO mice at 32kHz was 55□19 dB SPL. At 4 months, high frequency hearing loss progressed for all genotypes and significant difference between mice was observed at 16kHz and/or 22.64kHz. At 4 months, DEL17/KO, DEL37/KO and DEL44/KO mice had preserved hearing (31□5 dB SPL for 16kHz, 38□10 dB SPL for 22.64kHz), whereas DEL15/KO, DEL20/KO, DEL42/KO mice showed hearing thresholds (35□6 dB SPL for 16kHz, 56□20 dB SPL for 22.64kHz) comparable to the out-of-frame controls (42□7 dB SPL for 16kHz, 70□20 dB SPL for 22.64kHz)
Conclusions: By generating exon-deleted mouse models of Ush2a and testing their hearing, we showed that hearing was preserved in Ush2a mice that lacks exon 17, 37 and 44, respectively, at 4 months of age. The study identified Ush2a exons 17, 37 and 44 as the candidates for exon skipping strategy to rescue vision and hearing in the Ush2a mouse model.

SU95. Therapeutic Potential of ssAAV vs. scAAV in Inner Ear Gene Therapy

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Category: Gene Therapy
Background: Gene therapy using adeno-associated virus (AAV) is a powerful approach for treating inner ear diseases, with more than 40 preclinical studies and ongoing clinical trials demonstrating its potential. To fully achieve the potential of AAV-based therapies for hearing loss and balance disorders, several factors need optimization. One limiting factor in transgene expression is the conversion of single-stranded (ss) DNA to double-stranded (ds) DNA. Self-complementary (sc) AAV vectors can bypass this step and may improve expression efficiency in hair cells, but it remains unclear if this leads to superior therapeutic efficacy compared to ssAAV

vectors. This study aims to compare and evaluate the viral expression rates and therapeutic effects of ssAAV and scAAV in a mouse model of DFNB103 caused by CLIC5 mutations.
Methods: Synthetic AAV9-PHP.B vectors, single-stranded (ssAAV) and self-complementary (scAAV), both carrying the coding sequences of TurboGFP or Clic5, were generated and delivered into the inner ears of Clic5c.680T GREATER THAN C mice via utricle injection at P0. Immunostaining was used to quantify and compare the transduction rate between the two AAVs. Measurements of hearing function were performed using auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs), followed by behavioral assays such as Rotarod and open field tests to evaluate vestibular function. Sensory cell morphology in both the vestibular and auditory systems was assessed using immunofluorescence and scanning electron microscopy.
Results: Both ssAAV and scAAV demonstrated high expression efficiency in inner ear sensory cells, with scAAV.GFP demonstrating superior enhanced efficiency compared to ssAAV.GFP. Injection of either ssAAV or scAAV vectors encoding Clic5 rescued hearing in deaf Clic5c.680T GREATER THAN C mice by restoring Clic5 expression in hair cells. Compared to the untreated group, the treated mice had a higher survival rate of hair cells and reduced degeneration of hair bundle morphology. In the vestibular system, both ssAAV.Clic5 or scAAV.Clic5 rescued vestibular function, evidenced by decreased circling behavior and improved motor performance in treated mice. While the scAAV demonstrated enhanced transduction efficiency, the ssAAV showed a slightly more favorable trend for comprehensive therapeutic effects. However, this improvement was not statistically significant when compared to the scAAV.
Conclusions: Our findings demonstrate the feasibility of restoring Clic5 expression, reducing cell death and morphological degeneration, and rescuing auditory and vestibular function in Clic5-deficient mice. This was achieved using both ssAAV and scAAV. While scAAV demonstrated enhanced expression efficacy, this did not translate to significantly improved therapeutical outcomes in this model.

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SU96. Rational Design of a Lfng-Enhancer AAV Construct Drives Specific and Efficient Gene Expression in Inner Ear Supporting Cells

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Category: Gene Therapy
Background: Cell specific gene expression is crucial to ensure safe and efficacious gene therapies for the treatment of sensorineural hearing loss. Our approach combines an adeno associated virus (AAV) with high tropism for the inner ear and an enhancer sequence from the supporting cell specific gene Lfng.
Methods: To identify enhancer sequences, accessible elements (ATAC-seq) with high enhancer (H3k4me1), high active histone (H3K27ac), and low promoter histone (H3K4me3) marks were searched for at the Lfng locus in P1 supporting cells. Three candidate Lfng-enhancer sequences,

and the ubiquitous CBh promoter, driving the GFP reporter gene were packaged into the AAV-ie capsid. Neonatal mice were injected with AAV through the posterior semicircular canal and allowed to develop for 7-21 days. The mice were sacrificed, their cochleae and brains harvested and analyzed with immunofluorescence microscopy.
Results: AAV CBh-GFP transduced multiple sensory as well as non-sensory inner ear cell types and also transduced cells in the hippocampus, cortex, and cerebellum. One of the three Lfng-enhancers showed robust GFP expression in nearly all border cells, inner phalangeal cells, pillar cells, and all three rows of Deiter's cells, along the cochlear duct as well as in vestibular organ supporting cells. Significantly, no fluorescently labelled cells were detected in the brains of mice injected with this virus. To confirm our results and further test the construct's efficacy in driving gene expression, we designed an AAV CreER2 vector using this enhancer and injected it into neonatal Rosa Ai3 reporter mice. At 18 days, tamoxifen was given intraperitoneally, mice were sacrificed on day P21, and their ears and brains harvested. Reporter gene expression was strong in most supporting cell types indicating efficient Cre-recombination. Once more, no recombination was observed in the brain of injected mice.
Conclusions: We show that a Lfng enhancer sequence drives gene expression specifically in inner ear supporting cells while sparing the brain.

SU97. GJB2-GT, a Novel Adeno Associated Vector-Based Gene Therapy as a Treatment for the Autosomal Recessive Non-Syndromic Deafness 1A (DFNB1A)

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Category: Gene Therapy
Background: In the world, the estimated prevalence of severe or profound deafness in human is 1 out of 1000 neonates, and genetic factors account for half of the cases. Pathogenic variants of GJB2, the gene encoding for Connexin 26 (Cx26), are involved in 50% of congenital deafness and are mostly associated with an autosomal recessive non-syndromic DFNB1A. In the cochlea, GJB2 is largely expressed in the supporting cells (SCs) of the sensory epithelium, fibrocytes, and basal and intermediate cells of stria vascularis but not in sensory hair cells. It is hypothesized that Cx26 is essential for the recycling of potassium, which is essential for the proper functioning of sensory hair cells, but in vivo studies also suggest that Cx26 deficiency leads to cochlear developmental disorders. Gene therapy is a promising therapeutic strategy for autosomal recessive forms of deafness, and Adeno-Associated Vectors (AAVs) are being developed to this aim.
Methods: Here, we have developed GJB2-GT, an AAV vector for DNFB1A that offers broad coverage of Gjb2-expressing cells of the inner ear in both mouse and non-human primates and designed its specific expression cassette to allow detargeting hair cells. GJB2-GT was delivered into congenitally deaf conditional Gjb2 mutant mouse ears through the round window (RW). Efficacy on on-going cohorts, dose-response experiments, correlation between ABR responses

and transduction efficacy, early biodistribution and toxicology studies in mice were investigated. In parallel, GJB2-GT was administered to Non-Human Primates (NHP) using the surgical approach and medical injection device used in human. Early tolerability and biodistribution of GJB2-GT were assessed. Whole mounts and cryosections of injected inner ears were analyzed to assess the AAV tropism by immunoconfocal analyses and in situ hybridization.
Results: A single intracochlear injections of GJB2-GT into conditional Gjb2-mutant mouse inner ears lead to statistically significant improvement of hearing thresholds as early as 3 weeks post-injection in a dose dependent manner. In NHP, three weeks post-surgery, ABR measurements and DPOAE amplitudes remained contained within the normal hearing threshold range of NHPs indicating that GJB2-GT was well tolerated locally. Biodistribution was mostly limited to injected ears. In WT mice, GJB2-GT was also well tolerated. Lack of impact on ABR was demonstrated 6 months post administration together with long-term transgene expression. In mice and NHP, broad target cells coverage was observed, the vast majority of SCs were transduced along the tonotopic axis and no transduction was found in hair cells.
Conclusions: GJB2-GT efficiently targets the cells that naturally express GJB2 in the cochlea. GJB2-GT demonstrates promizing efficacy and safety data package, supporting its evaluation in IND-enabling studies as a novel therapeutic for treatment of DFNB1 in human.

SU98. Gene Replacement Therapy Rescues Hearing in a Mouse Model With an Mpzl2 Variant

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Category: Gene Therapy
Background: To date, 154 genes linked to nonsyndromic hearing loss have been identified, of which 87 are implicated in DFNB (autosomal recessive inheritance). Mpzl2 is expressed in the outer hair cells (OHCs), inner hair cells (IHCs), particularly in Deiter's cells (DCs), and pillar cells (PCs). Mutations in MPZL2 have been reported to cause DFNB111.
Methods: In order to develop a treatment strategy for individuals with DFNB111, we generated a knock-in (KI) mouse model harboring the MPZL2 p.Q74* mutation, which displayed slowly progressive hearing loss and disorganized supporting cell arrangement. We employed two adeno-associated virus (AAV) serotypes to target hair cells and supporting cells, delivering the human MPZL2 coding sequence through the round window membrane between postnatal days 1 and 3.
Results: Auditory assessments indicated that mice injected with AAV-MPZL2 caused worse auditory impairment than control mice. Targeting hair cells resulted in a decrease in OHCs, while targeting supporting cells led to reductions in IHCs, OHCs, and supporting cells. Controlling the expression level of human MPZL2 through a specific promoter restored auditory function in Mpzl2 KI mice without diminishing hair cells, supporting cells, or causing structural abnormalities at 4 weeks.
Conclusions: Our findings highlight the importance of selecting the target cells and precisely controlling gene expression in gene therapy.

SU99. Dexamethasone Nanocrystals-Embedded Hydroxypropyl Methylcellulose Hydrogel Increases Cochlear Delivery and Attenuates Hearing Loss Following Intratympanic Injection

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Category: Gene Therapy

Background: Sudden sensorineural hearing loss is often treated with intratympanic (IT) dexamethasone (DEX) injections. However, the conventional dexamethasone sodium phosphate has limited round window membrane permeability and short retention in the middle ear, reducing its therapeutic efficacy. A novel DEX nanocrystalline suspension (NS) embedded in a hydroxypropyl methylcellulose (HPMC) hydrogel aims to enhance cochlear delivery and hearing protection.

Methods: Hydrophobic steroidal nanocrystals were prepared using a bead milling technique and incorporated into a polysaccharide hydrogel. DEX NS embedded in a hydrogel (NS-G) system with HPMC (average molecular weight of 86,000 g/mol, 15 mg/mL) was characterized as follows: rod-shaped drug crystalline; LESS THAN 300 nm of particle size, and constant complex viscosity ≤ 1.17 Pa·s.

Results: Pulverization of the drug particles into submicron diameters increased drug dissolution, while the HPMC matrix increased the residence time in the middle ear cavity with a controlled release profile. The IT NS-G system provided markedly enhanced drug delivery to the cochlear tissue after 3 and 6 h of 87.2- and 385-fold higher, respectively, compared with that of dexamethasone sodium phosphate (DEX-SP), a water-soluble prodrug. In kanamycin- and furosemide-induced ototoxic mice, NS-G exhibited markedly enhanced hearing preservation across all frequencies (8–32 kHz) in the auditory brainstem response test compared to both saline and DEX-SP. Moreover, NS-G treatment resulted in improved anti-inflammatory effects, as evaluated via cytokine levels.

Conclusions: The IT administration of DEX NS-loaded HPMC hydrogels is a promising strategy for treating hearing loss.

SU100. Platelet-Rich Plasma as Neuroprotective Therapy Approach for the Inner Ear: In Vitro Study with Isolated Extracellular Vesicles

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Category: Inner Ear: Drug Delivery

Background: In the last years, cochlear implantation has been extended to patients with high frequency hearing loss but preserved residual hearing in the low frequencies. However, a large proportion of these patients lose their residual hearing after cochlear implantation. Thus, the performance of CI patients varies widely and generally depends on the number of remaining spiral ganglion neurons (SGN). This highlights the need to work on the development of new therapeutic options to preserve and protect residual hearing. Platelet-rich plasma (PRP) contains numerous cell-based growth factors such as bFGF (basic fibroblast growth factor), PDFGF (platelet-derived growth factor), VEGF (vascular endothelial growth factor) and TGF-β (transforming growth factor- β). In a previous study, we were able to show that the administration of human PRP significantly increased the survival rate of SGN in vitro and in addition, significantly longer neurites could be measured compared to the negative control (Stolle et al. 2017). For the planned allogeneic administration of PRP in the clinic, the cells should be removed from the PRP as a possible risk factor for a rejection reaction. Therefore, we isolated extracellular vesicles (EVs) from PRP and compared their neuroprotective effect with that of PRP.
Methods: From volunteer donors (2 female, 2 male), 8 mL of venous blood were freshly drawn and filled into the tubes of the RegenKit BCT3 (RegenLab) for the isolation of the PRP. Then, centrifugation for 5 min followed. Afterwards, the PRP was filtered and the EVs were isolated by size exclusion chromatography using the qEVoriginal 35 nm kit from IZON. The SGN were isolated from neonatal Sprague-Dawley rats (P3-5). The dissociated SGN were treated with various dilutions of PRP and PRP-EVs and were incubated for 48 hours. After that, the SGN were fixed, stained with neurofilament/DAB and the neuronal survival rate was determined. Moreover, the regenerative effect was investigated by measuring the length of the survived neurons.
Results: The PRP-EVs showed a neuroprotective effect at the same level as BDNF, the positive control of this experiment. The survival rate of the PRP-EVs is significantly increased compared to the negative control. In addition, a concentration-dependent behaviour of the PRP-EVs could be demonstrated. The effect of the PRP itself was significantly higher compared to the PRP-EVs isolated from it. Moreover, the neurite lengths after treatment with PRP-EVs, PRP and BDNF were significantly longer than in the negative control and the solvent control (PBS).
Conclusions: Autologous blood preparations such as PRP and products isolated from this like PRP-EVs could be used as neuroprotective and neuroregenerative agent in inner ear cells. A therapy for age-related hearing impairments and for preservation of residual hearing after cochlear implantation could be developed from this.

SU101. From Bench to Bedside: Vesicle-Enriched Secretome Fraction as Inner Ear Therapeutic During Cochlear Implantation

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Category: Inner Ear: Drug Delivery

Background: Following cochlear implantation, the performance of the device can be compromised by inflammation and fibrosis as a result of an immunological foreign body. In addition, patients often lose their residual hearing after cochlear implantation and there are no pharmacological therapies to compensate for this. A new medicinal product derived from umbilical cord mesenchymal stromal cells, vesicle-enriched secretome fraction (VSF) comprises a heterogeneous mixture of extracellular vesicles (EV), particles and soluble factors. We intend to use VSF for intracochlear application during device implantation to provide immunomodulatory and/or neuroprotective effects, as shown in preclinical experiments. Clinically, VSF has been used in the first named patient to demonstrate feasibility. This approach will now be extended to a first-in-human phase I/II clinical trial named ESCRT, which has been submitted to the European Medicines Agency (EMA) via the Clinical Trials Information System (CTIS).
Methods: Following a series of initial in vitro and in vivo experiments that support the biological concept, preclinical data were obtained in defined preclinical animal models for cochlear implantation trauma investigating biodistribution and toxicology in two different species. Preclinical safety was evaluated in NMRI mice after single intracochlear application of various doses of VSF under GLP compliance. Safety and efficacy were investigated in guinea pigs after cochlear implantation trauma and long-term safety was tested in guinea pigs after VSF application. Biodistribution of DiD-labeled vesicles was evaluated initially in guinea pigs and was extended under GLP-like conditions in NMRI mice. All data were compiled in various documents for clinical trial application.
Results: VSF was well tolerated and no abnormalities or drug-related clinical symptoms were observed at any time in either mice or guinea pigs. VSF improved hearing after implantation trauma in the guinea pig cochlear implant model. Six months after VSF application, ABR thresholds remained at a similar level compared to the control. Regardless of the species, DiD-labelled EV could be traced in a time-dependent manner and were detected at the applied cochleae (hair cells, supporting cells and spiral ganglion neurons) and in the kidney indicating a physiological degradation.
Conclusions: The process of taking a new drug candidate from the idea to the bench to the bedside of a clinical trial is a challenging one, starting with basic research for drug discovery, through preclinical testing, and resulting in the initiation of a Phase I/II clinical trial (ESCRT) for safety and efficacy.

SU102. Perilymph Duration of a Long-Acting Dexamethasone Formulation After Precise Microendoscopic Placement at the Round Window Membrane: A Fluidsim Model Based on Human Serum, Animal Serum, and Animal Perilymph Drug Concentrations

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Category: Inner Ear: Drug Delivery

Background: Inner ear disorders affect millions of individuals worldwide, yet there are no FDA-approved drug treatments for the most common forms of hearing loss and balance disorders. Systemically administered steroid therapy is not able to reach the inner ear at high concentrations, due to the small volume of the compartment and the blood-labyrinth barrier. Local delivery approaches are attractive for enhancing inner ear concentrations and reducing systemic exposure. SPT-2101 (6% dexamethasone) is a novel investigational treatment for Meniere's disease consisting of a long-acting gel formulation for precise intratympanic delivery to the round window membrane (RWM). Designed to overcome drug delivery shortcomings of previous inner ear therapies, SPT-2101 is placed minimally-invasively at the RWM under visualization with a microendoscope and remains in situ as a drug depot for an extended duration. Drug depot residence has been confirmed by MRI in clinical participants two weeks post-administration, and by middle ear visualization in guinea pigs four weeks post-administration. While extremely valuable for drug development efforts, collecting human perilymph is highly invasive, and only possible during cochlear implantation or other destructive procedures. The objective of this work is to estimate human dexamethasone perilymph concentrations from plasma concentration measurements over time following a single intratympanic administration of SPT-2101 using FluidSim modeling informed by corresponding animal pharmacokinetic data.
Methods: Plasma and perilymph dexamethasone concentrations were measured in guinea pigs and African green monkeys over 3 to 6 weeks post-intratympanic administration of SPT-2101. Plasma concentrations of dexamethasone were measured in nineteen adult Meniere's disease patients post-intratympanic administration of SPT-2101 in participating clinical sites across Australia. Measurements were taken at 1 day, 7 days, 14 days, and 28 days post SPT-2101 administration. FluidSim was trained on the correlations of animal plasma and animal perilymph levels, allowing the human perilymph drug time course for SPT-2101 to be predicted from measured human plasma dexamethasone concentrations.
Results: Plasma dexamethasone concentrations were detected for up to 28 days in guinea pigs and at least 21 days in African green monkeys. Perilymph dexamethasone concentrations were above estimated therapeutic levels for up to 35 days in guinea pigs and at least 21 days in African green monkeys. In human subjects, plasma dexamethasone concentrations were detected for one week in 18 of 19 participants, and two weeks post-administration in 9 of 19 participants. Animal plasma, perilymph and middle ear drug interrelationships were compared to FluidSim simulations, providing rationale for correlating dexamethasone concentrations in the respective compartments. Comparable simulations of human plasma concentrations predicted perilymph dexamethasone therapeutic levels are sustained in humans for 3-8 weeks, depending on the range of estimated minimum therapeutic concentration.
Conclusions: Dexamethasone eluted from SPT-2101 precisely delivered to the round window provides prolonged and durable estimated perilymph concentrations in clinical subjects.

SU103. In Vivo Determination of Gentamicin in Murine Inner Ear Perilymph After Local or Systemic Delivery

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Category: Inner Ear: Drug Delivery
Background: Intratympanic gentamicin is used clinically to treat Ménière's disease and reduce vertigo by ablating vestibular sensory function. Dosing in humans was empirically established due to difficulty measuring drug levels in very low volumes of perilymph in animal models. Thus, it is difficult to establish the inner ear pharmacokinetics of gentamicin to determine optimal therapeutic dosing and avoid ototoxicity. Here, we elucidate initial gentamicin pharmacokinetics in murine perilymph following intratympanic or intraperitoneal administration.
Methods: We previously developed a validated HPLC-Fluorescence method per USP guidelines to quantify gentamicin C-subtypes in very low volumes of artificial perilymph. For intratympanic delivery, 5 µL of a 30 mg/mL gentamicin solution was administered near the RWM of anesthetized mice. For systemic delivery, a 20 mg/kg gentamicin dose was administered into the lower right quadrant of the abdominal cavity. After administration, each mouse recovered for up to 24 hours before being anesthetized and collecting perilymph (1 µL) from the posterior semicircular canal. To estimate the pharmacokinetic parameters, non-compartmental and one-compartmental pharmacokinetic analyses were conducted using the observed drug concentrations in perilymph after intratympanic and intraperitoneal administration of gentamicin.
Results: After intratympanic administration, gentamicin concentrations in perilymph peak within 1 hour and decline steadily with loss of detection by 24 hours. Gentamicin C1 has the fastest clearance from perilymph, while C2 or C2a have the slowest clearance and thus the highest mean residence time in perilymph. After intraperitoneal administration, gentamicin concentrations in perilymph peak more slowly compared to intratympanic administration and decline steadily with a half-life of ~4 hours, and a mean residence time of ~13 hours. Subtype C1 has the lowest concentration in the perilymph and is removed from the perilymph at a faster rate compared to the other C-subtypes. Subtypes C2 or C2a have higher concentrations in the perilymph with higher mean residence time, half-life, and AUC compared to subtypes C1 and C1a.
Conclusions: A sensitive HPLC-fluorescence method revealed initial pharmacokinetics for gentamicin in perilymph, with distinct absorption and clearance profiles, after intratympanic or intraperitoneal administration. Intratympanic administration allows for rapid peak concentrations within perilymph, yet gentamicin is cleared more swiftly than after systemic administration. The gentamicin C-subtypes have different pharmacokinetic behaviors, with C2/C2a showing slower clearance and longer mean residence times, potentially enhancing the drug effect in the inner ear. Further studies are necessary to translate these findings into clinical practice and refine dosing strategies for inner ear drug delivery. However, understanding gentamicin pharmacokinetics in the inner ear will better optimize dosing strategies and improve therapeutic outcomes that further minimize the risk of adverse ototoxicity in clinical practice.

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SU104. Regeneration of Cochlear Synapses Following Intracochlear Delivery of Neurotrophin 3

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Category: Inner Ear: Drug Delivery

Background: Synaptic connections between sensory hair cells (HCs) and cochlear primary afferent neurons are particularly vulnerable to noise-induced trauma. Trophic factors, such as neurotrophin-3 (NT3), have been reported to induce synaptic regeneration when delivered exogenously, and a variety of methods have been studied to directly introduce them into the cochlea. While delivery of trophic factors at the round window (RW) niche has been reported, the bioavailability of these compounds relies on multiple factors, such as RW membrane permeability and drug distribution within the inner ear. A direct, intracochlear route may overcome these issues by providing direct access to the cochlea and allowing for more precise control over drug delivery. Herein, we hypothesize that this approach offers an alternative to effectively targeting the cochlea with lower dosage and volume requirement as compared to other methods of delivery.

Methods: We exposed 7-week CBA/CaJ mice to octave-band noise (8-16 kHz) for 2 hours at 98 dB SPL to induce synaptopathy in the 32 kHz range, as previously described (Kujawa and Liberman, 2009). After 24 hours, mice underwent a single intracochlear injection through the RW of 100nL of either artificial perilymph (AP), 5.31 ng/uL or 10.57 ng/uL NT3 at a rate of 200 nL/min. ABR and DPOAE thresholds were recorded 2 weeks after the injection. We evaluated HCs and synaptic ribbons in immunolabeled cochlear whole mount samples, and compared them to those of non-noise-exposed mice (controls). Statistical analyses were conducted using Prism GraphPad Software.

Results: ABR and DPOAE thresholds remained in the normal range, while suprathreshold wave 1 amplitudes were decreased at 32 kHz, consistent with synaptopathic noise damage. Whole mount confocal microscopy showed no loss of HCs. At 32 kHz, the mean synaptic counts in both NT3 dose subgroups (5.31 ng/uL or 10.57 ng/uL) were similar to controls (p=.5824 and p=.3909, respectively), and significantly higher than in the AP only group (p=.0017 and p=.0035, respectively). Additionally, the mean synaptic counts at 32 kHz between dose subgroups were similar (p GREATER THAN .9999).

Conclusions: Our findings suggest that low concentration intracochlear delivery of NT3 efficiently promotes synaptic regeneration in noise-exposed mice.

SU105. Biodegradable Inner Ear Implants for Controlled Release of Glucocorticoids in Combination With Cochlear Implants

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[1]*Martin Luther University of Halle-Wittenberg*

Category: Inner Ear: Drug Delivery

Background: Cochlear implants (CIs) have significantly improved treatment for individuals with severe hearing loss. Despite their success, challenges such as inflammation and fibrosis around the implant site limit their effectiveness. To address these issues, innovative drug delivery systems are being explored to enhance the performance and durability of CIs.
Methods: Biodegradable inner ear drug delivery implants (BIEDDI) were prepared by hot-melt extrusion. Different polymers were used for customization of release kinetics, mechanical properties, and degradation profiles. Various glucocorticoids, such as dexamethasone, dexamethasone phosphate, and triamcinolone were incorporated. In vitro drug release was measured over 90 days and the corresponding drug distribution in the human inner ear was simulated with FluidSim. High-resolution μCT was employed to assess the compatibility of BIEDDI with CIs, testing various dimensions for flexible insertion alongside CI electrode arrays.
Results: Controlled release profiles were observed, with dexamethasone and triamcinolone-loaded implants demonstrating release rates ranging from 10 ng/hour to 150 ng/hour over the course of 90 days. The inclusion of dexamethasone phosphate in the implants led to faster drug release, with complete release occurring within less than a month. FluidSim simulations indicated uniform intracochlear distribution of triamcinolone in human cochleas, with rapid drug penetration reaching the cochlear apex. In guinea pigs, the dexamethasone implant stayed intact post-trauma, with no damage detected. In human specimens, μCT confirmed space for both CI arrays and BIEDDI implants in the scala tympani.
Conclusions: This study demonstrates the overall suitability of co-administering BIEDDI and CIs for managing inflammation and fibrosis following cochlear implantation. No adverse effects on cochlear structures were observed via μ-CT imaging. Due to their biodegradable nature, there is potential for a renewal process by introducing a new BIEDDI after drug release and elimination of degradation products, if necessary. The ability to vary drugs and their concentrations during manufacturing offers a personalized medicine approach to control inflammation and fibrosis post-cochlear implantation.

SU106. The Nfi Family of Transcription Factors Instruct Radial Patterning and Cell Type Composition in the Developing Mouse Cochlea

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Category: Development: Cellular/Systems
Background: Development of the murine cochlear sensory epithelium is orchestrated by diverse sets of transcription factors, which play instructive roles in cell fate specification, differentiation, patterning, and maturation. One family of transcription factors, the Nuclear Factor I (NFI) family, has been studied in other sensory system paradigms, such as olfactory neuron receptor choice and temporal patterning in the developing retina. The NFI factors are expressed in the developing and postnatal cochlear sensory epithelium, but their function remains uncharacterized.
Methods: To study the function of the NFI factors in the mouse cochlea, Nfiaf/f;Nfibf/f;Nfixf/f (Nfia/b/xf/f) were crossed with mice carrying the Sox2CreERt2 transgene, allowing for inducible and cell type-specific knockout of the NFI factors in the cochlear sensory epithelium. Pregnant dams were injected with tamoxifen at embryonic day 12 (E12) and E12.5, and litters were

harvested at E18.5. Immunofluorescence microscopy, 5-Ethynyl-2′-deoxyuridine (EdU) labeling, and qPCR were used to profile changes in cell composition, proliferation, and maturation of cochlear hair cell (HC) and supporting cell (SC) subtypes.
Results: A Cre-dependent GFP reporter allele (CAG-Sun1/sfGFP) was used to confirm Cre expression in the Sox2 domain, and antibody labeling of NFIB confirmed knockout of the factors in the Sox2 domain. Knockout of Nfia/b/x beginning at E12 in the sensory epithelium resulted in broad patterning defects along the medial-lateral (radial) axis, resulting in misorientation and misalignment of outer HCs. Additionally, an increase in inner HCs and decrease in outer HCs was observed in knockout tissue compared with control samples.
Conclusions: This study establishes the NFI family of transcription factors as key regulators of patterning and cell identity in the developing murine cochlea. Ongoing experiments aim to determine whether loss of these factors delays the timing of cell cycle withdrawal and differentiation. Future studies will examine the necessity of these factors in hearing capability in adult mice and characterize the contribution of these factors to a larger gene regulatory network controlling cochlear sensory epithelium development.

SU107. PROX1 Controls Cellular Patterning and Maturation in the Lateral Compartment of the Mouse Organ of Corti

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Category: Development: Cellular/Systems
Background: The lateral compartment of the organ of Corti contains outer pillar cells, Deiters' cells, and outer hair cells. It is separated from the medial compartment by the tunnel of Corti and is exclusively innervated by type II Spiral Ganglion Neurons (SGNs). This aspect of the organ of Corti patterning is crucial for the development and function of the cochlear amplifier. Since the transcription factor Prox1 is expressed by SGNs and supporting cells located throughout the lateral compartment, we evaluated PROX1's contribution to Deiters' cell and pillar cell differentiation and outer hair cell innervation using a mouse model.
Methods: Prox1 function was tested in the organ of Corti using a Prox1 conditional knockout (CKO) in which LoxP sites flank two exons encoding the PROX1 homeobox domain. Cochlea-restricted Prox1 gene deletion was generated using Emx2-Cre, thereby eliminating PROX1 function in supporting cells while leaving expression in the SGN intact. Gene deletion was validated by in-situ hybridization and cellular phenotypes were evaluated by immunofluorescent labeling of wholemount and sectioned cochleae. Cell division was assayed by EdU incorporation while changes in gene expression were evaluated by bulk RNA-seq analysis and in-situ hybridization.
Results: In-situ hybridization confirmed loss of the targeted Prox1 exons while immunofluorescent labeling revealed persistent expression of mutant PROX1 protein which we used as a marker for mutant Deiters Cells and Pillar cells in the CKO. While Prox1 deletion did not affect outer hair cells, the patterning of lateral compartment supporting cells was disrupted by an increase in their number. This increase did not correlate with changes in EdU incorporation. Some genes associated with lateral compartment identity were downregulated, though persistent expression of others, including Prox1, suggesting that while their fate was

specified, cellular differentiation was incomplete. This was correlated with an incomplete tunnel of Corti formation and changes in phalangeal process extension. Some medial compartment genes were upregulated and expressed in the lateral compartment suggesting that medial cell characteristics may be repressed as part of lateral compartment differentiation. Finally, the Type II SGNs displayed peripheral axon pathfinding defects and frequently turned incorrectly towards the cochlear apex rather than the base.
Conclusions: Based on these findings, we conclude that the transcription factor PROX1 is required for supporting cell differentiation in the lateral compartment of the organ of Corti. PROX1 positively regulates gene expression in the lateral compartment and in its absence, some medial compartment genes are upregulated. Since gene deletion was restricted to the organ of Corti in these experiments we conclude that the axon pathfinding defects are secondary to the cellular changes we described in the lateral compartment.

SU108. The Insm1 Knockout Mouse as a Model for Identifying Morphogen Signaling Pathways Driving Inner Hair Cell Differentiation

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Category: Development: Cellular/Systems
Background: Inner hair cells (IHCs) and outer hair cells (OHCs), two types of mechanosensory cells in the cochlea, are critical for hearing, each distinguished by unique morphology, molecular function and specialized patterns of innervation. Damage to either cell type leads to permanent hearing loss, as neither can regenerate. Therapeutic approaches for specific hair cell regeneration are hindered in part by a lack of understanding of the signaling pathways that guide IHC vs. OHC fate.
Methods: We have previously shown that INSM1, a zinc-finger transcription factor transiently expressed in OHCs, consolidates their fate during development. In the Insm1 mutant mouse, approximately half of all OHCs lose their fate markers and undergo transdifferentiation into IHCs, acquiring the characteristic morphology, nuclear positioning and molecular markers of IHCs. This transdifferentiation occurs in a gradient, with medial OHCs (closer to the normal IHC row being more likely to transition, suggesting the influence of a graded morphogen that promotes IHC differentiation. We surmise that INSM1 normally represses responsiveness to this morphogen by OHCs. In the absence of INSM1, embryonic OHCs become receptive to this morphogen, leading to OHC-to-IHC conversion in a concentration dependent manner.
Results: To explore the hypothesis that this transdifferentiation gradient is mediated by an IHC-inducing morphogen, we utilized organotypic cochlear explants from developing Insm1 mutants. In this system, OHC transdifferentiation into IHCs mirrored in vivo observations, as marked by the loss of the OHC marker, BCL11b, and gain of the IHC marker, VGLUT3. By modulating candidate signaling pathways with activators or inhibitors, we showed that the degree of OHC-to-IHC transdifferentiation was modifiable. Specifically, perturbations in certain pathways led to near-complete transdifferentiation, or conversely led to minimal transdifferentiation depending on the signaling input being modified.
Conclusions: These findings indicate that the Insm1 mutant phenotype is not fixed, but can be influenced by altering signaling inputs, thus supporting the morphogen hypothesis. Additionally,

this approach has identified key signaling pathway components that are involved in IHC differentiation. Integrating these components into the broader understanding of cochlear development will ultimately inform strategies for inducing IHC or OHC specific regeneration.

SU109. Cross-Sectional Evaluation of Auditory and Vestibular Function in a Mouse Model of Down Syndrome

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Category: Development: Cellular/Systems

Background: Down syndrome (DS) is the most common chromosomal disorder worldwide, and hearing loss as well as balance deficits have been reported extensively in this population. Mouse models of DS are fundamental to studying the genotype-phenotype relationship in DS. The Dp16 mouse model of DS has a large segmental duplication of ~120 protein-coding genes from mouse chromosome 16 orthologous to human chromosome 21, leading to their triplication. Preliminary data indicate that the Dp16 mouse model on B6 background replicates human auditory and vestibular phenotypes of DS. Here, we report a cross-sectional study to characterize peripheral auditory and vestibular function in this mouse model.

Methods: Auditory brainstem response (ABR) thresholds and distortion product otoacoustic emissions (DPOAEs) were collected with a Tucker-Davis Technologies RZ6 system. Utricular and saccular function were assessed by vestibular sensory evoked potentials (VsEP) using bone-conducted stimuli. Thresholds, peak latencies and amplitudes were analyzed. Statistical analyses were performed to detect differences in auditory and vestibular function between wild-type [WT] and Dp16 littermates.

Results: We evaluated mice from 2 to 12 months old. Dp16 mice exhibited: (i) 25-45 dB higher ABR thresholds compared to WT controls which decreased at higher frequencies, suggesting frequency-dependent auditory dysfunction, and (ii) reduced DPOAE responses compared to WT controls. Vestibular testing revealed significant differences between groups starting at 3 months of age. Dp16 mice had higher mean thresholds (-4.0± 3.6 dB re:1g/ms) compared to WT controls (-13.5±1.12 dB re:1g/ms). Dp16 mice also had a higher and wider distribution of latency values, and lower amplitude (0.89±0.45 μV) compared to WT controls (1.17±0.14 μV). In both genotypes, age did not significantly affect vestibular amplitudes or latencies (p GREATER THAN 0.05).

Conclusions: Our study demonstrated significant auditory and vestibular impairments in Dp16 mice compared to WT mice. ABR threshold shifts were markedly elevated in Dp16 mice, along with diminished DPOAEs, both consistent with hearing loss. Dp16 mice also had lower VsEP amplitudes and longer latencies relative to controls, consistent with vestibular dysfunction. These results emphasize the presence of both auditory and vestibular abnormalities in the Dp16 mouse model of DS, akin to clinical phenomena in the DS population. Further studies with larger sample sizes, additional measures, and additional experimental conditions will test hypothesized mechanisms of hearing loss as well as investigate potential therapeutic interventions.

SU110. Towards an Artificial Niche for Stem Cells: Investigating the Effects of the Microenvironment on Differentiation of Inner Ear Organoids From Mouse Embryonic Stems

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Category: Development: Cellular/Systems

Background: Hearing loss is one of the most common forms of sensory impairment in humans, affecting more than 5% of the world's population. To date, our understanding of the mechanisms of hearing loss and the generation of therapies have been hampered by the lack of human in vitro models. Previous work showed that it is possible to generate inner ear organoids (IEOs) containing functional hair cells from mouse or human pluripotent stem cells through three-dimensional (3D) culture systems. However, their translation into an in vitro model for drug screening or developmental modelling is limited by low differentiation yield, lack of reproducibility and limited standardisation of the differentiation protocol.

Methods: This study aims to explore microenvironments for IEOs and otic neuron differentiation from mouse embryonic stem cells (mESCs) that could ultimately be combined with engineering devices for translational research. IEOs containing hair cells, supporting cells and neurons were generated with an Atoh1/nGFP mESC line (provided by S. Heller). The microenvironment was explored by culturing mESC aggregates in microwells or in 3D MatrigelTM, collagen or a combination of both.

Results show that microwells allow the control of the size of mESC aggregates but affect otic differentiation. Maintenance in gel droplet domes sustained the formation of IEOs and also supported the formation of ganglion-like neural structures in the surrounding matrix. Characterisation by immunostaining, electrophysiology and single nuclei RNA sequencing confirmed the presence of a population of functional neurons, often observed in clusters.

Results: Results show that microwells allow the control of the size of mESC aggregates but affect otic differentiation. Maintenance in gel droplet domes sustained the formation of IEOs and also supported the formation of ganglion-like neural structures in the surrounding matrix. Characterisation by immunostaining, electrophysiology and single nuclei RNA sequencing confirmed the presence of a population of functional neurons, often observed in clusters.

Conclusions: This work provides a step forward in the characterisation of a microenvironment suitable for development of IEOs combined with functional, presumptive otic neurons. Further work on this model is required to better understand otic neuron development in the context of IEOs and the production of stem-cell based in vitro models for investigating the mechanisms underlying sensory neural hearing loss.

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SU111. Identification of Regulators That Control the Tonotopic Specialization of the Mammalian Auditory Sensory Epithelium

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Category: Development: Cellular/Systems

Background: The auditory sensory epithelium is crucial for our ability to detect sound. Mechano-receptors termed hair cells within the spiral-shaped sensory epithelium are specialized to aid frequency selective sound detection. Features of tonotopic specialization include graded differences in the morphology, electrophysiology and gene expression of hair cells. The mechanisms that regulate their position-dependent features are unknown. Mice start hearing at 2-weeks of age and our lab has shown that manipulations of follistatin, an antagonist of TGF-β type signaling after birth disrupts frequency selective hearing, suggesting that hair cell-specific tonotopic specialization occurs late during postnatal development. Here, I propose to identify and functionally characterize key factors that regulate tonotopic specialization of hair cells and supporting cells during postnatal development.

Methods: To identify novel gene expression patterns along cochlear epithelium, we harvested the sensory epithelia of 2-day old wildtype mice and divided them into apical, mid and basal segments for bulk RNA sequencing. To identify the signals that maintain position-dependent gene expression, we analyzed position-dependent gene expression using qPCR after culturing apical, mid and basal segments of cochlear tissue or cells. Since one of the specializations is that only supporting cells in the cochlear apex show regenerative capacity, we also profiled the capacity of supporting cells to proliferate and generate hair cells.

Results: The bulk RNA-sequencing experiment identified a total of 1293 downregulated and 640 upregulated genes in the apical compared with basal cochlea. Motif analysis showed enrichment of helix-loop-helix family, homeobox family, and nuclear receptors family transcription factors in the promoter regions of differentially expressed genes. Notably, one of the top-enriched motifs belonged to the transcription factor Smad2, which is a downstream effector of TGFβ/Activin signaling and is negatively regulated by follistatin, a gene critical for position dependent cochlea development and tonotopic specialization.

Hair cells and supporting cells did not alter their tonotopic-specific gene expressions when cultured as explants. Also, consistent with previous reports only supporting cells located in the apex were able to re-enter the cell cycle and convert into hair cells. However, epithelial cells (supporting cells) cultured as organoids lost their tonotopic specialization. Position-dependent gene expression gradients were lost (e.g. Inhba, Fst) and supporting cells from apex, mid, and base re-entered the cell cycle and formed hair cells at similar rates.

Conclusions: This study provides valuable insights into the molecular mechanisms underlying tonotopic specialization of the auditory sensory epithelium. Our results suggest that neurons or mesenchymal cells play critical roles in maintaining the tonotopic features of supporting cells. Future studies will address the involvement of the newly identified transcription factors in tonotopic specialization.

SU112. Pre- And Post-Hearing Development of Glutamatergic Neurotransmission in the Dorsal Cochlear Nucleus of Mice

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Category: Development: Cellular/Systems

Background: The dorsal cochlear nucleus (DCN) is part of the cochlear nuclei complex in the auditory brainstem that performs the integration of acoustic information from the cochlear nerve and multimodal sensory information from the trigeminal and facial nerves, which is mostly conveyed by the parallel fibers. The 2 main neurons in DCN, the fusiform and cartwheel neurons, receive glutamatergic synapses from the parallel fibers. In rodents, hearing begins on the 12-14th postnatal day with the opening of the acoustic meatus, and in DCN, the electrophysiological properties of fusiform neurons change significantly after hearing onset. Our objective was to study the development of glutamatergic synapses from parallel fibers onto fusiform (PF-FUS) and cartwheel neurons (PF-CW) in the pre-hearing and post-hearing periods.
Methods: Brainstem slices containing the DCN of Swiss mice (P7-13, pre-hearing group; p14–P21, post-hearing group) were used to perform electrophysiological recordings of excitatory postsynaptic currents (EPSCs) evoked by stimulation of parallel fibers, in whole-cell patch clamp. We examined the amount of VGLUT2-positive synapses using immunofluorescence staging with confocal microscopy.
Results: We find that the PF-FUS synapse is stronger after hearing onset at P14 (amplitude: pre-hearing: -409 pA ± 79; post-hearing: -1092 pA ± 109, p LESS THAN 0.0001 unpaired t-test; area: pre-hearing: -1753 pA.ms ± 314; post-hearing: -4252 pA.ms ± 449, p LESS THAN 0.0001 unpaired t-test), but, we did not observe differences in EPSCs kinetics. In addition, 21% of fusiform neurons from the pre-hearing group did not respond to parallel fibers stimulation, while after hearing onset, all fusiform neurons were responsive to the stimulation. We did not observe differences in the paired-pulse ratio (PPR) and frequency and amplitude of spontaneous EPSCs (sEPSCs). The coefficient of variation analysis (1/CV2), showed a significant increase in synaptic strength (p LESS THAN 0.005, unpaired t-test) indicating that the locus of the rise in strength takes place in the postsynaptic neuron. For the PF-CW synapse, no difference in the glutamatergic transmission was found between the groups. All cartwheel neurons from both age groups responded to parallel fibers stimulation, and we also did not find differences in PPR or amplitude and frequency for sEPSCs. Immunofluorescence staining showed a decrease in VGLUT2-positive synapses in the post-hearing group, indicating excitatory synapse pruning in this stage of development.
Conclusions: These results show that glutamatergic neurotransmission from parallel fibers changes during postnatal development, being limited to fusiform neurons. We believe that these changes take place in the postsynaptic fusiform neuron.

SU113. Characterization of Supporting Cell Diversity in the Mammalian Utricle

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Category: Development: Cellular/Systems
Background: The mammalian inner ear consists of the cochlea, a spiral-shaped organ that confers our sense of hearing, and the vestibular system, which confers our sense of balance and acceleration. Our lab previously demonstrated that different subtypes of cochlear supporting cells are transcriptomically distinct at the single-cell level, reflecting their different roles in trophic and structural support. By contrast, relatively little is known about supporting cell diversity in the vestibular epithelia. While vestibular organs have a simpler architecture by comparison with the cochlea, they contain several different types of hair cells, as well as different patterns of innervation between hair cells and vestibular ganglion neurons. This points to the possibility that the population of vestibular supporting cells may also include multiple unique subtypes. The aim of this project is to characterize supporting cells in a vestibular organ, the utricle, to determine whether they represent a homogenous or heterogenous population.
Methods: To generate transcriptional profiles of utricular support cells we collected cells for analysis by single cell RNA-seq. Using the 10X chromium platform, we collected 4597 hair cells and support cells from adult mouse utricles between postnatal days 28-30. Transcriptional data was analyzed in R using Seurat v5. The data from each collection was normalized using SCTransform v2. Datasets were integrated using Seurat CCA integration, and quality checking was performed by overclustering to identify and remove small clusters of poor quality cells, or cells that were not supporting cells.
Results: Initial clustering analysis indicated hair cells and support cells clustered separately based on their identity. Isolation and analysis of the support cell clusters (2430 cells) revealed 5 transcriptionally distinct groups, each with a unique set of gene markers. In addition, many markers of these unique support cell groups are shared by sub-types of cochlear support cells, suggesting similarities in function.
Conclusions: In this study we have characterized the molecular diversity of support cells in the mature mouse utricle, using high throughput single cell RNA-seq techniques. Next, we will validate our findings, using in situ hybridization to label tissue sections for each group of support cells. We have also collected single cell RNA-seq data across multiple timepoints in utricular development, so we will conduct analyses to generate trajectory projections for the development of distinct utricular cell types and to identify candidate genes that might regulate their development.

SU114. Loss of TMC1/2 Function Induces Expansion of TMC1/2b+ Cells in the Zebrafish Inner Ear

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Category: Development: Cellular/Systems
Background: Detection of sound and head movement requires mechanoelectrical transduction (MET) channels at tips of hair-cell stereocilia. In vertebrates, the transmembrane channel-like (TMC) proteins TMC1 and TMC2 fulfill critical roles in MET. During normal development in

zebrafish, hair-cell progenitors within the supporting cell layer first express tmc1 and tmc2b and then initially express all three tmc1/2a/2b genes (tmc2 is duplicated in zebrafish) before migrating to the upper layer of the neuroepithelium.
Methods: We assessed the development of the inner ear endoorgans in a tmc1/2a/2b triple mutant larvae and discovered a potential developmental regulatory loop involving the tmc genes.
Results: We find that ectopic expression of tmc1/2b transcripts in peripheral cells occurs in tmc1/2b double and tmc1/2a/2b triple mutants in a gene dosage-dependent manner. Results from tmc single and double mutants reveal that expression of wild-type tmc1 or tmc2b is sufficient for suppressing ectopic expression of tmc1/2b. In addition, our experiments indicate that ectopic expression of tmc1/2b is not universal to all endorgans. Instead, it is specific to otolithic organs and progressively increases during development in tmc triple mutants. To determine whether tmc1/2b ectopic expression is caused by the absence of mechanotransduction and/or mislocalization of Tmc proteins, we examined expression in mechanotransduction mutants carrying strong alleles of tmie and tomt and did not observe ectopic expression of tmc1/2b. We also examined loss of function alleles for the known interacting partners of Tmc1/2 proteins such as Pcdh15a, Lhfpl5a and Cib2/3 auxiliary subunits and found no effect on tmc1/2b expression.
Conclusions: Our preliminary data suggest that loss of Tmc1/2b proteins specifically result in upregulation of tmc1/2b expression in peripheral cells. The mechanism is unclear and may involve a secondary role for Tmc1 and Tmc2b in signaling to peripheral cells to differentiate into hair cells. Whether ectopic expression involves canonical developmental or transdifferentiation pathways remains to be determined.

SU115. EMX2 Generates the Line of Polarity Reversal by Blocking STK32A-Dependent Regulation of GPR156 During Mouse Utricle Development

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Category: Development: Cellular/Systems
Background: Hair cells in the utricle are divided between two polarized groups with stereociliary bundles oriented in opposite directions to enable the detection of broad ranges of motion. The two groups meet at cellular boundary called the Line of Polarity Reversal (LPR). The LPR is positioned near one edge of the striola by the transcription factor EMX2. Both groups of hair cells are aligned along a common polarity axis established by core Planar Cell Polarity (PCP) proteins, but each group interprets that axis differently in order to generate their characteristic bundle orientation. We previously showed that EMX2 acts together with the receptor GPR156 and the kinase STK32A to guide this process and position the LPR. Here we use a series of genetic experiments to define the signaling network comprised of these molecules and how they position the LPR.
Methods: The functional hierarchy of EMX2, GPR156 and STK32A was tested through genetic epistasis experiments in which hair cells were evaluated in double knockout mice (DKO) with mutations in GPR156 and Stk32a or Emx2 and Stk32a. Phenotypes were evaluated in the developing utricle and cochlea using immunofluorescent labeling to visualize hair cells and stereociliary bundle orientation.

Results: We show that GPR156 reorients stereociliary bundles in hair cells expressing EMX2, and that this function is blocked by STK32A in hair cells located on the opposite side of the LPR. However, in Stk32a knockouts stereociliary bundles are misoriented and not reversed as would be predicted by this result. We demonstrate that this is a consequence of mislocalized GPR156 and ectopic function in the absence of STK32A because the phenotype is rescued in GPR156; Stk32a DKOs. We further show that EMX2 determines the position of the LPR by repressing Stk32a transcription. In Emx2 mutants STK32A is active in all hair cells. Consequently, GPR156 is blocked throughout the utricle and the LPR is lost. Consistent with this, we show that GPR156 can be restored and rescue the Emx2 mutant phenotype in Emx2; Stk32a DKOs. These interpretations were interrogated in the developing cochlea which expresses EMX2 and GPR156 but not STK32A. We show that in the Emx2 mutant cochlea, repression of Stk32a is released, allowing STK32A to block GPR156 localization and function. As a result, there is a PCP phenotype in the Emx2 mutant cochlea that can be rescued in Emx2; Stk32a DKOs.
Conclusions: Together these phenotypes support a functional hierarchy in which EMX2 positions the LPR by negatively regulating transcription of Stk32a. Since STK32A blocks GPR156 function, GPR156 is only able to reorient stereociliary bundles along the side of the LPR that also expresses EMX2. In contrast, for hair cells expressing STK32A on the opposite side, GPR156 is inactive and bundle orientation is likely directly guided by core PCP proteins.

SU116. Ctcf-Mediated Chromatin Remodeling in Cochlear Development: Implications for Sensory Epithelium Patterning and Hair Cell Maintenance

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Category: Development: Cellular/Systems
Background: The inner ear is a complex sensory organ responsible for hearing and balance, with the cochlea serving as a highly sensitive and organized sound-detecting structure. While genetic studies have provided insights into inner ear development, the role of epigenetic regulation, particularly higher-order chromatin organization, remains unclear. CTCF, a highly conserved 11-zinc finger protein, is known to play a pivotal role in global chromatin architecture and gene regulation, but its specific functions in inner ear development and maintenance have not been fully elucidated.
Methods: To investigate CTCF's role in inner ear development and function, we employed conditional knockout mouse models. We crossed Ctcf^fl/fl mice with Pax2-Cre mice to delete Ctcf in the developing otic epithelium, and with Gfi1-Cre mice to delete Ctcf specifically in developing hair cells. We analyzed the effects of CTCF deficiency on cochlear development, hair cell formation and maintenance, and otic neurogenesis through various stages of embryonic and postnatal development.
Results: In Pax2-Cre; Ctcf^fl/fl embryos, we observed extra rows of auditory hair cells in a shortened cochlea at E14.5 and E17.5, accompanied by massive and ectopic expression of sensory specifiers like Jag1 and Sox2. This indicated an expansion of the sensory domain in CTCF-deficient cochleae. In Gfi1-Cre; Ctcf^fl/fl mice, while no obvious developmental defects were seen until postnatal day 8, by 3 weeks, intermittent degeneration of outer hair cell

stereociliary bundles was observed, leading to profound hearing impairment. By 5 weeks, most hair cells had degenerated, with apparent defects in other organ of Corti structures. Furthermore, CTCF loss severely compromised otic neurogenesis, partly due to reduced Neurog1 expression. This reduction was associated with changes in histone modification at the Neurog1 gene's promoter and upstream enhancer.
Conclusions: Our findings demonstrate that CTCF plays essential and diverse roles in inner ear development and function. It is crucial for proper sensory domain specification during cochlear development, maintenance of hair cells and hearing function in the mature cochlea, and regulation of otic neurogenesis. CTCF appears to exert these effects through modulation of chromatin architecture and histone modifications, particularly at key loci such as Neurog1. These results highlight the importance of epigenetic regulation in inner ear biology and open new avenues for understanding and potentially treating hearing disorders.

SU117. FOXJ1 Plays a Critical Role in Vestibular Hair Bundle Formation

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Category: Development: Cellular/Systems
Background: Forkhead Box J1 (FOXJ1) is a transcription factor essential for ciliogenesis across various tissue types and species and shares common targets with the transcription factors RFXs. Kinocilia are critical for early development of hair cells, establishing polarity and orientation of stereociliary bundles. RNA-sequencing data have shown that Foxj1 is highly expressed in cochlear and vestibular hair cells (HCs). However, the specific role of Foxj1 in inner ear function, as well as hair bundle development and maintenance, remains undefined. Here, we test the hypothesis that FOXJ1 is essential for hair cell function and hair bundle development in the inner ear using a Foxj1 conditional knockout (cKO).
Methods: We validated Foxj1 expression in the inner ear using RNAscope™ fluorescent in-situ hybridization across different time points: E16, P2, and 1 month old in C57BL/6J wildtype mice. To investigate the role of Foxj1 in both the auditory and vestibular system, we generated a cKO mouse line (Foxj1flox/flox;Gfi1-Cre) to delete Foxj1 in HCs starting at embryonic day 16.5 (E16.5). Auditory function testing was performed at 1 month using auditory brainstem response (ABR) at 8, 16, 24, and 32 kHz frequencies in both Foxj1 cKO and control littermates. Cochlear and vestibular tissues were harvested for immunohistochemical analysis to quantify the number and morphology of HCs, kinocilia, and stereociliary bundles in controls and Foxj1 cKO at post-natal day 7 (P7) and 1-month-old.
Results: Foxj1 is highly expressed in cochlear and vestibular HCs, as well as in vestibular and spiral ganglion neurons during embryogenesis (E16) and at early postnatal days (P2). However, by 1 month of age Foxj1 expression is restricted to spiral ganglion and vestibular neurons and vestibular HCs. Deletion of Foxj1 in HCs at E16.5 did not result in gross morphological changes in cochlear HCs up to 1 month old of age. Furthermore, there were no significant difference in ABR thresholds between Foxj1 cKO mice and their wildtype littermates. However,

immunohistochemistry from utricles and saccules from P7 Foxj1 cKO reveals that the deletion of Foxj1 at E16.5 in vestibular HCs results in a shortened kinocilia when compared to their wild type littermates.
Conclusions: Our results suggest that Foxj1 plays a functional role in vestibular HC, likely related to kinocilia structure and function. We will share these results as well as results from ongoing VsEP testing to determine if loss of Foxj1 results in functional deficits.

SU118. Characterization of Let-7 as a Regulator of Supporting Cell Plasticity in the Murine Utricle

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Category: Development: Cellular/Systems
Background: Mammals are incapable of regenerating mechanosensory hair cells after damage. By contrast, hair cell regeneration is observed in non-mammalian vertebrates such as birds and fish throughout adulthood. The newly formed hair cells derive from surrounding supporting cells. Thus, recent approaches to stimulate hair cell regeneration in mammals have been focused on manipulation of supporting cells. While the complete cessation of progenitor activity is well documented for mature cochlear supporting cells, previous studies have shown that vestibular supporting cells retain some regenerative plasticity even in adult mammals. This study leverages the vestibular utricle as a model to interrogate the effect of let-7 miRNAs that are known to repress proliferation and pluripotency and therefore may be acting as regenerative barriers in the cochlea. Let-7 miRNAs are known to repress the expression of progenitor-associated genes and are part of a well-characterized negative feedback loop involving the RNA-binding protein LIN28B.
Methods: We use the doxycycline inducible let-7g transgenic mouse line to increase let-7 miRNA activity in undamaged whole-organ utricle explants derived from early postnatal mice (P5). We use stage P5 for our experiments as at this time point, supporting cells in the striola and transitional epithelium are still proliferative and are hypothesized to be closer to a progenitor-like stage. To decrease let-7 activity we overexpressed LIN28B using a doxycycline inducible LIN28B transgenic mouse model. To analyze proliferation, EdU was added to the culture medium for three days. We used immunohistochemistry to visualize the sensory epithelium then counted the number of EdU-positive supporting cells. The striola, extrastriola, and transitional epithelium were all analyzed independently to control for the differences in baseline proliferation between each ROI.
Results: Preliminary data demonstrates that let-7g overexpression in the murine utricle significantly reduces the amount of proliferating supporting cells in the striolar region. Overexpression of LIN28B does appear to have a mild positive effect on proliferation in the striolar region but does not increase supporting cell proliferation in the extra-striolar region where supporting cells rarely re-enter the cell cycle.
Conclusions: These findings suggest there may be dynamic patterns of gene expression that lead to regional sensitivity to the LIN28B/let-7 pathway. Further studies will focus on characterizing the spatial pattern of let-7 expression to gain insight into their role in development. Additionally, the capacity of let-7 miRNAs to repress hair cell regeneration will be investigated by increasing

let-7 activity in a neomycin-damaged utricle. We hypothesize that by increasing let-7 activity, the rate of hair cell regeneration will be significantly reduced. Overall, we find that let-7 miRNAs may be a key barrier to reprogramming supporting cells, and further studies are needed to understand the scope of their involvement.

SU119. Conditional Knockout of mGluR5 in Glutamatergic Pathways Disrupts the Development of Synaptic Excitatory Transmission Onto Mouse MNTB Neurons

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Category: Development: Cellular/Systems
Background: Glutamatergic transmission plays important roles in the development of sensory systems, partially via mGluR5 modulation. Consequently, a number of neurodevelopmental disorders involve mGluR5 misexpression and dysfunction. Previous studies on mGluR5 modulation in the auditory system, including our own, have used almost exclusively animal models with intact mGluR expression. However, the exact mechanism underlying mGluR5 contribution to developmental neuropathology remains largely unknown. To fill this critical gap, we propose to study the consequences of mGluR5 deficiency on the development of brainstem sound localization circuit.
Methods: Using the Cre-loxP system, we generated a conditional KO (cKO) mouse line in which mGluR5 on the glutamatergic pathways in the auditory brainstem was genetically eliminated, by crossing mGluR5-floxed mice with transgenic mice expressing Cre recombinases under the control of VGluT2 promotor. These two mouse lines were crossed with a ROSA26 reporter mouse line. Then, the mGluR5-loxP and VGluT2-Cre mice were crossed to generate F1 offspring (heterozygous), which were crossed to generate cKO and littermate control mice. Brainstem slices were prepared from mice at the ages of P30 (range 30-38). Using whole-cell patch clamp, we studied synaptic excitatory properties of MNTB neurons, at 35 C. t-test was used to detect differences between cKO and their WT littermates and/or age-matched controls.
Results: Elimination of mGluR5 in the glutamatergic pathways compromised the synaptic properties required for precise temporal processing in MNTB. In this experiment, 21 MNTB neurons were recorded from cKO mice and 31 neurons from WT mice. In response to afferent stimulation of the trapezoid body, MNTB neurons exhibited two distinct responses: all-or-none large EPSCs and graded smaller EPSCs. In mGluR5 cKO mice, there was a significant reduction in body weight ($p = 0.0039$) and a significant increase in the number of neurons exhibiting graded responses ($p = 0.0267$). Additionally, short-term plasticity of MNTB neurons showed marked differences in paired-pulse ratio ($p = 0.0078$). The amplitude of EPSCs induced by electrical stimulation decreased ($p = 0.0470$), and the latency of the responses increased (p LESS THAN 0.0001). The reduction in response amplitude may be related to the maturation of the calyceal synapse, while the prolonged response latency suggests a connection between mGluR5 and the development of myelin. Furthermore, the membrane capacitance was significantly reduced (p LESS THAN 0.0001), indicative of smaller cell size in mGluR5 cKO mice.

Conclusions: Glutamatergic pathway-specific cKO of mGluR5 disrupted the cellular properties required for temporal processing at the AVCN-MNTB synapse, suggesting a role of mGluR5 in synapse formation and maturation during development.

SU120. The Role of Strial Macrophages in the Development and Maintenance of the Blood-Labyrinth Barrier

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Category: Development: Cellular/Systems

Background: Immune-reactive macrophages are present in all functionally critical regions of the cochlea. However, the macrophages in the stria vascularis are particularly noteworthy due to their role in maintaining the integrity of the blood-labyrinth barrier (BLB). It is well established that macrophage depletion in adult mice disrupts the BLB and impairs hearing sensitivity. Following noise exposure, cochlear macrophages typically respond with increased cell numbers and activation, characterized by a transition to an activated status. However, macrophages in the stria vascularis often remain unaffected, retaining their ramified morphology, and may even show a reduction in cell number under certain conditions. Given the stria vascularis's essential role in maintaining cochlear microenvironment homeostasis and the endolymphatic potential, proper postnatal development of the stria vascularis is critical during the onset of hearing. Here, we investigate the role of strial macrophages during postnatal development using a macrophage depletion model.

Methods: CD11b-DTR mice (JAX #6000) and C57BL/6 mice (JAX #664) received multiple postnatal intraperitoneal injections of diphtheria toxin (DT, Sigma-Aldrich) or PBS, forming one experimental group and three control groups. Specifically, the mice were administered DT every other day (10 ng/g body weight, with an initial dose of 20 ng/g body weight) starting from postnatal day 6 (P6) and continuing for 4 weeks. At P35, the mice underwent comprehensive systemic and auditory assessments, including auditory brainstem response testing, flow cytometry analysis of peripheral blood and peritoneal fluid, immunofluorescence staining and immunoblotting of cochlear tissue, and BLB permeability assays to investigate the role of strial macrophages in BLB maintenance.

Results: Flow cytometry analysis revealed a significant reduction in monocytes and macrophages in the peripheral blood and peritoneal fluid, respectively, in DT-treated DTR mice, confirming the effectiveness of the experimental model. Auditory brainstem response (ABR) thresholds were elevated in higher frequency regions, particularly at 24kHz and 32kHz, in DT-treated DTR mice compared to controls. In the cochlea, a marked reduction in strial macrophages was observed in the basal coil, along with decreased ramification, fewer arborized processes, and reduced contact with capillary vessels. Furthermore, macrophage depletion in the basal coil of the stria vascularis resulted in diminished tight junction protein Occludin, increased BLB permeability to the azo dye T-1824, and reduced capillary density and branching index.

Conclusions: These results emphasize the critical role of strial macrophages in BLB development and hearing initiation, suggesting a potential mechanism by which macrophages regulate strial angiogenesis and BLB formation. This study further demonstrates the non-immune

functions of macrophages, particularly their role in vascular regulation, underscoring their indispensable contribution to establishing a fully functional BLB. These findings provide new insights into the mechanisms underlying sensorineural and age-related hearing loss, especially in the high-frequency range, and offer a deeper clinical understanding of these conditions.

SU121. Ptk7 is Required for Hair Cell Planar Polarity in the Utricle

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Category: Development: Cellular/Systems
Background: The Planar Cell Polarity (PCP) signaling pathway is crucial for the collective cell behaviors driving tissue morphogenesis, including processes such as neural tube (NT) closure, and alignment of cell polarity across the tissue plane. Disruption of PCP signaling in the inner ear leads to misorientation of hair cells in both the auditory and vestibular sensory epithelia. Core PCP proteins form asymmetric protein complexes across cell junctions to define the PCP vector, however how the PCP vector is regulated by tissue mechanics and junctional contractility is currently unknown. Our lab has shown previously that Ptk7, a pseudo-receptor tyrosine kinase (RTK), regulates both PCP and actomyosin contractility in inner ear hair cells (HCs). The goal of this project is to elucidate how Ptk7 couples junctional contractility with PCP signaling to align hair cell orientation in the utricle, leveraging a Ptk7 allelic series with predicted allosteric mutations or patient variants associated with birth defects.
Methods: Like typical RTKs, Ptk7 exists in an active or inactive conformation. We generated Ptk7 “kinase-dead” (KD) and patient-derived alleles targeting the Ptk7 conformational switch. Preliminary data indicates that HB misorientation in the utricle is significantly stronger than in the cochlea of Ptk7 KO mutants, suggesting PCP of utricular HCs is more sensitive to Ptk7 activity than cochlea HCs. To determine Ptk7 structure-function relationship for PCP signaling in the utricle, we analyzed HB orientation in the utricle of Ptk7 KD and patient alleles using whole mount immunofluorescence staining at E18.5/P0. To correlate changes in junctional contractility and PCP signaling, we stain for phosphorylated myosin light chain (pMLC), a readout for myosin activity and the core PCP protein Vangl2 in the Ptk7 mutant utricles.
Results: We have observed that the Ptk7 KD mutations give rise to partially penetrant NT defects. Preliminary analysis shows that KD mutations exhibit hair bundle misorientation, albeit less severe than Ptk7 KO. These results suggest that KD mutations are hypomorphic, and that the active RTK conformation is necessary for proper PCP regulation. In contrast, a patient mutation in the transmembrane domain demonstrated no NT defects or HB misorientation, indicating that the mutation is not pathogenic. The data on pMLC and Vangl2 immunolocalization are still being collected and will be presented.
Conclusions: These findings show that even though Ptk7 is a pseudo RTK, the active RTK conformation of Ptk7 is required for regulation of PCP signaling in the NT and in the utricle. We will further test the hypothesis that Ptk7 promotes Vangl2 localization by regulating junctional contractility.

SU122. Phospholipid Flippase ATP8A2 Localization is Impacted by Hair Cell Mechanotransduction and May be Necessary for Synapse Maintenance

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Category: Development: Cellular/Systems

Background: The establishment of functional connections between the sensory hair cells of the cochlea and spiral ganglion neurons (SGNs) is essential for hearing. The cochlea initially develops excessive neurons and synapses that must be removed prior to the onset of hearing in a process called pruning. We hypothesize that canonical "eat me" signal phosphatidylserine (PS) marks neurons and synapses to be removed during pruning. The localization of PS within the cell membrane is controlled by flippases, which shuttle PS from the external leaflet of the membrane to the inner leaflet. Flippase ATP8A2 is known to flip PS, is necessary for hearing function, and is expressed in SGNs and OHCs. We observed a marked shift in ATP8A2 localization within the cochlea during the pruning period, with a distinct concentration at outer hair cell (OHC) synapses and type II nerve fibers. This leads us to hypothesize that the PS flipping activity of ATP8A2, possibly driven by hair cell mechanotransduction (MET), is actively involved in pruning type II fibers and synapses.

Methods: We have generated several mouse lines to investigate ATP8A2 in the developing cochlea. Atp8a2-HA mice have an HA tag knocked-into the C-terminus of ATP8A2 for visualization of the protein. To test whether ATP8A2 localization is driven by MET, we knocked out Cib2 to generate a mouse line with hair cells that do not perform MET (Cib2-/-) and crossed this line to our ATP8A2-HA line to visualize the localization of ATP8A2 in MET negative mice. We generated a mouse with hypomorphic alleles of ATP8A2 (A2Δ33 mice) to test if the flippase function of ATP8A2 is necessary for pruning. To measure the effects of these genes on pruning of the presynaptic ribbons of OHCs, we performed immunostaining for CtBP2 and used confocal microscopy to quantify the number of synapses per OHC.

Results: ATP8A2 expression persisted in Cib2-/- mice, but its localization and targeting to the synapse were disrupted. In MET-negative mice, there was also a delay in the pruning of outer hair cell synapses. The phenotype of A2Δ33 mice was complex, with synapse numbers remaining unchanged during the pruning period. However, by P21, these mice exhibited a reduction in both synapse number and SGN density, accompanied by elevated auditory brainstem response thresholds.

Conclusions: These data suggest that ATP8A2 localization is driven by hair cell mechanotransduction and that OHC synapse pruning is disrupted in MET negative mice. Furthermore, ATP8A2 might be necessary for the maintenance of ribbon synapses and/or needed for the longevity of neurons and synapses. Future work will assess whether ATP8A2 loss of function can be compensated by other cochlear flippases such as ATP8A1.

SU123. Developmental Transformation of the Cochlear Sensory Epithelium Through Local ERK Signaling

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Category: Development: Cellular/Systems
Background: Formation of the sensory epithelium in the cochlea is achieved through tight coordination of cell transformation and cell loss. Among the most pronounced cellular changes in the developing cochlea occurs medial to hair cells as Kölliker's organ (greater epithelial ridge, GER) regresses to form the inner sulcus. The columnar epithelial cells that comprise this structure help create the tectorial membrane and induce spontaneous activity of hair cells before hearing onset, functions that are essential for proper development of the biomechanical properties of the cochlea and for inducing maturation of central auditory circuits. Previous studies have shown that changes in thyroid hormone signaling causes the GER to either regress prematurely or persist beyond the point of hearing onset, both of which are associated with elevated auditory brainstem response (ABR) thresholds, morphological defects in the tectorial membrane, and changes in cochlear tuning. However, the cellular mechanisms that control the gradual transformation of the GER are unknown. Here, we explored the contribution of ERK signaling to this process, as this signaling pathway has been implicated in controlling cellular patterning in diverse epithelia throughout the body.
Methods: To visualize dynamic changes in ERK1/2 signaling in the developing cochlea, we generated cochlear explants from early postnatal CD1 mice that ubiquitously expressed an ERK1/2 kinase translocation reporter (ERK-KTR) and recorded changes in localization of the mClover from the nucleus (unphosphorylated ERK1/2) to the cytosol (phosphorylated ERK1/2) (CAG-ERK-KTR-P2A-H2B-mRuby2). To define the role of this ERK1/2 signaling, we examined the effects of the ERK inhibitor PD0325901. To define upstream and downstream interactors of ERK1/2 in the cochlea, we inhibited EGFR and pCREB with PD153035 and 666-15.
Results: Longitudinal fluorescence imaging over hours in cochlear explants from prehearing mice revealed that ERK1/2 was transiently activated in small clusters of GER cells. Over time, these ERK events spanned the entire GER. Small molecule inhibition of either EGFR or MEK1 prevented phosphorylation of ERK1/2, as assessed by persistent nuclear localization of the ERK-KTR reporter. Strikingly, inhibition of ERK1/2 signaling resulted in rapid, widespread death of GER cells. To better understand the downstream effectors of ERK1/2, we inhibited pCREB, which did not affect translocation of ERK-KTR, but similarly caused rapid death of cells in the GER. Immunostaining cochleae isolated from early postnatal mice revealed similar focal activation of pCREB and ERK within the GER, indicating that similar signaling pathways are engaged in vivo.
Conclusions: Our studies indicate that transient ERK activation within supporting cells of the GER promotes their survival. The ability of EGFR and pCREB inhibition to phenocopy the response to ERK inhibition suggests that periodic, focal EGF release within the GER paces the regression of this structure to coincide with maturation of the sensory epithelium at hearing onset.

SU124. The Role of CWC27 in Cochlear Development

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Category: Development: Cellular/Systems
Background: CWC27 spliceosome-associated protein (CWC27) is a nuclear cyclophilin involved in pre-mRNA splicing. A large RNA protein complex, spliceosome, performs a stepwise pre-mRNA splicing by rearranging its components and recruiting small nuclear ribonucleoproteins (snRNA). The formation of successive complexes is named as E (early), A (pre-spliceosome), B (pre-catalytic spliceosome), B LESS THAN sup GREATER THAN act LESS THAN /sup GREATER THAN (activated spliceosome), B* (catalytically activated spliceosome), C (catalytic spliceosome), P (post-catalytic spliceosome), and ILS (intron lariat spliceosome). CWC27 is a component of a B LESS THAN sup GREATER THAN act LESS THAN /sup GREATER THAN complex.
The human CWC27 homolog mutation generating CWC27 transcript truncation causes retinal degeneration, skeletal dysplasia, and other developmental abnormalities (retinitis degeneration with or without skeletal anomalies: RPSKA). CWC27 mutant mice recapitulated the phenotypes of human CWC27 mutation, showing retinal degeneration with abnormal mRNA splicing, including intron retention and exon skipping, in retinal cells (Bertrand et al. 2021). The abnormal mRNA splicing was found in several inherited retinal disease genes. This study suggested that retinal degeneration in CWC27 mutants was caused by the mRNA splicing abnormalities.

Some patients with human CWC27 homolog mutation were reported to have hearing loss, suggesting that CWC27 and mRNA splicing are involved in developing hearing organs.

Methods: We produced Cwc27 null mice using CRISPR/Cas9-mediated homologous recombination of the mouse genome. We observed the histological morphology of the cochlea using Hematoxylin/Eosin staining.
Results: We confirmed the sequence of the Cwc27 genome. One base insertion was observed in the coding region of Cwc27 exon 1. This mutation causes a stop codon at the beginning of Cwc27 exon 1, which is supposed to be a null allele. The ratio of mice with wild-type, heterologous, and homologous alleles was 1: 2 :1 (n = 69, 141, and 74, respectively) at embryonic days (E) 13.5 and 1: 2: 0.25 (n = 141, 244, and 33) at postnatal days (P) 28, indicating that embryonic lethality happens in Cwc27 null mice.
The observation of homologous mutant mice at E18.5 showed the variation of gross morphologic phenotypes. Although two-thirds of mice had normal head morphology, one-third had cranium bifida, probably due to neural tube defects.

Regarding the cochlear morphology, the phenotype was dependent on the existence of cranium bifida. The mutant mice with cranium bifida showed smaller turns of cochlear ducts (around one turn) compared to those without cranium bifida, which had one and a half turns of cochlear ducts.

The development of cochlear turns and neural tube closure are reported to be related to planar cell polarity. The phenotypes of the head and cochlea suggest that CWC27 and mRNA splicing may control the planar cell polarity or other mechanisms related to neural tube closure and cochlear turn extension.

Conclusions: Spliceosome-associated protein, CWC27, regulates the cochlear turn extension through a similar mechanism controlling neural tube closure.

SU125. SHANK2 Shapes Hair Bundle Architecture Essential for High-Frequency Hearing and Long-Term Maintenance

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Category: Development: Cellular/Systems

Background: The precise architecture of the hair bundles in mammalian auditory hair cells is critical for efficient sound transduction. Each bundle is composed of actin-filled stereocilia arranged in a staircase pattern, forming a broad U-shape in inner hair cells (IHCs) and a V-shape in outer hair cells (OHCs). This arrangement develops during development by the lateral movement of the microtubule-based kinocilium, which is regulated by lateral surface proteins such as Gαi and GPSM2. While the role of these lateral surface proteins is well characterized, the mechanisms governing the medial side remain unclear. Our study identifies SHANK2, a protein associated with synaptic function and autism spectrum disorders, as a key player in establishing the U- and V-shaped bundle architecture from the medial side.

Methods: We used immunofluorescence to determine protein localization, and scanning electron microscopy to observe hair bundle morphology. Auditory function was assessed by measuring auditory brainstem responses and distortion product otoacoustic emissions. We investigated the role of SHANK2 in hair bundle formation and auditory function using systemic (Shank2–/–), hair cell-specific (Gfi1-Cre; Shank2lox/lox), and spiral ganglion neuron-specific (Bhlhe22-Cre; Shank2lox/lox) Shank2 knockout mutants and organ of Corti explant cultures.

Results: SHANK2 was specifically localized to the medial apical surface of developing hair cells, complementing the lateral localization of proteins such as Gαi and GPSM2. In Shank2–/– mice, the characteristic U- or V-shaped bundle architecture was disrupted, exhibiting a fragmented or wavy morphology, while the kinocilium position and staircase arrangement were preserved. Despite widespread bundle defects along the tonotopic axis, auditory function is specifically impaired at high frequencies, primarily due to compromised OHC amplification. Longitudinal studies further suggest that this unique bundle architecture is essential for maintaining hair bundle integrity and hearing function over time. Conditional deletion of Shank2 in hair cells recapitulated the auditory defects seen in systemic knockouts, whereas deletion in spiral ganglion neurons did not, highlighting the specific role of SHANK2 in cochlear hair cells. In addition, yeast two-hybrid screening identified RAP1, a small GTPase, as a potential SHANK2-binding partner in the cochlea. Inhibition of RAP1 caused mislocalization of SHANK2 and resulted in bundle defects similar to those observed in Shank2 mutants. Notably, RAP1 inhibition did not affect the localization of lateral proteins such as Gαi and GPSM2 or other medial proteins such as aPKCζ and PARD6B.

Conclusions: These results suggest that SHANK2, localized on the medial apical surface of cochlear hair cells, plays a critical role in establishing the characteristic U- or V-shaped hair bundle architecture, which is essential for high-frequency hearing and long-term maintenance of bundle integrity and hearing function.

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SU126. A New Genetic Model to Mark Pure Neonatal Cochlear Outer Hair Cells for Single-Cell Transcriptomic Analysis

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Category: Development: Cellular/Systems

Background: The mammalian cochlear sensory epithelium, the organ of Corti, has two types of mechanosensors, inner (IHCs) and outer hair cells (OHCs), the outer hair cells are responsible for sound amplification. One of the challenges in hearing field is getting pure cochlear OHCs at neonatal ages. Here, we generate a new mouse model Bcl11b-CreDHFR/+. Combining with Atoh1-Tdtomato/+, we manually pick pure OHCs for high-quality full-length transcriptomic profilings, at postnatal day 1 (P1).

Methods: Tamoxifen injection to pregnant female in CreER mouse models always results in dystocia. Here, we generate a new mouse model Bcl11b-CreDHFR/+ by which the Cre activity is temporally controlled by administration of trimethoprim (TMP), which does not lead to dystocia.

Results: Bcl11b, also known as Ctip2, is known to be a cochlear OHC marker, we characterized the detailed dynamic pattern of Bcl11b in OHC which started to be expressed from E15.5 to P7. After administration of trimethoprim into early postnatal Bcl11b-CreDHFR/+; Rosa26-lsl-tdtomato/+ mice (P0-P1), we noticed that not only outer hair cells were successfully induced, numerous induced cells also existed in the spiral limbus. By combining with Atoh1-Tdtomato/+, we established the Bcl11b-CreDHFR/+; Rosa26-lsl-EGFP/+; Atoh1-Tdtomato/+ mouse strain, after trimethoprim administration from E16.5 to P0, we obtained Tdtomato+/EGFP+ cells by FACS and subject to qPCR assay. Those cells were able to significantly enrich OHC gene Bcl11b, but dramatically deplete IHC gene Fgf8 and neuronal gene Mafb, supporting that the Tdtomato+/EGFP+ cells were OHCs with high purity. By manual picking, we performed single cell full-length transcriptomic analysis of pure OHCs at P1 via the smart-seq approach with further validation.

Conclusions: In conclusion, we have constructed a TMP-controlled Bcl11b-CreDHFR/+ mouse strain. The advantage of TMP over tamoxifen is manifest, and we can envision that the TMP/CreDHFR system would facilitate future temporal genetic lineage tracing analysis, especially the one starting at embryonic ages. Furthermore, the high-quality transcriptomic profiles of P1_OHCs would be a valuable dataset and benefit studies of OHC development and regeneration.

SU127. Absence of Sall1 Delays the Differentiation of Cochlear Outer Hair Cell

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Category: Development: Cellular/Systems
Background: The mammalian cochlea contains two types of sound receptors, outer hair cells (OHCs) and inner hair cells (IHCs). Both of them derive from the Atoh1+ sensory progenitors. It is known that Insm1/Ikzf2 and Tbx2 are critical for OHC and IHC development. However, it remains elusive what genes control velocity of their differentiation. In this study, we investigated the role of Sall1 in regulating the speed of HC, especially the OHC, development.
Methods: We utilized two genetic approaches: 1) Mosaic Sall1 mouse mutants by Crispr-stop; 2) the typical conditional Sall1flox knockout model, using different drivers include Atoh1-P2A-Cre/+, Gfi1-P2A-Cre/+ and Atoh1-CreER+. Immunostaining, auditory brainstem responses (ABR) and single cell RNA-seq assays were applied to decipher the functions of Sall1 in OHCs.
Results: Both RNA-seq and immunostaining assay showed that once it is turned on in HCs at ~E15, Sall1 is persistently expressed in HCs. Besides HCs, Sall1 is detected in SCs. Similar phenotypes were observed in different genetic mutants. Briefly, upon deletion of Sall1, the Sall1-/- OHCs differentiated slower than the control OHCs. It was evidenced by that Bcl11b was detected in Sall1-/- OHCs, but not in control OHCs at P7. Oppositely, Prestin was turned on in control OHCs, but was repressed in Sall1-/- OHCs at P4. This was further supported by single cell transcriptomic comparison between control and Sall1-/- OHCs. The Sall1-/- OHCs degenerated at adult ages, with more severe phenotypes occurring in the basal turns, resulting in hearing impairment. In addition, we performed gain-of-function study of Sall1. To our surprise, overexpression of Sall1 also led to HC degeneration.
Conclusions: Our findings uncovered the critical roles of Sall1 in cochlear HC differentiation and survival. Future studies will focus on deciphering the underlying molecular mechanisms.

SU128. Hair Cells Do Not Require Core PCP Proteins or GPR156 Signaling to Correct Their Orientation in Time

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Category: Development: Cellular/Systems
Background: Auditory function relies on hair cells (HC) that detect directional vibrations and are precisely oriented in the epithelium. Core PCP proteins are well-known to regulate HC orientation via intercellular communication at HC-support cell junctions. Inner HC (IHC) are severely misoriented in compound Fzd3; Fzd6 and Vangl1; Vangl2 mutants, as are outer HC in the third row (OHC3) in Vangl1; Vangl2 mutants. Strikingly, both compound mutants show generally inverted and not randomized IHC and OHC3 orientation, and HC orientation is progressively corrected at postnatal stages in conditional Vangl2 mutants.
G protein-coupled receptor GPR156 is polarized at apical junctions and signals via inhibitory G proteins (GNAI) to reverse the orientation of HC populations expressing the transcription factor EMX2. Interestingly, Gpr156 and Gnai mutants show defects complementary to Fzd3;6 and Vangl1;2 mutants, with inverted OHC1 and OHC2. Surprisingly however, OHC1-2 orientation is

not corrected in Gpr156 mutants. Core PCP proteins are unaffected in Gpr156 mutants, but GPR156 protein distribution is disrupted in Vangl2Looptail mutants. To address these similarities and differences, we investigated potential interactions between core PCP proteins and GPR156, and notably a possible role for GPR156 in the correction process.

Methods: We used confocal microscopy to track HC orientation and GPR156 distribution during development or along the tonotopic gradient of HC differentiation. We analyzed conditional Vangl1;2 and Fzd3;6 compound mutants as well as Vangl2; Tecta mutants that have no tectorial membrane. We performed GPR156-VANGL1/2 co-immunoprecipitation in HEK293 cells. We explanted semi-dominant Vangl2Looptail and Fzd3;6 cochleae at E17.5, cultured for 4 days with or without pertussis toxin to block GPR156 signaling, and compared HC orientation before and after culture.
Results: In both Vangl1;2 and Fzd3;6 mutants, HC orientation improved at postnatal compared to late embryonic stages, showing that close homologs are not required for correction. GPR156 enrichment was severely decreased in Vangl1;2 mutants and GPR156 and VANGL1/2 could be co-immunoprecipitated, suggesting that GPR156 could be a core PCP component. In Fzd3;6 mutants, GPR156 was enriched at normal levels but GPR156 crescents appeared to correct their orientation ahead of the basal body , suggesting a potential role for GPR156-GNAI in the correction process. Because HC orientation still improved in time in Vangl2; Tecta mutants, we concluded that the tectorial membrane is not required for correction. Next, we thus used cochlear cultures and successfully observed correction ex vivo in both Vangl2Looptail and Fzd3;6 mutants. However, blocking GPR156-GNAI signaling with pertussis toxin did not impair correction, instead improving its efficiency.
Conclusions: This work suggests that GPR156-GNAI acts complementarily to core PCP in defining HC orientation since GPR156 interacts with VANGL1/2 and counteracts rather than helps correction. It remains unclear why HC correct their orientation in core PCP but not Gpr156 mutants, and how correction occurs at the mechanistic level.

SU129. Prox1 Regulates Development of the Lateral Compartment of the Organ of Corti

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Category: Development: Cellular/Systems
Background: The Prospero homeobox 1 (Prox1) transcription factor plays a fundamental role in mouse inner ear development. In the cochlea, initial Prox1 expression is restricted to the prosensory cells located in the lateral compartment of the organ of Corti (oC). By embryonic day (E)16, PROX1 expression is absent in cells expressing outer hair cell (OHC) markers, but persists in lateral support cells (SC) until perinatal ages. The temporal and topographically restricted expression of Prox1 suggests that it regulates different aspects of development in OHC precursors and differentiated lateral support cells. Initial studies in mouse using a germline deletion of Prox1 resulted in perinatal lethality which prevented a complete assessment of the

role of Prox1 in the inner ear. By generating a conditional knockout (cKO) of the Prox1 gene, we demonstrated profound hearing loss in Prox1-cKO mice by P30 due to the loss of OHCs after the onset of hearing. In this study we investigated the early transcriptomic and anatomical changes in the cochlea due to the deletion of Prox1.
Methods: To generate a Prox1-cKO mouse, Cre recombinase was expressed in cochlear precursor cells from the Fgf20 gene (Fgf20-cre) to facilitate recombination of a reporter expressing TdTomato (Ai14) and the Prox1-flox allele. We used standard immunohistochemical protocols at different ages to validate the deletion of the Prox1 gene and to evaluate developmental changes in Prox1 expressing cells. We used scanning electron microscopy (SEM) to visualize the surface topography and composition of the oC in Prox1-cKO cochleae. Lastly, we conducted single cell RNA-sequencing (scRNA-seq) to characterize the transcriptomic changes that occur in response to the deletion of Prox1 in the cochlea.
Results: We confirmed the deletion of the Prox1 gene and the affected cell types by the co-expression of eGFP and TdTomato following Prox1-flox recombination. Expression of eGFP as a proxy for Prox1 expression confirmed the reported tonotopic gradient of expression in which Prox1 is upregulated from base to apex in late gestational ages, and downregulated in the same manner in postnatal ages. Our phenotypic analysis indicated similar changes in cochlea morphology to those reported in previous studies while novel postnatal changes included a failure of the tunnel of Corti to mature. Ongoing scRNA-seq analyses will identify transcriptomic changes underlying the altered phenotypes observed in the absence of Prox1.
Conclusions: These results provide evidence of a critical role for Prox1 in normal cochlear development. The SEM images reveal at higher definition the changes that occur at the surface of the sensory epithelium in the absence of Prox1. Our scRNA-Seq analyses will characterize the transcriptional targets of Prox1 in both HCs and SCs and should provide insights regarding the pathways that mediate the effects of Prox1 in distinct cochlear cell types.

SU130. Injury-Induced Remodeling of the Actin Cytoskeleton in the Vestibular Maculae of Mice

Mark Warchol*[1]

[1]*Washington University School of Medicine*

Category: Regeneration
Background: Cell-cell junctions in the sensory epithelia of the inner ear contain filamentous actin bands that provide mechanical support between adjoining cells. It has also been proposed that the width and stiffness of these structures regulates the capacity for hair cell regeneration (e.g., J Burns et al., J Comp Neurol 2008). Cellular junctions in the (highly regenerative) vestibular maculae of birds possess relatively thin actin cables, while junctional bands in the vestibular organs of mammals are much thicker. Hair cell injury in utricles of birds also leads to the formation of filamentous actin phagosomes within the sensory epithelium that engulf the debris of dying cells. The role of these structures in regeneration is not known, but similar structures are also present in the mammalian utricle. The present study characterized changes in junctional and intraepithelial actin structures in the utricles of mice following ototoxic injury.
Methods: Mice (Bl/6:129 background, 7-8 weeks old) received a single 4 mg/gm injection of 3,3'-iminodipropionitrile (IDPN) and were allowed to recover for 3-56 days. In other

experiments, organotypic cultures of chick utricles were treated for 16 hr in 0.5 mM neomycin. After fixation, fluorescent labeling and confocal imaging were used to visualize hair cells and actin filaments. Quantification was carried out using Volocity and Fiji/ImageJ software.
Results: Treatment with IDPN resulted in a progressive loss of vestibular hair cells in both the striolar and extrastriolar regions of the mouse utricle. This injury was accompanied by significant changes in the structure of the junctional actin bands, with many bands between adjoining supporting cells becoming significantly thinner. Prior studies have also described thick rings of filamentous actin in the lower stratum of the sensory epithelium (e.g., T Kaur et al. Front Cellular Neurosci 2015, S Bucks et al., eLife 2017), which were assumed to be phagosomes. However, we observed no change in either the morphology or numbers of these structures at any time after IDPN ototoxicity. Genetic deletion of MerTK (which induces the formation of phagosomes in other cell types) had no effect on the actin rings in the mouse utricle. In contrast, ototoxic injury in organotypic cultures of utricles from P10 chicks led to a dramatic increase in the numbers of actin phagosomes, most of which enclosed pyknotic nuclei.
Conclusions: The thick actin bands at cell-cell junctions in the mouse utricle have been proposed to limit regenerative ability. Our data suggest that ototoxic injury to the mouse utricle leads to greatly reduced thickness of many junctional bands, but does not affect the numbers or morphology of putative phagosomes. In contrast, injury to the avian utricle does not affect junctional actin, but triggers the formation actin phagosomes that engulf the remnants of dead hair cells.

SU131. Understanding the Spatial Transcriptome of the Regenerating Zebrafish Inner Ear

Clarke Bagsby*[1], Erin Jimenez[1]

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Category: Regeneration
Background: Hair cells are mechanosensory receptors that reside in the inner ear of all vertebrates. In mammals, hair cells are unable to regenerate after loss or damage causing permanent impairments to hearing. However, non-mammalian vertebrates, such as zebrafish, are able to completely regenerate hair cells after loss or damage. To understand the molecular responses that occur during hair cell regeneration in zebrafish, we previously performed transcriptomic and epigenomic analysis at single-cell resolution on adult zebrafish inner ears undergoing hair cell regeneration. Although we were able to identify the gene regulatory network for hair cell regeneration, our single-cell analysis lacks spatial information. To identify gene-expression responses during hair cell regeneration at spatial resolution, we are performing three-dimensional spatiotemporal transcriptomics to reconstruct the adult zebrafish inner ear undergoing regeneration.
Methods: To gain spatial information of the molecular dynamics during hair cell regeneration, we are generating spatiotemporal transcriptomic maps of adult zebrafish inner ear across three timepoints of the regenerative process using Slide-seq technology. Slide-seq is a method for transferring RNA from tissue sections onto a surface covered in DNA-barcoded beads with known positions, allowing the locations of the RNA to be inferred by sequencing. To study adult zebrafish hair cell regeneration in inner ear tissues, we employed the Tg(myo6b:hDTR) zebrafish

which enables targeted and inducible hair cells within adult inner ear tissues. Using Slide-seq on non-regenerating and regenerating adult inner ears from zebrafish, we are localizing cell types identified by scRNA-seq datasets withing the regenerating inner ear, characterizing the spatial gene expression patterns, and integrating this spatial data with our previous omics datasets. We are currently collecting and analyzing spatial transcriptomic data to integrate the information with previously collected single-cell data.
Results: We are currently collecting and analyzing spatial transcriptomic data to integrate the information with previously collected single-cell data. Previous single-cell transcriptomic studies on the regenerating adult inner ear from zebrafish revealed a temporal regulation of gene expression operating in a switch-like manner in each cell type. We expect to see similar patterns in our Slide-seq data.
Conclusions: Our findings will provide the first spatial transcriptomic map of both the homeostatic inner ear and regenerating inner ear of zebrafish. This will validate the bioinformatic data performed previously and provide functionality to the gene regulatory networks for further understanding of how regeneration is coordinated in the inner ear of zebrafish.

SU132. Open Board

SU133. Organoids to 'Cure' Deafness? A Scoping Review on the Ethics of Using Organoid Technology to Treat Deafness

Esther Fousert*[1], Heiko Locher[1], Martine de Vries[1], Nienke de Graeff[1]

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Category: Regeneration
Background: Hearing loss affects millions of people globally, yet no effective biological treatments are currently available. As a result, patients must rely on technological aids such as hearing aids or cochlear implants. A key barrier to developing therapies is the lack of accurate in vitro models of the human inner ear that can simulate inner ear diseases and support treatment validation. Organoid technology is a promising new biomedical tool in the search for the cause and solution of deafness. Organoid technology, which involves growing human tissues from stem cells to mimic organs, offers a promising new tool for investigating hereditary deafness, evaluating drug efficacy, and developing personalized treatments. However, past novel attempts to treat deafness have faced ethical concerns, particularly regarding their impact on the Deaf community. As organoid technology advances, it is crucial to consider the societal and ethical implications of this emerging field.
Methods: While there is existing literature on the ethics of organoids or the ethics of deafness separately, no comprehensive analysis addresses the ethical concerns of using organoids specifically for treating deafness. To fill this gap, we conducted a scoping review and synthesized 107 articles from bioethics and biomedical databases using the search terms “organoids and ethics” and “deafness and ethics”. This allowed us to identify the specific ethical challenges of applying organoid technology to address deafness.

Results: The ethical concerns in this context echo broader debates surrounding deafness treatments, such as medicalization, disability, and identity. These questions take on new significance as we shift from technological aids to potential biological treatments. Additionally, organoid technology also introduces new ethical dimensions. These include the transition from experimental science to clinical practice and its integration into the current healthcare system. With that come questions of justice, equal access to treatment, and the broader societal costs associated with its use and development. As well as how to prioritize which inner ear diseases receive attention.
Conclusions: Our ethical analysis of organoids as a potential solution for deafness highlights key challenges that must be carefully navigated. We propose a framework for addressing these issues and set an agenda for future discussions. Moving forward, we will test our recommendations through qualitative research with patients, clinicians and the wider community.

SU134. Hair Cell-Like Cells Generated in Mature Cochleae by Adenovirus-Mediated Expression of Gfi1, Atoh1, Pou4f3 With or Without Six1

Matthew Averyt[1], Lin Yang[1], Valeria Mas[1], Sunita Singh[2], Lisa Beyer[1], Diane Prieskorn[1], Andrew Groves[2], Yehoash Raphael*[1]

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Category: Regeneration
Background: Using transgenesis and culture work, several labs have shown that a combination of transcription factors that play a role in hair cell (HC) development is superior to Atoh1 alone in inducing transdifferentiation of supporting cells into new HCs. Here we test the combinatorial approach in vivo in the mature Pou4f3-DTR mouse, where an injection of diphtheria toxin (DT) leads to degeneration of all HCs leaving behind supporting cells that appear morphologically differentiated.
Methods: Mature Pou4f3-DTR mice (N=20) were deafened by injecting diphtheria toxin (DT). Adenovirus vectors containing gene inserts for Gfi1, Atoh1, Pou4f3, Six1 (GAPS) (N=10) or Gfi1, Atoh1, Pou4f3 (GAP) (N=10) with a reporter gene were injected into the scala media of the cochlea. The contralateral ear served as a control. Mice were given the vector at the same time as the DT injection. Animals were prepared for analysis one month after adenovirus injection. Cochlear whole-mounts were labeled with antibodies against Myosin VIIa, as a hair cell marker, and phalloidin. Presence of new hair cell-like cells (HCLC) was assessed and quantified using epi-fluorescence or confocal microscopy.
Results: Both groups (GAP and GAPS) exhibited HCLCs with large variability in the numbers between animals. All animals from both groups had Myosin VIIa positive cells in the experimental (left) ear. Initial qualitative observation revealed that GAPS group animals included 4 out of 10 animals with a large number of HCLCs. In the GAP group, 5 out of 10 animals had a large number of HCLCs. In both groups, HCLCs were located within the auditory epithelium, in areas of the deaf organ of Corti as well as medial or lateral to this area.
Conclusions: The GAP cocktail of transcription factor transgenes appears to convert non-sensory cells to HCLCs in a similar way to the GAPS vector. The variance of the samples is high and therefore more animals are needed to define differences between the outcomes of the two gene cocktails.

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SU135. Genetic Landscape of Hearing Loss in Argentina: Comprehensive Molecular Studies and Preclinical Research Using the Zebrafish Model

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Category: Genetics B: General

Background: Hearing loss (HL) affects nearly 10% of the global population, with over half of the cases attributed to genetic factors. Congenital HL is observed in 1 in 500-1000 newborns, often manifesting as non-syndromic cases (70%) and exhibiting an autosomal recessive mode of inheritance (80%). It is mostly related to genetic variants in the GJB2 and GJB6 genes; however, over 100 genes are associated with HL. The whole-exome sequencing (WES) technique has emerged as a cost-effective molecular diagnostic tool to analyze all related genes at once. However, challenges persist, particularly in detecting novel missense variants, which may hinder genotype-to-phenotype correlations. This study aimed to uncover the genetic causes of HL in a cohort from Argentina and validate novel variants identified.

Methods: Sanger sequencing and GAP-PCR for GJB2 and GJB6 were performed on 1,000 patients with HL. Further screening of 100 undiagnosed patients with moderate HL for STRC gene deletions was carried out using multiplex ligation-dependent probe amplification. Subsequently, WES analysis was performed in 80 families (68 non-syndromic cases and 12 patients with extra-cochlear signs). A non-syndromic familial case exhibiting a novel missense variant in the MYO6 gene underwent protein modeling with AlphaFold2, followed by functional validation in zebrafish using a knockdown phenotype rescue assay.

Results: The diagnosis rate from the initial screening of GJB2 and GJB6 was 15.5% for sporadic cases and 36% for familial cases. The STRC analysis led to a 6% diagnosis rate for moderate cases. Regarding the WES analysis, a 44% diagnosis rate was achieved, with half of the identified variants being novel. A novel variant in MYO6 was identified in a familial case with postlingual HL. Variant pathogenicity was predicted by in silico analysis through protein modeling, revealing that the aminoacid change alters the protein's surface electrostatic charge from a positive to a predominantly negative region. Therefore, in vivo functional validation in the zebrafish model was carried out, demonstrating that the mutated mRNA was unable to

recapitulate the wild-type zebrafish phenotype, proving the deleterious effect of the MYO6 variant.
Conclusions: Integrating zebrafish analyses into diagnostic frameworks enhances the precision and clinical relevance of genetic findings, paving the way for more targeted therapeutic interventions in the field of HL. This work demonstrates the suitability of our algorithm for HL genetic diagnosis in the cohort studied and underscores the importance of a combined strategy, integrating in silico and in vivo studies to identify candidate variants, analyze their pathogenicity, and enhance understanding of the pathophysiology of hearing impairment.

SU136. Multi-Omics Analysis for Elucidating the Mechanism of Supporting Cell Reprogramming During Chick Cochlear Hair Cell Regeneration

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Category: Regeneration
Background: Although mammalian cochlear hair cells (HCs) do not regenerate, chick cochlea can regenerate spontaneously, originating from supporting cells (SCs) after HCs loss. However, the mechanism of hearing regeneration in the chicken remains largely unknown. We have been researching using various methods to elucidate the chick cochlear HC regeneration mechanism. First, we established the HC regeneration model of the chick cochlea explant culture system, in which the direct conversion of SCs to HCs is the predominant path. Then, we performed bulk RNA-seq focusing on the early process of gene expression changes in SC direct conversion. (Matsunaga et al., 2020). In addition, we analyzed gene expression changes during the direct conversion from SCs to HCs at the single-cell level. The results indicate that the reprogramming of SCs to the precursor state occurs before differentiation into HCs (Matsunaga et al., 2023). In this study, we performed an integrated analysis of bulk RNA- and ATAC sequencing to elucidate the SC reprogramming mechanism to search for enhancer regions of target genes and upstream transcription factors (TFs).
Methods: We performed RNA- and -ATAC seq using our explant culture model, in which HCs are entirely lost by exposure to streptomycin (SM) for 48 h. Time course samples for RNA- and ATAC-seq are SM0 (before SM exposure), SM24 (a 24-h exposure to SM), and SM48 (48-h exposure to SM). Twenty-five to 35 basilar papillae were used for one batch and prepared in duplicate for both analyses. RNA sequencing was performed by Illumina-Nova-seq-6000, and differentially expressed genes were identified by DEseq. Open chromatin lesion of ATAC analysis detected from peak call by MACS3, and we created the novel enhancer score index for selecting the appropriate enhancer lesion and detected an enhancer candidate region for each expressed gene within a range of ±100 kbp from the transcription start site. We performed TF-binding motif scanning to investigate the candidate upstream TFs using Find Individual Motif Occurrences (FIMO). Motif enrichment analysis was performed using Gene Set Enrichment Analysis (GSEA) to test our enhancer score index's validity.
Results: RNA-seq identified 24,888 genes, and ATAC-seq identified 80,366 open chromatin lesions. We determined one enhancer candidate locus for each gene using our enhancer score index and created the database of TF-bindings motifs for enhancer candidate loci of all expressed genes using FIMO. GSEA indicated that highly identified TF-binding motifs for upregulated genes in SM24 included well-known TFs associated with cochlear development and

maintenance, suggesting the utility of our enhancer score index. Using this workflow for predicting upstream TFs for SC reprogramming-associated genes, we identified several TFs that played roles in chromatin remodeling in the nervous system.
Conclusions: We conducted an integrated analysis of RNA and ATAC-seq using our original workflow to elucidate the mechanism of SC reprogramming during chick cochlear HC regeneration.

SU137. Lentiviral Transduction of the Avian Inner Ear

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Category: Regeneration
Background: The chicken serves as an excellent model organism for studying natural hair cell regeneration. However, the lack of genetic tools hinders functional investigation of candidate genes and signaling pathways in the inner ear. While transgenic birds can be generated using integrating retroviruses, the process is complex, inefficient, and costly. As a simpler alternative, we propose using integrating lentivirus to infect exposed ectodermal tissues during the otic placode stage. Post-hatching, we focus on characterizing virus-mediated gene expression in the inner ear of day 7 chicks (P7).
Methods: Fertilized chicken eggs were incubated until embryonic day 2 (E2), at which point a window was created in the eggshell to visualize the embryo and allow for injection into the amnion. For preliminary testing, diluted phenol red was injected to assess post-hatching survival rates. To confirm stable post-hatch expression following lentiviral transduction, E2 embryos were transduced with a lentivirus encoding a membrane-targeted green fluorescent protein (memGFP) and nucleus-targeted H2B-mCherry. Additionally, an inducible lentivirus was designed with an inverted fluorescent reporter cassette flanked by lox sites and a Cre-ERT2 cassette, allowing for transgene expression upon tamoxifen administration at P1 and analysis at P7.
Results: Injection of diluted phenol red at E2 into the amnion yielded in a 42% hatching success rate. Current experiments are assessing the hatching rate of transduced embryos and evaluating stable transgene expression post-hatching.
Conclusions: We aim to develop and refine a lentiviral transduction method targeting the chicken otic placode to enable conditional gene expression or knockdown in the mature chicken inner ear. Lentiviral delivery of fluorescent reporter proteins will label the basilar papilla, allowing for the characterization of different supporting cell subpopulations and validating the transduction strategy. In the future, this system can be employed to test the roles of candidate genes in hair cell regeneration through misexpression, CRISPRi, or shRNA knockdown approaches.

SU138. Organoid-Derived Otic Neuronal Progenitor Cells for Restoration of the Denervated Cochlea

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Category: Regeneration

Background: Harnessing the potential of progenitor cells for regeneration of the auditory nerve evolved as a new approach to the treatment of cochlear neuropathy e.g. the loss of spiral ganglion neurons (SGN) in the past decade. Learning that CI performance is closely linked to the number of healthy SGN in the cochlea has evoked the desire to replace SGN to improve hearing restoration in this patient population. However, not only the generation of transplantable cells, but also the establishment of a robust model for auditory neuropathy and the grafting of cells into the cochlea remains challenging. Here, we utilize a well-established model of Ouabain induced SGN-loss in rodents and transplanted animals with otic neuronal progenitor cells (ONPs) under 4 different conditions of immune suppression.

Methods: We generated otic organoids by patterning human iPSCs embedded in collagen hydrogel towards an otic fate by BMP, TGF, WNT, SHH, and RA signaling in a stage-specific manner similar to in vivo development. Since we aim to graft SGNs expressing channelrhodopsin (ChR) for optogenetic stimulation, we integrated the red-shifted f-Chrimson variant with an enhanced yellow fluorescent protein (EYFP) tag by CRISPR/Cas9 genome engineering. Adult Mongolian gerbils were deafened by administration of Ouabain to the round window (RW). After a regeneration period of 8-12 weeks to await intracochlear inflammation, ONPs were transplanted to the cochlea by an intramodiolar injection approach. Finally, acoustic auditory brainstem responses (aABR) and optical ABR (oABR) were performed. During a 5 week period after cell transplantation immunosuppression approaches were tested: (1) gelatine sponge with dexamethasone (4 mg/0.5 ml) and cyclosporine A (5 mg/0.1 ml) positioned at the RW; (2) bi-weekly subcutaneous dexamethasone injections (40 mg/kg); (3) daily subcutaneous cyclosporine A injections (15 mg/kg); (4) combination of bi-weekly subcutaneous dexamethasone injections (40 mg/kg) and daily injections of cyclosporin A (10mg/kg).

Results: Ouabain administration significantly increased the thresholds of aABR and reduced the number of cochlear SGNs compared to the contralateral control side. We successfully detected EYFP-positive neural cells localized in the modiolus in 3 of 28 animals, partially showing SGN-like bipolar morphology, as well as positive Parvalbumin-staining. Regime (1) and (3) did not lead to successful intracochlear transplantation of ONP cells (n = 8), regime (2) revealed intramodiolar GFP-positive cells in n=2 animals (n = 8). Regime (4) resulted in n=1 positive animal. We ascribe the latter success to the application of dexamethasone. So far, oABR of transplanted gerbils remained negative.

Conclusions: Ouabain administration to the cochlea and subsequent SGN loss induce immune response and scaring in the cochlear modiolus detrimental to cell transplantation. However, we found that a systemically administered immunosuppression is required for successful engraftment of ONP. Further experiments will aim at reducing immune response and enhancing outgrowth of transplanted cells to the cochlear nucleus.

SU139. Sox 10 Haploinsufficiency Increases Hair Cell Reprogramming in Mature Non-Sensory Cells

Alissa Buck*[1], Melissa McGovern[1]

[1]*University of Pittsburgh*

Category: Regeneration

Background: Sensory hair cells in the organ of Corti detect sound and convert it into chemical signals that the brain can interpret. Hair cells are surrounded by supporting cells and other non-sensory cells that provide structural support and ion recycling. Damage from ototoxic insults- loud noises, aging, etc.- can lead to hair cell death and permanent hearing loss because hair cells do not naturally regenerate. Recently, we demonstrated that non-sensory cells of the mature cochlea are reprogrammed into hair cell-like cells by the expression of Atoh1, Gfi1, and Pou4f3. Reprogrammed hair cells share similar characteristics to endogenous hair cells, however, they lacked innervation from the spiral ganglion and developed disorganized stereocilia bundles that appear immature. It is possible that hair cell identity has not been completely activated and molecules within non-sensory cells are restricting reprogramming.
Sox10 is a transcription factor that is expressed throughout the cochlea during development and becomes restricted to non-sensory cells as the cochlea matures. Sox10's role for cellular identity in the ear and on the floor of the cochlear duct are not well understood, however, Sox10 mutations in humans can result in deafness due to malformation of the stria vascularis and a shortened cochlear duct. Preliminary data suggest that Sox10 haploinsufficiency promotes the formation of ectopic inner hair cells and permits the formation of ectopic sensory patches in non-sensory regions postnatally. Taken together, data suggests that Sox10 is involved in regulating cellular identity in the mammalian cochlea.

Methods: We investigated whether Sox10 haploinsufficiency improves hair cell reprogramming from non-sensory cells in the mature cochlea. Expression of Gfi1, Atoh1, and Pou4f3 was induced in non-sensory cells using Rosa26loxP-stop-loxP-Gfi1-Ato1-Pou4f3 (Rosa26GAP) mouse line. Recombination was induced in non-sensory cells with Fbxo2CreERT2 and targeted cells were fate-mapped using Rosa26loxP-stop-loxP-tdTomato (Rosa26tdTomato). One copy of Sox10 was eliminated using Sox10LacZ mice. Tamoxifen was injected to activate tdTomato and GAP expression at six weeks of age and the cochlea are collected at nine weeks.
Results: Preliminary data suggests that Sox10 haploinsufficiency increases the number of reprogrammed hair cells in non-sensory regions of the cochlea.
Conclusions: Hearing loss is a prevalent disability that is often caused by the loss of sensory hair cells that are unable to regenerate. Current treatment options include cochlear implants and hearing aids; however, hearing is not restored for all patients, nor are the cochlea's biological structures restored. The use of gene therapies for hair cell regeneration is a promising candidate for hearing restoration, making it critical to understand the molecular and genetic regulation of cellular identity. Preliminary data suggests that overcoming Sox10-mediated control of cellular identity will be important for hair cell reprogramming and understanding how Sox10 controls non-sensory cell identity will advance the development of therapeutic options for hearing restoration.

SU140. Isoform Diversity in the Mouse Cochlea and Utricle

Sarath Vijayakumar*[1], Mi Zhou[1], Guanfang Xie[1], Venkatlaxmi Chettiar[1], Khushboo Patel[1], David He[1], Litao Tao[1]

[1]*Creighton University*

Category: Genetics A: Genomics and Gene Regulation

Background: The majority of genes in the mammalian genome produce multiple isoforms of mRNA. These isoforms are generated through mechanisms such as alternative splicing (AS), alternative transcription start/stop sites, and intron retention. AS, the predominant isoform regulatory mechanism, plays a crucial role in cell differentiation, cell fate determination, and brain development. It is estimated that over 60% of multi-exonic genes in mice undergo AS, facilitating the generation of multiple functional (or non-functional) isoforms from a single gene locus. Although isoforms of some genes such as Whrln, Ush1c, and Myo7a have been identified in the cochlea, the full diversity of isoforms in the cochlea and vestibular end organs remains relatively unexplored. In this study, we attempted to identify these isoforms in the adult mouse cochlea and utricle.

Methods: The cochlea and utricle were microdissected from 6-week-old CBA/J mice. Total RNA was isolated from the samples using the Quick-RNA Microprep kit (Zymo Research). Full-length cDNA synthesis and amplification for long-read sequencing were conducted using the TeloPrime Kit V2 (Lexogen). Long-read RNA sequencing was performed on the PacBio SEQUEL IIe platform. PacBio raw reads were analyzed using the Iso-Seq workflow in the SMRT Link pipeline. TALON and SQANTI3 were employed for transcriptome annotation and isoform classification. Bulk RNA sequencing reads obtained from individually picked hair cells and supporting cells were assembled de novo using Trinity and subsequently utilized for downstream processing.

Results: In the cochlea, over 90,000 isoforms were identified. Twenty percent (20,709) of the identified isoforms were classified as full splice match (FSM). The majority, approximately 75% (72,786) of isoforms, were classified as novel in catalog (NIC; 30,169) or novel not in catalog (NNC; 42,617). In the utricle, 35,000 unique isoforms were identified. These were classified as FSM (27%; 10,718), NIC (6%; 2,257), and NNC (10%; 4,158). The identified isoforms were cross-validated with de novo assembled transcriptome from short-read RNA sequencing. Genes of interest were selected for further validation. The Gfi1 and Pou4f3 isoforms were of particular interest. We identified a novel short isoform of Gfi1 that is predominantly expressed in the outer hair cells. The long and short Gfi1 isoforms were confirmed by qPCR. The validation of protein isoforms and of other genes is still underway.

Conclusions: We combined long-read and short-read RNA sequencing in the cochlea and utricle to identify novel isoforms. Gfi1 isoforms appear to be differentially expressed in cochlear outer hair cells. These findings suggest the possibility of a new mechanism for cell fate determination and maturation. We believe that this information on transcript diversity can contribute to ongoing hair cell regeneration endeavors in the field.

SU141. A Novel Chd7+/CreERT2 Mouse Model to Study Contributions of CHD7 to Inner Ear Development and Function

Jennifer Skidmore*[1], Donna Martin[1]

[1]*University of Michigan*

Category: Genetics A: Genomics and Gene Regulation

Background: Proper development of the mammalian inner ear requires a precise sequence of signaling and epigenetic events to control gene expression. These events are coordinated by chromatin remodeling proteins including CHD7, which acts upstream of sex determining region Y-box 2 transcription factor Sox2 in proneurosensory cells. Chd7 and Sox2 are both necessary for the proper development of vestibular and auditory structures. Prior studies have shown that SOX2-lineage cells contribute to the formation of multiple cells and tissues in the ear, but there are no detailed reports investigating the contributions of CHD7-lineage cells to otic structures. Here we describe the generation and characterization of a novel Chd7+/CreERT2 mouse model to test the timing, specificity, and functions of Chd7 in the ear.
Methods: Chd7+/CreERT2 mice were developed in collaboration with the University of Michigan Transgenic Core using CRISPR/Cas9 technology to insert a CreERT2 recombinase cassette at the ATG site in Chd7 exon 2. To test for functionality of the allele, crosses were established between male and female Chd7+/CreERT2 mice or between Chd7+/CreERT2 and Chd7+/Gt mice. Litters were collected at embryonic day 10.5 (e10.5) and stained with anti-CHD7 antibody. To assay early Cre expression, crosses were established between Chd7+/CreERT2 and Zsgreen1 reporter mice. ` was administered by intraperitoneal injection at e8.5 or e9.5, tissues harvested at e10.5, and whole embryos imaged under fluorescence. To test for leaky Cre activity, a litter of Zsgreen1;Chd7+/CreERT2 embryos was collected at e10.5 in the absence of tamoxifen administration. To characterize inner ear Cre activity, crosses were established between Chd7+/CreERT2 and Zsgreen1 reporter mice with tamoxifen delivery at e12.5 and dissection at e14.5, followed by whole mount fluorescence examination, cryo-sectioning, and staining with anti-CHD7 antibody.
Results: No CHD7 was detected by antibody staining in e10.5 Chd7 CreERT2/CreERT2 or Chd7 CreERT2/Gt embryos, confirming that Chd7CreERT2 is a null allele. No Cre reporter activity was detected in Chd7+/CreERT2 embryos in the absence of tamoxifen, confirming that Cre was not leaky or spuriously expressed. Zsgreen1 reporter expression was present in the e10.5 Chd7+/CreERT2 otocyst after in utero delivery of tamoxifen at e8.5 or e9.5, signifying Cre activity in CHD7 lineage cells in the early otocyst. Abundant CHD7 lineage cells were also observed in the E14.5 cochlear ganglion and sensory epithelium after tamoxifen treatment at E12.5, confirming CHD7 lineage cells contribute to proneurosensory cell development in the inner ear.
Conclusions: Chd7+/CreERT2 mice express Cre from the Chd7 locus and exhibit heterozygous loss of Chd7 function. These mice are a useful tool for determining the contributions of CHD7 to development and function of specific cells and tissues in the inner ear and will help in the rational design of therapies for hearing and balance disorders.

SU142. Evaluating Pendrin Correctors in a Mouse Model of Pendred Syndrome and DFNB4

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Category: Genetics A: Genomics and Gene Regulation
Background: Mutations in the SLC26A4 gene, which encodes the anion exchange protein Pendrin, cause hearing loss in both Pendred syndrome and non-syndromic DFNB4. Pendrin

plays a critical role in ion transport and maintaining inner ear homeostasis, essential for normal hearing function. Mutations such as H723R, common in East Asian populations, result in progressive hearing loss that begins early in life. Despite advances in understanding these genetic mutations, no curative treatments are available for Pendred syndrome or DFNB4. The aim of our study is to assess the efficacy of Pendrin correctors, which are designed to restore proper protein folding and function, using a mouse model that closely mimics human genetic mutations.
Methods: To evaluate Pendrin correctors, we used a specifically designed Tg(E);Tg(R);hH723R(+);Slc26a4(-/-) mouse model. The Tg(E);Tg(R) system is doxycycline-inducible, allowing temporal control of gene expression. In this model, hH723R(+) represents human Pendrin (hPDS) knock-in and Slc26a4(-/-) represents mouse Pendrin (mPDS) knock-out. Pendrin correctors were administered via mouse intratympanic injections to evaluate their ability to restore Pendrin function. After treatment, whole-mount immunofluorescence imaging was conducted to visualize Pendrin expression within the cochlea. Immunofluorescence staining assessed the localization and expression levels of Pendrin across cochlear regions, focusing particularly on hair cells responsible for hearing.
Results: Imaging and staining studies revealed a significant increase in Pendrin expression in Tg(E);Tg(R);hH723R(+);Slc26a4(-/-) mice treated with Pendrin correctors. Restored Pendrin expression was prominent in key cochlear areas, particularly in hair cells, and correlated with improved hair cell survival, demonstrating the effectiveness of the corrector compounds. In contrast, minimal Pendrin expression and hair cell protection were observed in Tg(E);Tg(R);hH723R(-);Slc26a4(-/-) mice.
Conclusions: Pendrin correctors enhanced Pendrin expression in the Tg(E);Tg(R);hH723R(+);Slc26a4(-/-) mouse model, providing evidence for their potential efficacy in treating Pendred syndrome and DFNB4. The significant difference in Pendrin expression between hH723R(+) and hH723R(-) mice underscores the need for gene-specific therapeutic interventions, especially for individuals with hearing loss caused by SLC26A4 mutations.

SU143. CHD7 Enriched Silencers Promote Neuronal Differentiation

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Category: Genetics A: Genomics and Gene Regulation
Background: Spiral ganglion neurons (SGNs) that reside within the cochlea are critical for conveying sound information. The loss of SGNs contributes to hearing loss. Guiding fate-restricted progenitors into SGN-like neurons holds promise for hearing restoration by repopulating lost SGNs. A deeper understanding of the molecular mechanisms that drive SGN differentiation will advance stem cell therapies. Using the immortalized multipotent otic progenitor (iMOP) cells, we identified that the chromodomain helicase DNA-binding protein 7 (CHD7) displayed genome-wide enrichment at different cis-regulatory elements including enhancers. I interrogated CHD7 functions at enhancers to govern cell-type-specific gene expression and neuronal differentiation.
Methods: To investigate the role of CHD7, shRNA knockdown was performed. Cleavage Under Targets and Tagmentation (CUT and Tag) was utilized to identify the genome-wide binding sites

of CHD7 in proliferating iMOPs and iMOP-derived neurons. Molecular marks were used to define promoters (H3K4me3), enhancers (EP300), and insulators (CTCF). Bioinformatic analysis of CHD7 enrichment revealed a large number of enhancers in proximity to genes implicated in neuronal differentiation. CRISPRi (interference) was used to probe the function of these regulatory regions. Using Mir9-2 as an exemplar, I measured the effect of inactivating the regulatory regions around the Mir9-2 gene and detected its transcript levels using a dual fluorescence reporter.
Results: Knockdown of Chd7 shortened neurite length compared to the control. CUT and Tag analysis showed that CHD7 is enriched at promoters, enhancers, and insulators in proliferating iMOPs and iMOP-derived neurons. The identification of CHD7 binding at neuron-specific enhancers indicates that CHD7 may regulate gene expression during neuronal differentiation. Unexpectedly, inactivating putative enhancers near the Mir9-2 gene increased Mir9-2 transcript levels, indicating that the identified regions correspond to functional silencers that repress gene expression.
Conclusions: Knockdown of Chd7 implicates its role in neuronal differentiation. CUT and Tag analysis shows CHD7 enrichment at cell-type-specific regulatory elements. Inactivating CHD7-enriched regulatory elements suggests that some CHD7 functions at silencers during neuronal differentiation.

SU144. TNFRSF25: From Genetic Analysis of DNA Methylation in Human to a Mouse Model With Hearing Loss

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Category: Genetics B: General
Background: Current estimates reflects that nearly half a billion people worldwide are affected by hearing loss (HL). Epigenetics has been established as a mediator if the interaction between genes and the environment. These modifiers have been associated with hearing impairment, evocative of a role in the auditory system. One well-characterized epigenetic marker in human genome found to affect the inner ear is DNA methylation. Here we present a report examining the involvement of DNA methylation in humans and confirmed by a mouse model for a new gene for HL. Our study has used an association analysis of DNA methylation signatures in Age-related hearing loss patients (ARHL) and mouse models to identify novel candidate genes for hearing loss.
Methods: We conducted a hospital-based case-control study of DNA methylation in adults with presbycusis. Hearing measurements were obtained from each participant to establish the audioprofiles. A quantitative interrogation of methylation sites across the genome was achieved using the Illumina Infinium® Methylation EPIC Beadchip array. This assay measures CpG loci across relevant genomic regions including CpG islands and promoters. A C57BL/6J-Tnfrsf25em1C/Cya knockout was generated by CRISPR /Cas-mediated genome engineering to study the Tnfrsf25 gene function and validated our preliminary findings.
Results: Our data demonstrate a robust association between patients' hearing thresholds and CpG sites methylation in hearing-related genes. CpG sites located in ESPN and TNFRSF25

indicates an increase in methylation at each frequency as the patients' hearing declines. The mutant C57BL/6J-Tnfrsf25em1C/Cya and C57BL/6J wildtype mice were divided based on age and their hearing ability was evaluated by measuring the auditory brainstem response (ABR) thresholds. A baseline ABR test at 4 and months shows a significant hearing thresholds difference between the homozygote mutants and the wildtypes mice. Furthermore, by RT-PCR we have shown that the tnfrsf25 protein is highly expressed in the cochlea.
Conclusions: In this study we probe for the role of DNA methylation in hearing loss by examining the methylation status across the genome of ARHL patients and age-matched controls. We have established a correlation between patients hearing measurements and methylation status of hearing genes. We have generated a Tnfrsf25 knockout mouse model showing by ABR that the mice are deaf. We are examining whether the Tnfrsf25 deletion can cause morphological changes in the Tnfrsf25 mouse model.

SU145. Association of Glial Cells in Hearing Loss in the Zebrafish Spen Mutant

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Category: Genetics B: General
Background: In humans, de novo mutations in split ends (SPEN) are associated with childhood intellectual disabilities, psychiatric disease, postural control impairment, impaired hearing and vision. The cause of the neurological symptoms in patients is not understood. Using a forward genetic approach, we identified a novel zebrafish mutant harboring a nonsense mutation in spen, offering an opportunity to explore central dysfunction in a vertebrate model of the disease.
Methods: The mutation was identified using bulk RNAseq combined with RNAMapper analysis. Immunohistochemistry, live calcium imaging and in situ hybridization were used to characterize the phenotype of spen mutants. Behavioral tests, including auditory evoked behavioral response (AEBR), vestibular induced eye movement (VIEM), vestibulospinal reflex (VSR), and optomotor response (OMR) were conducted.
Results: Mutant spen larvae have severe postural defects and hearing loss. The mutant phenotype also includes development defects in the jaw and behavioral deficits in the optomotor response, suggesting the visual system is impaired. Although the balance defects are pronounced in spen mutants, selective deficits in vestibular reflexes are observed. Surprisingly, VIEM is not significantly different in spen mutants, suggesting that all components of this pathway including utricular hair cells and VIIIth nerve afferents are functional. In contrast, the VSR is decreased regarding maximum tail angle. These results suggest that the vestibular defects are central in origin in spen mutants. Zebrafish use saccular hair cells for hearing, and we observed a modest reduction of sound-evoked calcium transients in saccular hair cells and afferent neurons. However, AEBR is severely reduced, suggesting that an auditory central processing defect may contribute to this phenotype. Using phosphorylated ERK levels as an indicator for brain activity, we observed an abnormally increased signal in the midline radial glial population in spen mutants. This astrocyte glia cell type has been previously implicated in sensory-related suppression of trunk motor output. We hypothesize that hyperactivity of this circuit shuts down

motor responses to sensory input such as auditory tones. Our preliminary experiments with an FDA approved drug that is known to target a component of the circuit showed a striking decrease in radial glial hyperactivity in spen mutant fish. In addition, this drug also partially rescued AEBR and VSR in spen mutants. These results showed hyperactivity of radial glial cells is associated with sensorimotor defects in spen mutants.
Conclusions: Collectively, our data indicate that spen mutation causes deficits that occur in the sensing and/or processing of auditory/vestibular cues, and the hyperactive radial glial cells are associated with sensorimotor defects in spen mutants. Treatment with this FDA approved drug or related drugs may offer a promising avenue of therapy for SPEN related motor dysfunction in human patients. Future efforts will focus on pursuing which part/component of the circuit accounts for the sensorimotor defects in spen mutants.

SU146. Candidate Therapeutic Approaches Identified From Transcriptomic Analyses of Mice Carrying Human MIR96 Mutations

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Category: Genetics B: General
Background: Progressive hearing loss is a common problem in the human population, with no effective therapeutics currently available. However, it has a strong genetic contribution, and investigating the genes and regulatory interactions underlying hearing loss offers the possibility of identifying therapeutic candidates. Mutations in regulatory genes are particularly useful for this, and one example is the microRNA miR-96, a post-transcriptional regulator which controls hair cell maturation. Humans carrying heterozygous mutations in miR-96 exhibit hearing impairment, and mice homozygous for Mir96 mutations are completely deaf, but different mutations result in different physiological, structural and transcriptional phenotypes.
Methods: We have carried out bulk RNAseq on two lines of mice carrying different human mutations knocked-in to Mir96. We took several bioinformatic approaches to compare the differentially expressed genes and identify candidate targets. We compared the whole transcriptome data to data from DrugMatrix, to find drugs with opposing effects on the transcriptome. We chose a candidate drug and tested it on one of the mouse lines, administering the drug in the water provided to the mice and using repeated auditory brainstem response tests to assess the progression of their hearing loss.
Results: Transcriptomic analyses on these two mouse lines revealed a wide range of misregulated genes in both mutants which were notably dissimilar, reflecting the different phenotypes in the two lines. We identified multiple potential targets from our different bioinformatics analyses, but there was no good overlap between them, which meant we were not able to compile a shortlist of candidate proteins to target. However, we identified a candidate drug to test from the whole transcriptome comparison to DrugMatrix profiles, and found that it delayed the progression of hearing loss in heterozygous mice.

Conclusions: Our work adds further support for the importance of the gain of novel targets in microRNA mutant phenotypes, and offers a proof of concept for the identification of pharmacological interventions to maintain hearing.

SU147. Oligogenic Approaches to Whole Exome Sequence Analysis of a Large, Well-Phenotyped Cohort of Older Adults

Morag Lewis*[1], Jennifer Schulte[2], Bradley Schulte[2], Judy Dubno[2], Karen Steel[1]

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Category: Genetics B: General

Background: Age-related hearing loss (ARHL) is a common, heterogeneous disease with a considerable genetic contribution, but identifying the underlying genes has proven to be a challenge. Over 200 genes are known to be involved in human hearing impairment, but it is likely that many more remain to be found. One explanation for the difficulty in identifying candidate genes, even in large cohorts with good phenotype data, is the potential for ARHL to result from combinations of variants in multiple genes. It is thus important to assess digenic and oligogenic contributions to age-related hearing loss.

Methods: Here we present several oligogenic analyses of exome sequence data from a large cohort with detailed phenotyping. The first approach involved methods designed for genome-wide association studies (GWAS) and common variants, for example PLINK and MAGMA, some of which also use gene-set analyses to prioritise candidate genes. The second used network-based approaches (eg HetRank), which use gene and protein interaction data to identify common variants within subnetworks. Third, the ORVAL variant analysis resource was used, which predicts candidate variant combinations from exome data on a per-exome basis. Finally, machine learning approaches were tested to identify which variant combinations were most useful for correct prediction of phenotype from genotype.

Results: The GWAS-based approaches resulted in few candidate genes, and this is probably due to the relatively small size of the cohort (n=839) compared to traditional GWAS cohorts for which these tools were designed. However, multiple candidate genes were identified from the analysis methods designed for interrogating combinations of more rare variants from exome sequencing. These included known human deafness genes (eg OTOF, TNC), genes involved in hearing impairment in mice (eg ARHGAP21), and novel candidate human deafness genes (eg MYOF, TYK2, both of which were linked to hearing impairment in our previous study on the UK Biobank).

Conclusions: In conclusion, it is important to be able to include oligogenic variants when investigating the genetic basis of a common, heterogeneous condition like age-related hearing loss. The tools for carrying out this sort of analysis are still being developed, and none of the methods tested were a precise fit for the phenotype and genotype data from our cohort, suggesting that there is scope for developing better tools for this purpose. Nonetheless, the gene lists obtained include promising candidates for further study.

SU148. Gender Differences in Gene Profiling in the Sexually Immature Murine Cochlea

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Category: Genetics B: General

Background: Sex-biased gene expression is known to influence various biological processes. Previously, we reported sex differences in cochlear gene expression profiles in sexually mature mice (Ye et al., Hearing Research 2024). We demonstrated that male-biased genes are oriented towards mitochondrial energy production and regulatory control of gene expression, while female-biased genes are associated with mechanotransduction and synaptic transmission. However, it is not yet clear how these differences emerge during postnatal cochlear development. The current study aims to determine if these differences arise during sexual immaturity and how they progress as maturation occurs.

Methods: CBA/CaJ mice were divided into three age groups: 7, 14, and 21 days postnatal (PND7, PND14, and PND21), with 8 male and 8 female mice in each group. For PND7 mice, sex was identified by genotyping. After the mice were sacrificed, their cochleae were collected and dissected. The cochlear tissue, including the lateral wall, sensory epithelium, and osseous spiral lamina, was harvested, and total RNA was extracted for RNA-sequencing analysis.

Results: The study revealed age-dependent differences in gene expression between sexes. In the PND7 group, 1,169 male-biased and 906 female-biased genes were identified. By PND14, the number of sex-biased genes decreased to 5 for each sex but increased slightly at PND21 to 25 male- and 19 female-biased genes. Analyses using DAVID, GSEA, and Gene Ontology demonstrated sex-specific effects across various biological functions in all groups. In female mice, half of the top 20 female-biased genes were involved in mitochondrial energy production, while the other half impacted RNA regulation and protein synthesis in all age groups. By PND21, several female-biased genes were also associated with immune-related processes. For male mice, the top 20 genes influenced a broader range of biological functions. Genes related to structural development and maintenance were consistently present, though their proportion varied: 10% at PND7, 50% at PND14, and 35% at PND21. Regarding synaptic communication and mechanotransduction, 90% of the top 20 genes played prominent roles at PND7, but this percentage dropped to 45% at PND14 and 5% at PND21. Additionally, genes affecting RNA regulation and protein synthesis were absent until PND21, when 40% of the top 20 genes contributed significantly to those functions.

Conclusions: Our studies, along with previous findings, show that sex-biased gene expression patterns shift from one sex to the other as mice age. In juvenile males, genes related to mechanotransduction and synaptic transmission were prominent but became female-biased as the mice matured. Similarly, genes involved in mitochondrial energy production were female-biased before sexual maturity but shifted to male-biased afterward. These changes suggest that development and sexual maturity both play key roles in shaping and modifying sex-specific traits in the cochlea in response to environmental cues.

SU149. Diverse Genetic Profiles in Hearing Loss Patients With Enlarged Vestibular Aqueduct: A Small Cohort Study Using a Targeted Exon Sequencing Panel

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Category: Genetics B: General

Background: Enlarged vestibular aqueduct (EVA) is an inner ear malformation that accompanies hearing loss and accounts for about 12% of sensorineural hearing loss in children and adolescents. Mutations in genes SLC26A4, FOXI1, GJB2, POU3F4 and KCNJ10 are currently associated with non-syndromic EVA. The purpose of this study was to investigate the genetic and auditory profiles of a small cohort of EVA patients using a next generation DNA sequencing assay, which utilizes a 24 gene targeted exome panel.

Methods: Patients with EVA were recruited from Boys Town National Research Hospital, Omaha NE, USA. All participants underwent air- and bone-conduction audiometry to quantify hearing and collection of a blood sample to allow for genetic analyses. A 24 gene exon-sequencing panel was completed for each patient.

Results: Twelve EVA patients (6 male, 6 female) were recruited for this preliminary cohort study. The average age of patients was 28.5 years (range: 8 – 75) and average age at diagnosis was 18.2 years (range: 1 – 69). Eleven out of 12 (91.6%) patients had EVA in both ears and one patient had unilateral EVA for a total of 23 ears with EVA. Seven out of 12 patients (58.3%) had bilateral cochlear dysplasia (incomplete partition type II) and the remaining 5 had normal cochlear anatomy. Of the 23 ears with LVAS, 3 (13%) had normal hearing, 3 (13%) had mild hearing loss, 2 (8.7%) had moderate hearing loss, 4 (17.4%) had moderately severe hearing loss, 8 (34.8%) had severe hearing loss and 3 (13%) had profound hearing loss. These data highlight the heterogeneous nature of the hearing loss noted in individuals with EVA.

Nine of 12 EVA patients (75%) had variants in SLC26A4. No variants were found in FOXI1, POU3F4 and KCNJ10. Variants in GJB6, associated with non-syndromic hearing loss, were also found in 75% of patients. Notably, 91.6% (11 of 12) of patients had two specific variants in gene CEP250, linked to atypical Usher syndrome. Although EVA is traditionally considered a chromosomal recessive monogenic condition, only one patient in the cohort exhibited a homozygous likely pathogenic (LP) mutation in SLC26A4. Three patients had heterozygous LP mutations, while five carried heterozygous variants of uncertain significance (VUS) in this gene. Six of nine patients with SLC26A4 variants had multiple mutations in the gene. Importantly, all patients had genetic variants across multiple genes associated with hearing loss, emphasizing the genetic complexity of the condition.

Conclusions: Overall, these findings indicate the diverse nature of the genetic and auditory profiles in EVA. These findings warrant a large-scale multicenter approach using auditory phenotyping coupled with a whole exome sequencing strategy to better understand genetic and auditory variations in EVA.

SU150. Investigating the Potential to Reverse Hearing Loss Caused by Myo7a Mutations

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Category: Genetics B: General

Background: Myo7a encodes an unconventional myosin which is expressed in the sensory hair cells of the inner ear. Myo7a is located at the upper tip-link density, where it is implicated in generating tension in the tip-link to optimise the opening probability of the transduction channels. In humans, mutations in the MYO7A gene result in a range of syndromic and non-syndromic hearing disorders, the most serious of which is the deaf-blind disorder Usher syndrome type 1B. Previously studied Myo7a mouse mutants, such as the shaker1 mutants, exhibit profound deafness accompanied by severe balance defects. Here, we characterise a new tm1a allele of Myo7a. The tm1a allele is a 'knockout-first' design with a LacZ-containing transcription disruption cassette between exons 9 and 10. The inclusion of FRT sites in the tm1a disruption cassette enables the activation of Myo7a gene expression via removal of the cassette by a tamoxifen-inducible Flpo recombinase enzyme. Using this approach, we have investigated whether the hearing impairment caused by the Myo7a LESS THAN tm1a GREATER THAN allele is reversable. This will shed light on the potential for successful gene therapy for hearing loss caused by MYO7A mutations.

Methods: Auditory-evoked brainstem response (ABR) recordings have been carried out at frequencies ranging from 3-42kHz on Myo7a LESS THAN +/+ GREATER THAN , Myo7a LESS THAN +/tm1a GREATER THAN and Myo7a LESS THAN tm1a/tm1a GREATER THAN mice (n≥5 for each genotype) at P14, P28, P56, P98, and P182. Whole-mounts of the organ of Corti from Myo7a LESS THAN +/+ GREATER THAN and Myo7a LESS THAN tm1a/tm1a GREATER THAN mice at P28 were immunolabelled (n=4 for each genotype) for calretinin and prestin for inner (IHC) and outer hair cell (OHC) quantification respectively. Finally, ABR recordings were also performed on Myo7a LESS THAN +/tm1a GREATER THAN and Myo7a LESS THAN tm1a/tm1a GREATER THAN mice (n≥6 for each genotype) at P28, P42, and P56 following administration of tamoxifen, and thus activation of Myo7a gene expression, at P4 or P28 respectively.

Results: The Myo7a LESS THAN tm1a/tm1a GREATER THAN homozygote mice were profoundly deaf from P14, with no ABR waveforms measured up to a 95dB stimulus at all frequencies tested. Myo7a LESS THAN +/tm1a GREATER THAN heterozygote mice had similar ABR thresholds to Myo7a LESS THAN +/+ GREATER THAN littermate controls up to the oldest age tested of P182. Myo7a LESS THAN tm1a/tm1a GREATER THAN mice also exhibited an obvious balance defect which appeared to worsen with age. Immunolabelling analysis revealed a significant degeneration of OHCs, but no degeneration of IHCs, in Myo7a LESS THAN tm1a/tm1a GREATER THAN mice at P28 compared to littermate Myo7a LESS THAN +/+ GREATER THAN controls. Overall, no discernible improvement in ABR thresholds of Myo7a LESS THAN tm1a/tm1a GREATER THAN mice have been observed up to at least P56 following Myo7a activation at either P4 or P28.

Conclusions: Myo7a LESS THAN tm1a GREATER THAN is a recessive allele which causes profound deafness in mice from P14 along with balance defects. Activating the Myo7a gene as early as P4 did not improve ABR thresholds in homozygous mutants, indicating MYO7A may not be a good gene therapy candidate, however this requires further study.

SU151. Can Hearing Loss in Pex3 Mutants With Synaptic Defects Be Reversed?

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Category: Genetics B: General

Background: Peroxisomes are ubiquitous organelles crucial for redox homeostasis, ether phospholipid synthesis and β-oxidation of fatty acids. Peroxin (PEX) genes are important for peroxisome biogenesis and loss of PEX genes are responsible for peroxisome biogenesis disorders (PBD). One of the common features observed in PBD patients is sensorineural hearing impairment. Pex3 is important for peroxisomal membrane protein import, and strongly expressed in the spiral ganglion and widely around the cochlear duct, including sensory hair cells. Recently, mice carrying a mutation in Pex3 were found to have early-onset progressive high frequency hearing loss, including a defect in synapse connection to inner hair cells at higher frequency regions. Currently, we are using a genetic approach to explore the possibility of reversing the hearing loss in Pex3 mutant mice. This method involves activating Pex3 gene transcription upon tamoxifen injection at different ages after the onset of hearing loss and recording the auditory brainstem responses (ABR). This study will help to understand if peroxisome-associated hearing loss can be reversed and it's potential in developing gene therapy interventions to reverse the hearing loss associated with peroxisomal disorders.

Methods: Pex3 LESS THAN +/+ GREATER THAN , Pex3 LESS THAN +/tm1a GREATER THAN , and Pex3 LESS THAN tm1a/tm1a GREATER THAN mice with or without the tamoxifen-inducible Flpo transgene that encodes Flp recombinase to remove the transcription-disrupting cassette of tm1a alleles were assessed using ABR recordings at P28. This was followed by tamoxifen injection at P28 to activate Pex3 gene expression, with subsequent ABR assessments at P42, P56, and P98. To study synaptic connections, whole mounts of the organ of Corti from P98 mice were immunolabelled using Ribeye for pre-synaptic ribbons and GluR2 for post-synaptic densities (n≥6 for each genotype).

Results: The Pex3 LESS THAN tm1a/tm1a GREATER THAN homozygote mice hearing threshold was elevated at P28 at high frequency ranges from 30-42kHz and was comparable to the hearing thresholds of Pex3 LESS THAN +/+ GREATER THAN and Pex3 LESS THAN +/tm1a GREATER THAN littermate control groups only across the 3-24 kHz range. However, following the tamoxifen injection, there was no improvement in threshold. Additionally, the threshold became elevated at lower frequencies (12-24 kHz), indicating that hearing loss is progressive and continues to worsen with age as in the original Pex3 LESS THAN tm1a GREATER THAN mutants. Whole mount analysis of the organ of Corti showed reduced synaptic connections in the higher frequency region (30-42kHz) in homozygotes with or without Flpo compared to controls. Overall, there was no improvement in ABR thresholds of Pex3 LESS THAN tm1a/tm1a GREATER THAN mice, at least until P98, following tamoxifen injection at P28.

Conclusions: Pex3 is a crucial peroxisomal protein essential for the overall health of the peroxisome organelle. Activation of the Pex3 gene at P28 is insufficient to reverse the elevated hearing thresholds observed in homozygous mutants. Future experiments will focus on activating the Pex3 gene at an earlier time point to determine if this intervention can be sufficient to reverse the hearing loss.

SU152. Rare Missense Variants in Constrained Regions in the OTOG Gene Support a Founder Effect in Southern European Population in Familial Meniere Disease

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Category: Genetics B: General

Background: Meniere's disease is a chronic inner ear disorder defined by episodic vertigo associated with sensorineural hearing loss and tinnitus. The prevalence of familial MD (FMD) is 9% in Southern Europeans compared to 6% in East Asians. A large genetic heterogeneity has been observed in FMD, OTOG being the most common mutated gene, with a compound heterozygous recessive inheritance in 6 unrelated families We hypothesize that an OTOG-related founder effect would explain the higher prevalence of FMD observed in the Iberian population. Therefore, the present study aimed to compare the allele frequency (AF) and distribution of OTOG rare variants across different populations.

Methods: Coding regions with high constraint (low density of rare variants) in OTOG coding sequence in Non-Finnish European (NFE) were retrieved from gnomAD database v.2.1. Missense variants (AF LESS THAN 0.01) were selected from a 100 FMD patients' cohort, and their population AF was annotated using gnomAD v2.1. A linkage analysis was performed, and odds ratios were calculated to compare AF among different populations (NFE, N = 56,885), African/African American (AFR, N = 8,128), East Asian (EAS, N = 9,197), South Asian (SAS, N = 15,308), Latino/Admixed American (AMR, N = 17,296), and global populations (N = 125,748).

Results: Thirteen missense variants were observed in 13 FMD patients (Fig.1), with two variants (rs61978648, rs61736002) shared by 5 individuals and one variant (rs117315845) shared by 2 individuals. The results confirm the observed enrichment of rare missense variants in the OTOG gene in families. Furthermore, 8 variants were enriched in the NFE population, and six of them were in constrained regions. Structural modeling predicts the 5 missense variants could alter the otogelin stability.

Conclusions: Several variants reported in FMD are in constraint regions suggesting a founder effect. and Since OTOG is the most common gene in FMD, this could explain the high prevalence of MD in the European population.

Funding. University of Sydney (B3413 Grant), American Tinnitus Association (G227177)

SU153. Characterization of Kdm6a Conditional Knockout Mice as a Preclinical Model for Kabuki Syndrome Type 2

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Category: Genetics B: General

Background: KDM6A (lysine demethylase 6A) is a histone 3 lysine 27 demethylase that plays a critical role in regulating developmental gene expression across various tissues, including neuronal cells. This X-linked gene consistently escapes X chromosome inactivation across tissues and species, and pathogenic variants in KDM6A cause Kabuki Syndrome Type 2 (KS2). KS2 is characterized by distinctive facial features, skeletal abnormalities, gross motor delay, intellectual disability, and, in some cases, hearing loss. Despite progress in understanding KDM6A's broader functions, its specific role in cochlear development and auditory function remains unclear. Our study aims to develop a preclinical model for KS2, with a focus on associated hearing and balance deficits, to uncover how KDM6A impacts cochlear development and auditory function.
Methods: To confirm Kdm6a overexpression in the inner ear, we first performed next-generation sequencing (NGS) on young male and female CBA/CaJ mice. We then generated conditional knockout (cKO) mice lacking Kdm6a in the central and peripheral nervous systems (Kdm6aflox/flox; Nestin-Cre or Kdm6a cKO) along with control mice (Kdm6aflox/flox). Body weight and length were recorded weekly. Auditory brainstem response (ABR) thresholds were measured at 8, 16, and 32 kHz, and motor coordination and balance were evaluated using the rotarod test starting at 4 weeks of age. Cochlear whole mounts from 4-week-old control and Kdm6a cKO male mice were immunostained for KDM6A, hair cells (anti-MYO7A), and nuclei (DAPI). Confocal z-stack images were acquired for analysis.
Results: Pairwise differential expression analysis revealed significant overexpression of Kdm6a in the inner ears of female CBA/CaJ mice compared to males (fold change = 2.06), which was further validated by qPCR. Confocal imaging of wild-type cochleae showed prominent KDM6A expression in outer hair cells (OHCs), with additional detection in inner hair cells (IHCs) and spiral ganglion neurons (SGNs). PCR genotyping confirmed the absence of Kdm6a in the tail tissue of Kdm6a cKO mice. These cKO mice exhibited reduced body weight and shorter body length compared to age-matched controls. Preliminary auditory brainstem response (ABR) tests at postnatal day 28 (P28) indicated normal hearing in Kdm6a cKO mice, but impaired motor coordination and balance compared to control mice.
Conclusions: Our differential expression and qPCR analyses indicate that Kdm6a escapes X-inactivation and is overexpressed in the inner ears of female mice. Confocal imaging confirmed KDM6A expression in OHCs, IHCs, and SGNs. By approximately P30, Kdm6a cKO mice exhibit reduced body weight, shorter body length, and impaired motor coordination and balance, mirroring symptoms observed in KS2 patients. These findings suggest that KDM6A plays a critical role in inner ear development. Ongoing studies aim to determine whether Kdm6a cKO mice experience accelerated or progressive hearing loss.

SU154. Assessing Auditory Brainstem Response Changes Due to Aging in Macaque Monkeys

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Category: Aging

Background: While it is well documented that age influences auditory processing, the neural coding consequences, such as adaptation, variability and synchrony, are thus far unclear. These details can help clarify the physiological bases of the changes that occur in the system. Aging causes hair cell and ribbon synaptic loss/dysfunction, hearing in noise difficulties, increases in audiometric thresholds, and other auditory processing changes. While studies in humans are informative, they conflate accumulated noise exposure with aging. To avoid this potential confound, we studied non-invasive physiological measures in a cohort of aged rhesus macaques, which are phylogenetically very close to humans, and compared the results with a young cohort.
Methods: ABRs (vertex-to-mastoid) were measured in anesthetized aging rhesus macaques (Macaca mulatta, 26–35 years old, n = 10, 7 male) and compared with our database of young (6–9 years old, n = 18, 4 female) macaques with normal hearing. We analyzed responses to broadband stimuli (clicks, chirps). Specifically, we measured the recovery of response (amplitude and latency) to a 100 μs click (70, 80, and 90 dB SPL) after a preceding click at the same SPL (inter-click intervals, ICI, 10 – 1 ms). Responses were obtained to two repeats of either 512 (chirps) or 1024 (all others) stimulus presentations for each condition. We calculated the variability to individual clicks, and averaged increasing numbers to estimate the variability of the responses.
Results: Recovery of click responses varied with ICI. As ICIs were reduced, the responses to the second click decreased in amplitude while latencies remained roughly stable. This trend was consistent across different waves, and was observed in both aging and young monkeys, but aging macaques ABR waves showed lower amplitudes across all ICIs compared to younger monkeys. When we changed the number of repeats to average, we found that the latency variability was not different between younger and older macaques. In addition, response amplitudes changed with differing group sizes in similar ways for both younger and older macaques, indicating that variability isn't strongly impacted by aging. Measuring the distribution of widths of waves I, II, and IV at an intermediate group size where we averaged 64 trials showed that the distribution of widths of waves I and IV was changed significantly (p LESS THAN 0.05) due to aging, with younger monkeys showing more narrow waveforms in Wave I and towards higher widths in Wave IV.
Conclusions: While ICI reduction resulted in decreased amplitude, this decrease was significantly larger in younger monkeys, indicating a slower recovery in aging monkeys. The equal variability suggested that auditory neural element variability does not vary greatly with age. The wave width results suggest changes in neural synchrony in aging monkeys.

SU155. No Evidence for Cochlear Dysfunction in Ageing Barn Owls

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Category: Aging
Background: In mammals, the sensory hair cells, neurones, and endolymph-generating tissue of the inner ear are all vulnerable to degenerative processes that collectively lead to a typical progression of age-related hearing loss. This can be further accelerated by acoustic and ototoxic insults. In contrast, birds are renowned for their ability to regenerate sensory hair cells and re-innervate them, leading to an impressive functional recovery after such insults. The same

regenerative processes have been suggested to be at work during normal ageing and are believed to underlie the remarkable preservation of behavioural threshold sensitivity shown for old starlings and barn owls – a phenomenon that has been dubbed "ageless ears". However, it remains unknown whether the auditory periphery is truly "ageless" or whether in behavioural tests the central auditory system might potentially be compensating for, and thus masking, peripheral deficits.
Methods: Here we contrast several common, functional cochlear metrics between young-adult (up to 4.5 years of age) and old barn owls (9 – 12y): a) Endocochlear potential, b) CAP thresholds, and c) CAP suprathreshold amplitudes, an established metric for age-related, afferent neuropathic changes in mammals. Finally, d) auditory-nerve neurophonic amplitudes (after Verschooten and Joris, 2014, JARO 15:767-787), providing a correlate of fine-structure phase-locking.
Results: The endocochlear potential was measured in 7 young-adult and 6 old owls. It was not significantly different between the age groups (median young-adult: +34 mV, median old: +32 mV). For 5 young-adult and 6 old owls, CAP thresholds were determined in 1 kHz-steps between 1 and 10 kHz. Thresholds were not significantly different between the age groups for any of the frequencies. Similarly, auditory-nerve neurophonic amplitudes, assessed over the same frequency range, did not differ significantly between the age groups. Surprisingly, however, CAP suprathreshold amplitudes were increased in old barn owls at all frequencies, compared to young adults, suggesting increased recruitment of fibres and/or improved temporal onset synchrony.
Conclusions: These findings support the notion that birds have truly ageless ears, that is, they are able to preserve cochlear function to an advanced age – even in the barn owl, a species with extremely sensitive hearing and a specialised cochlea. Ongoing analysis explores single-unit responses in the auditory nerve and will obtain counts of afferent neurones, to probe for mechanisms behind the intriguing observation of improved CAP suprathreshold amplitudes in ageing barn owls.

SU156. L-Ergothioneine Shows Sex-Specific Benefits in Reducing Age-Related Hearing Loss: Improved Signal-in-Noise Detection in Male Mice

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Category: Aging
Background: The amino acid, L-ergothioneine (EGT), has immense potential as an anti-aging therapeutic, and also shows promise for the treatment of various diseases, including neurological disorders. Previously, we reported that long-term treatment with EGT in old CBA/CaJ mice resulted in significant hearing improvements in male mice but not in females, compared to vehicle controls. These results were measured using electrophysiological techniques (auditory brainstem response – ABRs and Distortion Otoacoustic Emissions – DPOAEs), but no confirmation of improvement in perceptual measures of age-related hearing loss (ARHL) was done. Here, we report that aged male CBA/CaJ mice receiving EGT for 4 months demonstrated

significantly improved performance in a signal-in-noise detection task using reflex modification of the acoustic startle response (ASR), while female mice did not.
Methods: Mice 14 months of age from CBA/CaJ stock from Jackson Labs were administered daily injections of 35 mg/kg EGT in saline for one week. Thereafter, mice received weekly injections of 42 mg/kg until the end of the study. At Baseline, and at 2 and 4 months, mice were tested in a battery of ASR prepulse inhibition (PPI) tests, assessing the animal's ability to detect tonal signals in the presence of a 65 dB SPL wide-band background noise. This signal-in-noise test used narrow-band prepulse signals at 8, 16 and 32 kHz, ranging from 65 to 77 dB SPL in 3 dB steps, equating to +0, +3, +6, +9 and +12 dB signal-to-noise ratios (SNR). The prepulses preceded a 110 dB SPL wideband startle elicitor pulse. Startle activity was quantified as the voltage change recorded from piezoelectric pressure sensors and a non-supervised machine-learning program classified true startle and non-startle trials. Control tests consisted of the same prepulse sequences without the background noise. The dependent measure was PPI of acoustic startle, calculated as 1 minus the ratio of startle amplitude in prepulse trials to the startle amplitude on non-prepulse trials. Hence, the greater the startle inhibition, the greater the PPI, which was used as an indicator of acoustic signal detection.
Results: A non-parametric analysis (SheirerRayHare test) of PPI values for each frequency, using factors of time point and SNR revealed significant main effects of both factors, in male mice at all three frequencies, but not for the corresponding Control (no background noise) tests. Similar analysis of the female cohort revealed significant effects of SNR, but not of time point, for any frequency. Thus, after EGT treatment male mice, but not female mice performed significantly better at the signal-in-noise detection task, compared to baseline.
Conclusions: These findings suggest that EGT could be a valuable naturally derived therapeutic agent for influencing the progression of ARHL.

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SU157. Abnormal Histopathological Features in the Aging Vestibular System of an Alzheimer's Mouse Model

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Category: Aging
Background: The vestibular system is crucial for our sense of balance, detecting head motions, and regulating gaze and posture. As seen with other systems, vestibular aging results in reduced function, and is the leading cause of fatal falls among the elderly. It is also associated with social isolation, a suspected contributing factor to Alzheimer's disease (AD). Studies suggest there is a strong correlation between the vestibular system and spatial memory, with some reports showing improved cognitive function after vestibular stimulation. Thus, we hypothesize that vestibular degeneration occurs in patients with AD at an accelerated rate which may be evident prior to the onset of cognitive decline. To investigate this question, we used the APPNL-F/NL-F mouse model which is a knock-in line where the amyloid precursor protein (APP) is altered to incorporate two mutations found in human AD patients. APPNL-F/NL-F mice exhibit

overproduction of amyloid-beta without overexpression of APP, making it a model for sporadic AD. Our previous results showed minimal change in hair cell and stereocilia density with age, but abnormal anatomical features, such as extracellular spaces around hair cells and splayed or elongated stereocilia. Here we further investigated these abnormal features and also examined the calyx which is a specialized terminus of afferent fibers that envelop type I hair cells.
Methods: Temporal bones were collected from male and female APPNL-F/NL-F and C57Bl/6J mice between 2 and 18 months of age. Immunofluorescent staining was performed using the hair cell marker, myosin VIIa, with type II hair cells identified by Sox2 labeling and type I hair cells identified using secreted phosphoprotein 1 (SPP1). Stereocilia bundles were labeled using phalloidin, kinocilia were labeled using acetylated αtubulin, the cuticular plate was labeled using βspectrin II and innervation was mapped using βtubulin. Lastly, we used Thioflavin S and AmyloGlo to investigate presence of possible amyloid-beta plaques.
Results: APPNL-F/NL-F utricles have elongated and splayed stereocilia bundles as early as 5 months of age, but this phenotype did not appear in C57Bl/6J mice until 14 months of age. At most ages, some APPNL-F/NL-F vestibular hair cells were surrounded by extracellular spaces, which may be evidence of neuronal swelling or amyloid-beta plaque deposition. This phenotype was not observed in C57Bl/6J utricles at any of the ages examined. Using βtubulin, preliminary results suggest degeneration of calyces on some type I hair cells, but there was no evidence of calyx swelling in these extracellular spaces. We also observed that some type II hair cells appear to have abnormal innervation in aged APPNL-F/NL-F utricles. Examinations of amyloid-beta plaques using Thioflavin S and AmyloGlo are underway.
Conclusions: The APPNL-F/NL-F mouse line exhibits several vestibular pathologies including stereocilia degeneration and alterations in the innervation of hair cells, including degeneration of the calyx.

SU158. Identification of Functional Biomarkers Associated with Age-Related Cochlear Synaptopathy

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Category: Aging
Background: Age-related hearing loss (ARHL), or presbycusis, is the predominant neurodegenerative disease of aging. Recent preclinical studies suggest that early pathology due to aging involves a loss of synaptic connections between the sensory inner hair cells of the cochlea and spiral ganglion neurons. This cochlear synaptopathy can occur without measurable changes in audiometric thresholds leading to the use of the term “hidden hearing loss”. Several non-invasive physiological measures have been tested for detecting human hidden hearing loss including auditory brainstem response (ABR) and electrocochleography (ECochG). A decreased ABR suprathreshold wave peak 1 (wave-1) amplitude is associated with cochlear synaptopathy in animal studies, however similar measurements of human ABR/ECochG waves to detect HHL have produced inconsistent results. The purpose of this study is to improve the sensitivity of ABR measures by modifying the ABR stimulation paradigm and establishing new functional markers of cochlear synaptopathy in mice using machine learning (ML) platforms.

Methods: Paired click ABR measures were tested every month in 40 mice (20 M/F) from 8 to 11 months of age. ABRs were obtained using alternating polarity, paired-click stimuli of 0.1 msec duration presented at an inter-click interval of 7 msec at intensities of 70 and 90 dB SPL. ABR analyses included wave peak amplitudes, wave peak latencies and left curvature (lC), right curvature (rC), peak curvature (pC) quantifications for waves -1, -2, -3 and -4. All measurements were acquired from both click 1 (C1) and click 2 (C2) evoked waveforms. Inter-click measurements were acquired by calculating the ratio and differential between C1 and C2 measures within each recording while inter-intensity measurements were acquired by calculating the ratio and differential between 90 and 70 dB stimulation intensity levels within each animal. Classification reports of three machine learning models were compared: random forest, support vector machine naïve Bayesian.
Results: All three ML models demonstrate high accuracy in predicting the age of animal using paired ABR recordings. The random forest model yielded the highest precision at .91. Metrics of highest importance to the model included inter-click measurements for wave-1 and wave-4.
Conclusions: Our multi metric approach based on ML and paired click ABR measures can be used to detect age-related cochlear synaptopathy. In the future, we plan to apply similar approach to study age-related hearing loss in humans.

SU159. Increased Listening Effort and Decreased Speech Discrimination at High Presentation Sound Levels in Older Individuals With Hearing Loss

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Category: Aging
Background: Paradoxically, increasing the sound intensity during listening may lead to worsened speech understanding, especially in noise. This is known as the "Rollover" phenomenon. There have been few studies which investigate how rollover occurs differentially in acoustic listeners, and in those with and without hearing loss. There is mounting evidence that listening effort plays an important role in the neural processing of speech understanding in challenging listening conditions. This can be directly quantified with objective measures such as pupil dilation and cortical auditory evoked potentials (CAEPs). However, there have been no studies which utilized these methods simultaneously in conjunction with an auditory task to investigate the interactions of rollover with aging and hearing loss. Tracking these measures over time simultaneously will allow us to understand the correlative links between neural processes and listening effort as well as when and how they contribute to the observed reduced behavioral performance seen in rollover.
Methods: We aimed to recruit participants across the adult lifespan and without (NH) and with hearing loss (HI) to perform a speech discrimination two-alternative forced-choice task. Minimal word pairs were presented both in quiet and in six-talker babble noise (0 dB SNR), while varying across sound intensities ranging from 35-85 dB SPL. CAEP measures of the P1-N1-P2 complex (amplitude, latency) as well as pupil area are tracked across stimulus and response phases and quantified simultaneously with behavioral responses in percentage correct during the speech discrimination task.

Results: We expect to find that CAEPs and listening effort are modulated by sound level and the effect of rollover will be mainly present when background noise is added to the stimulus. We will compare these effects across subject groups and expect that rollover will be exacerbated by age and hearing loss in acoustic listeners. It is expected the greatest effect will be in older listeners with hearing loss, reflected in CAEP measures and pupillometry results. The expected results will be a novel finding considering it would demonstrate the neural and effort bases of why aging and hearing loss can compound the effects of rollover. These results will help demonstrate broad clinical implications for aging and hearing loss and how sound level can negatively affect speech understanding to differential extents for acoustic listeners, especially for those with hearing loss in the presence of background noise.
Conclusions: The project systematically tests the effects resulting from rollover for different age and hearing groups, which provide deeper insight into understanding age-related processing deficits with and without hearing loss in challenging listening conditions. Finally, the results of this project could broadly influence how to design future hearing devices and interventions that maximize hearing abilities for those affected by hearing loss.

SU160. Envelope Following Responses Constrained to the Auditory Midbrain Exhibit Evidence of Envelope Hyperresponsivity in Older Adults

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Category: Aging
Background: Aging is associated with a loss of neural inhibition that leads to neural hyperactivity in the central auditory system, often called central gain. One manifestation of central gain is an over-representation of stimulus envelopes, or envelope hyperresponsivity. Envelope hyperresponsivity is well-documented at the cortex in humans but is thought to originate in the midbrain. However, the extent of subcortical envelope hyperresponsivity in older adults is poorly understood because it is difficult to isolate neural signals from purely subcortical neural generators using scalp-measured EEG responses. This study tested the feasibility of using source-constrained methods to test the hypothesis that neural responses to stimulus envelopes in the auditory midbrain (inferior colliculus, IC) showed evidence of envelope hyperresponsivity in older adults relative to younger adults.
Methods: Participants were normal-hearing younger adults (age 18-30) and older adults (age 50+) with clinically normal hearing or mild-to-moderate hearing loss. Scalp-measured envelope following responses (EFRs) were collected in response to transposed tones with modulation frequencies of either 40 Hz or 150 Hz. Because hyperresponsivity and central gain is thought to manifest in quiet conditions but not in noisy ones, responses were measured in quiet (Q) and then normalized to responses elicited by the same stimuli in noise (N), and a Q/N ratio was calculated. To quantify subcortical hyperactivity relative to the periphery, Q/N ratios from the IC were normalized to Q/N ratios obtained from click-evoked compound action potential (CAP) of the auditory nerve. All stimuli were presented at 80 dB SPL or its peak equivalent in isolation (Quiet condition) and in +10 dB SNR gaussian noise. Participants' T1 images (MRI) were used to source-constrain EFRs to the bilateral IC. The strength of the response at the modulation

frequency (SNR) and stimulus-to-response correlation coefficients were then extracted from IC-EFRs to assess response strength and temporal regularity.
Results: IC-EFR waveforms in quiet were stronger (higher SNR) and more temporally regular for 40 Hz than 150 Hz modulation and there were no age differences, consistent with prior studies using scalp-measured EFRs. CAP N1 amplitudes in Quiet were larger for younger than older adults, also consistent with prior studies. Q/N ratios for both subcortical (EFR) and peripheral (CAP) responses did not differ between groups. However, when subcortical Q/N ratios were normalized to peripheral Q/N ratios, older adults exhibited higher stimulus-to-response correlations than younger adults.
Conclusions: Results establish the feasibility of using source localization to study subcortical EFRs, even for stimuli that are traditionally considered cortical in origin. Preliminary results also suggest that neural envelope responses at the auditory midbrain of older adults are more temporally regular than those of younger adults, but only when stimuli are presented in isolation and when individual variability is accounted for, suggestive of subcortical neural hyperresponsivity in older adults.

SU161. Age-Related Ultrastructural Changes in the Inferior Colliculus of an Alzheimer's Disease Model

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Category: Aging
Background: Accumulating evidence demonstrates that hearing loss is a modifiable risk factor for Alzheimer's disease (AD). Specifically, central gain (increased excitability) in the auditory system may be an early biomarker to diagnose dementias like AD. During normal aging, GABAergic synapses are downregulated in the auditory midbrain (inferior colliculus: IC). This loss of inhibitory synapses likely underlies the commonly reported downregulation of GABAergic neurotransmission that drives central gain during old age. We sought to determine if IC synapses in an AD model are also downregulated and undergo ultrastructural changes in a manner reminiscent of normal aging.
Methods: We assessed 3xTG mice at presymptomatic (2 months), emerging (8 months), and established disease-ages (15-19 months). We used transmission electron microscopy to characterize inhibitory and excitatory synapses, presynaptic terminal areas, post-synaptic targets, active zone lengths, and vesicle pools. Ultrathin sections (~50 nm) were placed on 300 Ni mesh grids and stained with uranyl acetate. A random 400 µm2 montage was taken with SerialEM from each grid. Synapses with symmetric synaptic junctions and pleomorphic vesicles were interpreted as inhibitory. Synapses with asymmetric junctions and round vesicles were interpreted as excitatory. Postsynaptic targets comprise somas, dendrites of three calibers (LESS THAN 0.05 µm, between 0.5 and 1.5 µm and GREATER THAN 1.5 µm) and spines.
Results: We analyzed 1,544 inhibitory and 1,519 excitatory synapses. Our primary finding was that the density of inhibitory synapses was reduced by ~50-55%, and that this reduction was present at emerging and established disease-ages. The density of excitatory synapses was also reduced by ~25-30% but not until an establish disease age. The average area of presynaptic

inhibitory and excitatory terminals increased (~24%) between presymptomatic and an established disease age. Interestingly, the loss of inhibitory synapses during emerging and established disease-ages was predominantly those that targeted medium sized dendrites. Meanwhile, the density of excitatory synapses that targeted large dendrites nearly tripled at an established disease age. Lastly, while the average number of total vesicles in each presynaptic terminal did not change, the number of vesicles bound to, or near, the presynaptic membrane declined from ~6 vesicles to ~3 vesicles.
Conclusions: Unlike normal aging, our data demonstrates that 3xTG mice undergo an earlier and more robust downregulation of inhibitory synapses in the ICc. Excitatory synapses were downregulated in a manner similar to normal aging. Conspicuously, inhibitory synapses favored targeting smaller dendrites, while excitatory synapses robustly targeted larger dendrites at an established disease age. Both changes could contribute to greater central gain. We conclude that 3xTG mice undergo several ultrastructural changes within their ICc that may lead to hearing deficits before advanced stages of AD.

SU162. Investigating the Impact of Neural Encoding and Cognition on Time-Compressed Speech Perception

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Category: Aging
Background: Speech perception is a complex process that involves the integration of auditory signals from the periphery to the cortex. Age-related changes in the auditory system, including reduced inhibitory neurotransmitters and alterations in neural processing, can lead to decreased temporal precision and increased central gain, affecting the ability to process rapid speech. Previous studies have found that older adults have more difficulty understanding time-compressed (TC) speech, and they exhibit reduced midbrain response amplitudes and an overrepresentation of speech stimuli in the cortex compared to younger individuals. We hypothesize that neural and cognitive measures are factors in the perception of TC speech.
Methods: Our current study compared peripheral (auditory nerve), central (midbrain and cortical processing), and cognitive contributions to perceptual performance. To achieve this, we obtained time compressed (TC) thresholds for 50% correct speech recognition. We also recorded electrophysiological responses to time-compressed speech stimuli in three participant groups: young individuals with normal hearing (YNH, n=29), older individuals with normal hearing (ONH, n=30), and older individuals with hearing impairment (OHI, n=25). We measured Wave I amplitude of the auditory brainstem response (ABR) and frequency-following responses to a monosyllabic word in unprocessed (no TC) and 40% TC conditions. We also measured envelope tracking to 10-minute story segments (no TC and 40% TC) to assess cortical responses to more ecologically valid stimuli. Finally, we incorporated cognitive assessments to evaluate the influence of processing speed (Pattern Comparison Test), attention (Flanker Test), and working memory (List Sorting task) on the TC speech thresholds.
Results: As expected, the older listeners had lower (worse) TC thresholds than the YNH listeners, and OHI listeners had lower TC thresholds than ONH listeners. Our results showed that

older listeners had reduced phase locking in the midbrain but enhanced reconstruction accuracy in the cortex relative to younger listeners. Reconstruction accuracy was enhanced in the TC compared to no TC conditions. Preliminary results for determining contributions to perceptual performance showed that Wave I amplitude is a significant predictor of poorer perceptual performance, whereas other neural measures were not. Additionally, processing speed was another predictor of TC speech thresholds.
Conclusions: This study underscores the significant role played by both neural and cognitive factors in the ability to process rapid speech. Our research outcomes contribute to a better understanding of how neural deficits and cognitive performance impact recognition of fast speech, paving the way for targeted auditory training to enhance speech comprehension and improve social participation among the aging population, regardless of their hearing status.

SU163. Physiological Functional Connectivity Changes During Difficult Listening in Older and Younger Adults

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Category: Aging
Background: Listening in difficult, noisy conditions affects the cortical neural circuits that underlie speech comprehension. These directional circuits convey neural signals between cortical regions, encode information related to processing of the stimulus, and are characterized by their dominant frequency band, e.g., delta band or theta band.
Methods: We utilize the Network Localized Granger Causality (NLGC) framework applied to magnetoencephalography (MEG) data to simultaneously estimate neural currents in cortex and the graph network that connects current sources to one another. This directional connectivity is analyzed in multiple non-overlapping regions that span the entire cortex. Additionally, a Temporal Response Function (TRF) analysis is performed on the estimated current sources to probe hierarchical processing of speech features among network-connected current sources and to determine to what extent these circuits convey signals that temporally track the stimulus.
Results: We elucidate how these circuits change as listening conditions become increasingly adverse, and we reveal differences in regional connectivity between older and younger individuals.
Conclusions: Broadly, we estimate the connectivity of the cortical neural circuits in physiological frequency bands that are involved in processing speech, and we examine how the circuits change with age and listening difficulty. We also demonstrate how to combine these circuits with established TRF analysis to localize hierarchical processing of speech. We present results on a listening data set, but note that the methods are widely applicable to most MEG data sets.

SU164. Ergothioneine Consumption Shows Improvements in Hearing in Older Adults: An Analysis of Nhanes Data

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Category: Aging

Background: Ergothioneine (EGT) is a powerful antioxidant, helping to neutralize free radicals that can cause cellular damage, aging and chronic diseases. Our initial studies showed that EGT, a potent dietary antioxidant found in mushrooms and other vegetables, has protective effects on age-related hearing loss (ARHL) in CBA/CaJ mice. To continue, we analyzed one of the top human health databases: National Health and Nutrition Examination Survey – NHNAES, examining correlations between consumption of dietary EGT and ARHL progression in humans. Our hypothesis: people consuming more mushrooms would have better hearing thresholds than counterparts of the same age.

Methods: Mushroom Intake: The NHANES is a series of surveys conducted by the Center for Disease Control (CDC) annually. To determine whether a participant ate mushrooms, a 24-hour dietary recall was studied to determine food intake. All USDA NHANES food codes that contained mushrooms as an ingredient were used to determine if participants ate mushrooms. Hearing Measurements: The NHANES also conducted a series of medical examinations, including audiometry tests – pure-tone audiogram thresholds, tympanometry, acoustic reflexes, wideband reflectance, and other qualitative hearing health questions. Various pure tone average (PTA) thresholds across multiple years (2003-2020) were used as the hearing measurements.

Statistical Analysis: The final data set analyzed was restricted to a minimum age of 30 and a maximum age group of 80. The post hoc two-tailed survey-weighted t-tests were applied across comparisons of the groups who ate mushrooms vs those who did not. Post hoc testing was done only after verifying that a main effect was statistically significant across age groups, PTA average, and whether the food was eaten or not, with ANOVA tests.

Results: PTA thresholds averaged across hearing frequencies (500-8000 Hz) and at individual frequencies show ARHL, where thresholds become worse with age; confirming the progression of ARHL in our subject age groups. A multivariable regression model (unadjusted) was used to determine the associations between mushroom consumption and PTA thresholds, with an alternative hypothesis that PTA thresholds (for mushroom intake) will be lower than PTAs for no mushroom intake. A strong correlation for mushroom consumption was observed for PTA averages and individual frequencies, especially for males. Further analyses are ongoing to expand the scope of this NHANES human database analysis, such as developing models adjusted for various confounding variables (e.g., noise exposure, cardiovascular health, diabetes, etc.) and including other hearing measures.

Conclusions: A limitation of our study is that besides EGT, mushrooms contain other antioxidants (glutathione), vitamins, and minerals (such as copper and selenium), that may contribute to hearing benefits. Our results provide important clues about the potential preventative effects of EGT for ARHL prevention and other neuroprotective treatments.

Supported by: NIH-NIDCD R21 DC021546-01 grant and the American Hearing Research Foundation – Muench Chairman Grant 2023.

SU165. Hearing Acuity and Musicianship Differentially Affect Mismatch Negativity and Memory Precision

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Category: Aging

Background: Musical training and age-related hearing loss are each associated with changes to auditory perception and domain-general cognitive abilities. Research has demonstrated perceptual and cognitive advantages (such as inhibitory control and episodic memory) from musical engagement that persist to older age. Research has further demonstrated worse auditory perception and cognitive abilities with age-related hearing loss. How hearing acuity interacts with the effects of musical engagement on perception and cognition, and whether hearing loss counteracts the benefits afforded by music training in older age, are unclear. We therefore investigated if and how musicianship and age-related hearing loss differentially affect precision in perception and memory in older adults.

Methods: Twenty-six older amateur and professional musicians (62–85 years, 13 females) and 25 older non-musicians (61–82 years, 16 females) were administered a novel task of auditory memory precision while electroencephalography (EEG) was recorded. The auditory mismatch negativity (MMN), an early event-related potential of change detection, was measured using a passive auditory oddball paradigm with standard and deviant pure-tone sequences differing in pitch contour. After exposure, all participants completed an incidental memory test for old targets amongst similar lure sequences (matched for frequency but differing in contour) and dissimilar foil sequences (differing in frequency and contour). Hearing acuity was assessed using pure-tone audiometry.

Results: Older musicians and non-musicians showed relatively equivalent pure-tone thresholds. Compared to older non-musicians, older musicians showed enhanced MMN amplitudes and better memory discriminability for old targets compared to new lures and foils. Greater age-related hearing loss showed attenuated MMN amplitudes, but did not interact with musicianship. Findings suggest better precision in perception and auditory memory performance in older adult musicians as compared to non-musicians.

Conclusions: Given age-related declines in both perception and memory, findings suggest contributions of musical engagement to cognitive reserve in support of healthy neurocognitive aging. These benefits to perception and cognition from musical engagement were retained while accounting for individual differences in age-related hearing acuity. Future research is necessary to examine the causal mechanisms of perceptual and cognitive benefits associated with music training.

SU166. Influence of Single-Nucleotide Polymorphisms of the NF-E2-Related Factor 2 Gene on Age-Related Hearing Loss in the General Japanese Population From the Iwaki Health Promotion Project

Akira Sasaki*[1], Takashi Kasai[1], Shuya Kasai[1], Shiori Miura[1], Shinichi Goto[1], Ryoko Yotsuyanagi[1], Tatsuya Mikami[1], Yoshinori Tamada[1], Ken Itoh[1], Atsushi Matsubara[1]

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Category: Aging

Background: The transcription factor NF-E2-related factor 2 (NRF2) is a master regulator of detoxifying and antioxidant genes, playing an important role in cellular defense mechanisms against oxidative stress. Recently, single-nucleotide polymorphisms (SNPs) of the NRF2 gene might be reported to be associated with several diseases. For example, we showed that a decrease in NRF2 activity caused by SNP was associated with an age-dependent increase in vascular stiffness, and Shimoyama et al. reported an association between NRF2 SNPs and cardiovascular mortality in hemodialysis patients. Focusing on the association with hearing, NRF2 has been reported to be associated with age-related hearing loss (ARHL) in animal models. However, the effect of NRF2 SNPs on ARHL in the general population has not been reported, and this study aimed to evaluate the effect of NRF2 SNP-617 on ARHL in the general Japanese population.
Methods: This study included participants from an annual large-scale epidemiological survey of the Iwaki Health Promotion Project. The genotypes of NRF2 SNP-617 were determined via whole-genome sequencing, and we separated the AA group (homozygotes for the minor allele) and CC+CA group (homozygote CC, heterozygote CA). Regarding the hearing, participants with an average hearing of GREATER THAN 25 dB at 0.5, 1, 2, and 4 kHz were included in the hearing loss group, while the remaining participants were included in the control group. Multiple logistic regression analyses were used to evaluate influencing on the hearing loss separately for each sex using possible factors that contribute to hearing loss including whether the CC+CA or AA group as independent variables.

Results: Among the 306 CC+CA and 31 AA group male subjects, 22 (7.2%) and six (19.4%) had hearing loss, respectively, with a significant difference.
Multiple logistic regression analysis showed that the risk of hearing loss in the male subjects was significantly higher in the AA group than that in the CC+CA group (p=0.031, odds ratio=3.437, 95% confidence interval=1.116–10.580).

Meanwhile, there was no significant association between hearing loss and NRF2 SNPs among the female subjects.

Conclusions: The genotypes of NRF2 SNP-617 AA carriers were significantly associated with the development of ARHL among the males aged 30–59 years. NRF2 SNP-617 was suggested to be involved in ARHL same as several diseases.

SU167. Exploring the Correlation Between Blast-Induced Hearing Loss and the Progression of Alzheimer's Disease

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[1]*University of Miami*

Category: Aging

Background: Exposures to hazardous noise causes irreversible injury to the structures of the inner ear, leading to changes in hearing and balance function with strong links to age-related cognitive impairment. While the role of noise-induced hearing loss in long-term health consequences, such as progression or development of Alzheimer's Disease (AD) has been suggested, the underlying mechanisms and behavioral and cognitive outcomes or therapeutic solutions to mitigate these changes remain understudied. The goal of this study to characterize the association between blast exposure, hearing loss, and the progression of AD pathology, and determine the underlying mechanisms.
Methods: Wild-type (WT) and 3xTg-AD mice, a well-established experimental model of AD pathology were acquired for the study. Female and male mice of 4 months of age were randomly assigned at the beginning of the study to no blast or blast-exposed groups. Blast injury was carried out in an ecologically valid oxyacetylene gas tube. Auditory brainstem response (ABR) and cervical vestibular evoked myogenic potential were used to assess functional outcomes while behavioral assessments were performed with the use of novel object recognition, water maze, open field over 2 months period. Western-blot and immunohistochemistry analysis were performed to quantify the expression levels of amyloid beta (Aβ), Tau, and inflammasome proteins in the peripheral auditory system and multiple brain regions including auditory cortex and brainstem following blast exposure.
Results: Our results show that blast injury led to auditory sensorineural cell loss with a consequent combination of temporary and permanent hearing and balance impairment. Mice behavioral analysis revealed that both WT- and 3xTg-blast mice animals had challenges with the novel object recognition. In open field, 3xTg blast mice exhibited significant increases in fecal boli when compared to WT mice while 3xTg animals (sham and blasted) showed a decrease in overall mean speed, total distance traveled and mobility while time spent at the center of the arena and frequency zone transition center-border increased noticeably. In addition, Aβ, Tau and inflammasome proteins were elevated in cochlea as well as brainstem and cortex of 3xTg mice suggesting an involvement of pyroptosis related mechanisms in the impact of hearing loss on the onset and progression of cognitive decline in AD pathology.
Conclusions: Together our findings suggest a strong association between early hearing loss and progression of AD pathology. Preventive and therapeutic strategies aimed at attenuating hearing loss could be beneficial in delaying the onset of Alzheimer's disease.

SU168. Memory-Guided Attention in Hearing Loss and Aging

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Category: Aging
Background: Age-related hearing loss (ARHL) is well known to predict cognitive decline in older adults, but the reason for this relationship is not understood. Some hypotheses suggest that reduced sensory input may strain limited cognitive resources or engenders compensatory cortical reorganization at the expense of other key cognitive functions such as long-term memory. (Slade et al., 2020, Trends in Neurosci. 43(10):810-21) One function of long-term memory is to guide attention, and memory-guided auditory attention tasks involve hippocampal networks that are known to be affected by both ARHL and age-associated cognitive decline (Billig et al., 2020,

Progress in Neurobiol. 21:102326; Zimmerman et al. 2019, Sci. Rep. 9(1):8138.) Here we test the prediction that aging and hearing loss are associated with deficits in behavioral correlates of memory-guided attention.
Methods: 53 adults aged 40–80 years (M = 69) with varying pure-tone thresholds and no evidence of cognitive impairment participated. The study consisted of training and testing phases. During training, participants learned to associate 40 short background sound clips (e.g., ocean shore) with the spatial location of a pure-tone target (left or right ear) that occurred after the background. Some clips were neutral and did not have a probe tone. As a test of memory-guided attention 1 hour after training, participants heard the same background clips during training, but all clips had a probe tone. Reaction times (RTs) were measured in response to the probe tone in both neutral and target trials. We expected faster RTs for probes in learned trials versus neutral trials if memory-guided attention was intact. A separate test examined participants' explicit recall for the probe tone location when they were cued only by the background clips heard during training.
Results: At a group level, participants showed evidence of implicit memory-guided attention by responding faster to learned probes compared to novel probes. Explicit recall, however, was at chance level. Pure-tone average thresholds and age did not correlate with implicit or explicit recall of targets.
Conclusions: Results suggest that auditory memory-guided attention is preserved in aging and in mild-to-moderate hearing loss. It is possible that deficits could arise in more severe cases of hearing loss. Findings are relevant to understand the increased risk of dementia and Alzheimer's disease associated with hearing loss in late life (Lin et al., 2011a; Livingston et al., 2017).

SU169. The Detection of Biomarkers for the Development of Age-Related Hearing Loss Using Metabolomics in the Japanese General Population

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Category: Aging
Background: Environmental and genetic factors have been reported as causes of age-related hearing loss (ARHL); however, there are still few reports of useful biomarkers. Recently, metabolomics has been used to elucidate the causes of various pathologies. We focused on metabolome analysis, handled large amounts of data using an AI-based machine-learning platform, and investigated the prediction of ARHL using metabolomics.
Methods: This study included participants from an annual large-scale epidemiological survey of the Iwaki Health Promotion Project conducted in 2016 and 2017. A total of 1393 participants were included in the analysis. Because the onset of hearing loss with aging occurs at high frequencies, participants with average hearing at 4 and 8 kHz greater than 25 dB were included in the hearing loss (HL) group, whereas the remaining participants were included in the control group. Propensity score matching by age, sex, and mitochondrial DNA haplogroups resulted in 117 participants, each in the HL group and 117 in the control group. We analyzed items containing metabolomics measured in this project. The 228 items were analyzed by an AI-based

machine-learning platform (DataRobot Inc.) to identify items highly associated with hearing loss. Multiple logistic regression analysis was performed with the top nine items as the independent variables from DataRobot analysis, and the presence or absence of hearing loss as the dependent variable.
Results: The result of multiple logistic regression analysis showed that low histidine levels were significantly associated with the high ratio of high-frequency hearing loss. On the other hand, high levels of monoethanolamine and 2-hydroxyvaleric acid significantly correlated with a high incidence of high-frequency hearing loss.
Conclusions: Mitochondrial dysfunction is thought to play an important role in the development of ARHL.
Previous reports described that low levels of histidine, and high levels of 2-hydroxyvaleric acid and monoethanolamine are associated with reduced mitochondrial dysfunction, which may lead to apoptosis. Present results might indicate these metabolites result in early-stage hearing loss at high frequencies, ARHL and prove to be valuable tools in the prediction of aging.

SU170. Language Learning and Musical Activities in Older Adults With Hearing Loss: Cumulative Effects on Cognitive Function and Psychosocial Wellbeing

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Category: Aging
Background: Maintaining cognitive function and psychosocial well-being is paramount to healthy aging. Previously in our Bilingualism and Aging Lab, randomized controlled training studies demonstrated that older adults can experience benefits to their cognitive function and psychosocial well-being by learning a new language or playing a musical instrument. Additionally, a retrospective study among older adults showed that second-language learning and musical training across the lifespan both improved cognitive reserve, which is intertwined with cognitive function and psychosocial well-being and a proxy for resilience to dementia. The study moreover found that cumulative second-language learning and musical training improved cognitive reserve more than either of the two taken alone.
However, these prior studies only included participants with normal hearing, while hearing loss affects over 50% of those over 65, and more than 90% of those over the age of 80. Moreover, hearing loss has documented detrimental effects to cognitive function and psychosocial well-being. Considering the findings of the previous training- and retrospective studies, activities such as language learning, musical training or the two combined may have a protective effect on older adults with hearing loss. Therefore, we aim to examine the extent to which second language learning and musical training can enhance cognitive function and psychosocial well-being in older adults with hearing loss. A cumulative design will allow us to evaluate the effects of language learning and musical training both separately and together.

Methods: Participants will be older adults (60+) with no exclusion based on hearing, i.e., both untreated or treated hearing loss.

Over one year, participants will successively engage in a 3-month choir and 3-month sign language lessons. The order of presentation will be counterbalanced in order to assess choir and sign-language effects both separately and cumulatively. A do-nothing control group will participate in the testing schedule only in order to rule out test-retest effects. Initially 116 participants are planned. Bayesian sequential analysis will be used to assess outcomes and adjust participant numbers after preliminary analysis.

Our primary outcomes are (1) the Color-Shape Switching Task to assess cognitive flexibility, as a proxy for cognitive function and (2) semi-structured interviews to assess the psychosocial impact of sign language and choir.

Results: Data collection is planned to start in November 2024.
Conclusions: Ultimately, this research endeavors to provide valuable insights into the benefits of language learning and musical training for older adults with hearing loss, contributing to the enhancement of cognitive and psychosocial well-being in this population. Our main outcomes will shed light on cumulative benefits of language learning and musical training, to see whether the findings from lifelong experience also translate to new experiences that can be created in older adulthood, especially in participants with hearing loss, to preserve cognitive function and psychosocial well-being.

SU171. Therapeutic Advancement of NHPN-1010 for Addressing Chronic Noise-Induced Tinnitus in Rats

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Category: Tinnitus
Background: Tinnitus has been theorized to be the result of hyperactivity in the central auditory system, one cause of which is loss of inhibition. In prior studies, we correlated lower levels of GABA type A receptor α1 (GABAA-R α1) in the dorsal cochlear nucleus with presence of a tinnitus percept in rats after an acoustic overexposure. Choline acetyltransferase (ChAT), a biomarker for efferent nerve termini, is of interest in the innervation of outer hair cells (OHCs). NHPN-1010, a Phase II-ready drug combining HPN-07 and NAC, has shown promise in decreasing the incidence of tinnitus in this model. In this study, we examined NHPN-1010's efficacy in treating tinnitus when administered 4 weeks post-exposure.
Methods: Young male Sprague-Dawley rats (n=66) whose hearing had been assessed via auditory brainstem response (ABR) were sedated and exposed to 2 hours of 108 dB SPL, 8-16 kHz octave band noise in a reverberation chamber. Four weeks post-exposure, tinnitus percepts were evaluated at 9.3, 16, 20, and 24 kHz with Gap Pre-Pulse Inhibition of the Acoustic Startle Reflex (GPIAS) testing, where subjects with tinnitus index scores increased beyond the 95% confidence interval of the companion control group (n = 34) were considered to have tinnitus at any targeted frequency. Rats with tinnitus percepts were administered a two-week regimen of 300 mg/kg twice daily NHPN-1010 (n=18) or saline (n=18). Eight weeks post-treatment, both groups repeated GPIAS, ABR, and had cochleae and brains harvested for inner hair cell (IHC)

ribbon synapse (RS) counts and measurement of OHC ChAT silhouettes and GABAA-R α1 immunostaining.
Results: In comparison to testing conducted prior to treatment, suprathreshold ABR wave I recordings were normalized in NHPN-1010-treated rats while wave V amplitude was maintained, and tinnitus index scores decreased in the NHPN-1010 treatment group (p LESS THAN 0.05) but not in the saline group (p = 0.76). The incidence of tinnitus percepts at 16 and 24 kHz declined in the NHPN-1010 treatment group (50% to 28% and 67% vs 56%, respectively), while in the saline group more animals exhibited a percept (28% to 50% and 56% to 78%). Significant restoration of IHC RS densities was measured at high frequency tonotopic positions, and noise-induced reductions in OHC ChAT silhouette areas were reversed in the NHPN-1010 group relative to saline-treated animals. GABAA-R α1 was also consistently up-regulated in the DCN in NHPN-1010-treated rats compared to saline treated controls (p LESS THAN 0.05).
Conclusions: These results demonstrate possible pathways for NHPN-1010's efficacy in treating tinnitus. Upregulation of GABAA-R α1, restoration of IHC RS density, and restoration of OHC ChAT silhouette areas could all play roles inhibiting central auditory system hyperactivity and ameliorating tinnitus. This Phase II-ready drug could represent a major innovation in tinnitus intervention, addressing signature pathophysiological aspects of this disorder.

SU172. Objective Functional Biomarkers to Find Druggable Targets for Tinnitus and Hyperacusis

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Category: Tinnitus
Background: Currently, conflicting views on a neural correlate of tinnitus hinder the development of effective diagnosis and therapy for tinnitus (Knipper et al. Rüttiger, 2020. J Neurosci). Although hyperacusis often co-occurs with tinnitus, it is until now considered neither in clinical diagnosis nor for targeted, individualized therapies.
Methods: We used objective functional biomarkers (PTT, ABR, fMRI, EEG) in patients.
Results: The co-occurrence of hyperacusis worsens the tinnitus percept.
Conclusions: We challenge the hypothesis that co-occurrence of hyperacusis worsens tinnitus percept towards a disease state that requires treatment including strategies to compensate for deficits in fast auditory processing.

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SU173. Comparative Study of Tinnitus Suppression Effect of Cochlear Implant and Bone Conduction Implant in the Patients With Asymmetric Hearing Loss and Single-Sided Deafness

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Category: Tinnitus

Background: Implantable hearing devices like cochlear implants (CI) and bone conduction implants (BCI) are commonly used for hearing rehabilitation in patients with asymmetric hearing loss (AHL) or single-sided deafness (SSD). This study aimed to compare the impact of CI and BCI on tinnitus in AHL/SSD patients who experience significant tinnitus.

Methods: This retrospective study included adult AHL/SSD patients with notable tinnitus who received either CI or BCI between 2017 and 2023. Data on clinical characteristics, pre- and post-operative audiological test results, and tinnitus assessments (Tinnitus Handicap Inventory, THI; Visual Analog Scale, VAS) were collected and analyzed.

Results: Among 33 AHL/SSD patients with severe tinnitus (THI ≥ 18), 16 underwent CI and 17 received BCI. In the CI group, all four VAS categories (loudness, awareness, annoyance, and impact on life) and THI scores showed significant improvement. In the BCI group, VAS scores for annoyance and impact on life, as well as THI scores, significantly improved, but no significant changes were observed in VAS loudness or awareness. A linear mixed model analysis demonstrated that reductions in VAS scores for loudness, awareness, and annoyance were significantly greater in the CI group than in the BCI group. Additionally, the cure rate for tinnitus was notably higher in the CI group (62.5%) compared to the BCI group (11.8%) at 6 months postoperatively.

Conclusions: Both CI and BCI were effective in reducing tinnitus symptoms in AHL/SSD patients. However, CI proved to be more effective in tinnitus suppression and achieved a higher cure rate, making it a more favorable first-line treatment option for tinnitus in these patients.

SU174. Intermodulation Distortions in a Mouse Cochlea

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Category: Otoacoustic Emissions

Background: Cochlear amplification is highly nonlinear which results in production of distortions in mechanical responses. For instance, when two or more tones are presented, the cochlea generates significant number of intermodulation distortions (e.g. cubic and quadratic difference tones), some of them becoming audible. Such distortions, while generated near stimulus tonotopic place, can propagate within the cochlea to their own, more apical, tonotopic location, presumably stimulating local auditory nerve fibers and consequently, the entire ascending auditory pathway. For instance, it is a common finding that afferent cells of mouse auditory pathway tuned to low frequencies (~8-20 kHz), can often respond to natural vocalizations with spectral energy at much higher frequencies (GREATER THAN 30 kHz). However, it is unknown whether the cochlea can produce distortions spanning such a wide range

of frequencies. Here, we study cochlear distortions, as measured in mouse ear canal (otoacoustic emissions, OAEs) as well as in cochlear mechanics (at 9 kHz location) in response to high frequency multitone stimulation (zwuis). We compare whether multitone stimuli can reliably produce intermodulation distortions, either in the OAEs or in cochlear vibrations at low frequencies. We compare the proportion of low-frequency neurons in mouse inferior colliculus that respond to same multitone stimulus, and responses to ultrasonic vocalizations (USVs). We use Zwuis multitone stimulus as it delivers many frequencies at the same time without overlap of higher-order distortion products (up to 3rd order), thus allowing for detailed analyses of all possible intermodulation products that are mathematically derived based on the frequency content of the zwuis.
Methods: The zwuis stimuli were designed to contain 15 frequency components in range of 10-20 kHz, 20-40 kHz, and 30-60 kHz. The OAEs were measured in anesthetized wild type mice, with an ultrasonic microphone and speakers sealed to the ear canal. Mechanical data were obtained in similar conditions from apical turn of a mouse cochlea with optical coherence tomography.
Extracellular recordings were obtained in the inferior colliculus of awake mice for zwuis with 20-40 kHz and 30-60 kHz bandwidth, pure tones from 4-92kHz, and pre-recorded ultrasonic vocalizations (USVs).

Results: Preliminary results indicate that wild-type mice produce robust distortions in the ear canal in response to all zwuis stimuli, however we rarely saw responses at frequencies expanding more than ~1-1.5 octaves below the zwuis bandwidth. Mechanical data show multiple distortions at 9-kHz location with 20-40 kHz zwuis bandwidth. Neural data show that more units responded to 20-40 kHz zwuis (14/70), than 30-60 kHz one (9/70).
Conclusions: We show that mouse cochlea can produce robust intermodulation distortions in response to high frequency zwuis multitone complexes. Such complexes can also evoke neural activity in low-frequency cells of inferior colliculus, which also show responses to complex (harmonics containing) USVs.

SU175. Effects of Contralateral Square-Wave Stimulation on Distortion Product Otoacoustic Emissions

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Category: Otoacoustic Emissions
Background: The outer hair cells (OHCs) are innervated by medial olivocochlear (MOC) efferents, which suppresses the movement of the OHCs by sound stimulation. This response is called the medial olivocochlear reflex (MOCR), and many measurements have shown that contralateral acoustic stimulation reduces the level of otoacoustic emissions (OAEs). To date, white noise and broad-band noise have often been used as the contralateral stimulation (Moleti, et al., 2023; Moulin, et al., 1993), but it is not clear what type of noise has a significant effect on OAE levels. In this study, the changes in distortion product otoacoustic emission (DPOAE) level were measured when square waves were used for contralateral stimulation, and the frequency of the contralateral stimulations that most influenced DPOAE levels was investigated.

Methods: It has been reported that the effect of OCR is greater when the sound pressure of the two stimuli (primary tones) that induce DPOAEs is lower (Yakunina et al., 2018). Therefore, we focused on the frequency stability of the primary tones and the most prominent 2f1-f2 component of DPOAEs, and averaged the sound pressure waveform in the ear canal by selecting an appropriate time window length. This reduced the noise floor during PDOAE measurement and shortened the measurement time. Using this measurement method, we measured the change in DPOAE level when a square wave (frequency 10-300 Hz, equivalent sound pressure approximately 60 dB) was input as a contralateral stimulus in subjects in their 20s with normal hearing.
Results: Although there were individual differences, it was found that DPOAEs tend to be easily suppressed by a square wave with a frequency of 100 Hz. In addition, the amount of suppression was greater when the sound pressure of the primary tones was lower, and there were cases where the suppression was more than 10dB.
Conclusions: It was suggested that contralateral low-frequency square wave stimulation suppresses DPOAEs, and that the effect varies depending on the frequency. By using contralateral stimulation, which has a large suppressive effect, it may be possible to simultaneously evaluate OHCs and contralateral IHCs in clinical practice. In the future, it will be necessary to select more effective measurement parameters and confirm individual differences.

SU176. Characterization of SSBP1-Mutation-Associated-Hearing Loss in Patient-Derived OTIC Organoids

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Category: Development: Human Subjects
Background: A recent study identified a novel disease-causing mutation in SSBP1, which encodes a mitochondrial single-stranded DNA-binding protein, in a patient with a distinct phenotype of sensorineural deafness. This mutation in humans was found to disrupt mitochondrial DNA maintenance, leading to compromised oxidative phosphorylation. However, the underlying mechanisms linking SSBP1 defects to hearing impairment remain unclear. Similarly, recent advances in otic organoids, which recapitulate the development and function of the inner ear, provide a valuable platform to investigate the pathogenic mechanisms of genetic disorders associated with hearing loss. In this study, we aimed to elucidate the role of SSBP1 in otic development and the consequences of SSBP1 mutation on inner ear function using a patient-derived otic organoid model.
Methods: We generated induced pluripotent stem cells (iPSCs) harboring the SSBP1 mutation identified in the hearing loss patient. A gene-corrected variant of the mutant cells was developed to serve as an isogenic control. The mutant and corrected iPSCs were then differentiated into otic organoids using a well-established protocol and compared to otic organoids from healthy embryonic stem cells. The otic organoids were subjected to comprehensive phenotypic analysis

using immunofluorescence and functional analysis using multi-electrode array electrophysiology.
Results: We found that the SSBP1 mutant otic organoids exhibited a range of structural and functional defects compared to the isogenic and healthy controls. Notably, the mutant organoids displayed reduced expression of key otic lineage markers such as myosin VIIa, Pou4f3, and Sox2, impaired stereocilia development, and compromised electrophysiological properties. Further investigations revealed that the SSBP1 mutation also affected the development of spiral ganglion neurons.
Conclusions: These findings suggest that SSBP1 plays a crucial role in the differentiation and maturation of both hair cells and auditory neurons and that disruption of this gene leads to severe auditory dysfunction. The otic organoid model developed in this study represents a valuable platform for investigating the pathogenesis of genetic hearing disorders and could facilitate the development of targeted therapies.

SU177. Hearing at Home: The Auditory Environment of Young Children With Hearing Loss

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Category: Development: Human Subjects
Background: The early years of life play a vital role in language development, making it essential for children to have adequate access to spoken language during this period. Since these early years are primarily spent in the home environment, the acoustic properties of that environment significantly influences the amount of access children have to auditory information. This study aims to examine the hours of hearing aid use and the characteristics of the acoustic home environment of young children with hearing loss and hence provide insights into the access these children have to language at home.
Methods: Children with bilateral sensorineural hearing loss using hearing aids, under the age of 4 years, were included. The study is ongoing, with 16 participants aged between 10 and 47 months old (mean 31.7, SD 10.5), experiencing hearing loss ranging from 40 to 70 dB HL (mean 50.9, SD 8.8) included so far. Acoustic measurements of background noise levels and reverberation times were conducted in the children's home living rooms. Each child was provided with seven sets of hearing aids to use for seven consecutive days, along with LENA environmental analysis device for monitoring on both a weekday and weekend day. Parents maintained a logbook indicating what children where doing, whether they were using their hearing aids, and their location at the time. The hearing aids recorded daily usage and acoustic scenes, while the LENA device provided insights into the sounds and spoken language around a child, allowing for the calculation of signal-to-noise ratios (SNRs) in the home environment.
Results: There is significant variability in daily hours of hearing aid usage both between and within individual children, with an average of approximately eight hours of use per day. The duration of use ranged from five to thirteen hours. Data logging from hearing aids indicated that the surrounding environment was noisy for roughly 25% of the time. We will present the

acoustic characteristics of the living room at home. Lastly, we will present the SNR of the language spoken around the children throughout the day.
Conclusions: Preliminary results indicate that children are exposed to varying acoustic environments at home, where they face challenges regarding auditory access to speech. Important outcomes will be discussed to provide a comprehensive understanding of the children's home environments.

SU178. Does Selective Attention to a Target Speaker Reveal a Cortico-Cochlear Loop?

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Category: Psychoacoustics
Background: When differentiating between competing speakers, the human auditory system must segregate one auditory stream from another. Studies have shown that voice pitch is a dominant monaural cue for the segregation of voices. Still, the physiologic mechanisms of auditory stream segregation are unclear. Given that the cochlea is influenced by top-down feedback, attention may steer the cortical pitch estimator, allowing for efferent processes to adjust the gain of cochlear outer hair cells (OHCs) in a tonotopic manner. The aim of this study was to determine the existence and role of this cortico-cochlear loop on speech perception in diotic listening by evaluating the effects of attention on non-invasive electrophysiological measures of cochlear function.
Methods: The cochlear microphonic (CM) was measured from an electrode placed on the tympanic membrane. Stimuli consisted of 12 mixtures of two naturally-spoken, 2-second-long sentences, one uttered by a female and one uttered by a male speaker. Each sentence was comprised of 5 digits: an onset digit "0", a carrier sequence "555" and a target/foil digit (i.e., "1"). Digits from the male and female speaker were interleaved. Participants were asked to press a button when they heard the target digit ("1") uttered by the female for half the session and uttered by the male for the other half of the session. The target number occurred during 10 percent of the stimulus presentations. Participants completed two sessions, where the order of attend female/attend male was counterbalanced across sessions. To evaluate the effect of attention on cochlear responses, the signum operation was performed on the difference between two CM spectrograms, one obtained while attending the male and one while attending the female. This difference spectrogram was evaluated for the differences in the attended speaker's harmonics compared to the harmonics of the competing speaker.
Results: Preliminary results reveal an enhanced response to the attended speaker compared to the competing speaker in the time-frequency representation of the CM signal. This enhancement reverses direction when participants are asked to attend to the competing speaker. These results are consistent with activation of the medial olivocochlear bundle, which reduces cochlear gain by increasing OHC current. This efferent induced change in current manifests as an increase in CM magnitude.
Conclusions: These results suggest that a cortico-cochlear loop may contribute to stream segregation of voices in a speech mixture.

SU179. Probing for Diplacusis in Individuals With Endolymphatic Hydrops

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Category: Psychoacoustics

Background: The stiffness gradient along the cochlear partition determines where the traveling wave peaks, thereby creating the frequency-place map or tonotopic organization. Diplacusis is the perceptual anomaly where the perceived pitch of a tone is different between ears. Endolymphatic hydrops (EH), a condition that most commonly presents unilaterally, is hypothesized to create a stiffened the cochlear partition, altering the tonotopic map, particularly in the apical half of the cochlea where hydrops is most pronounced. Thus, if the traveling wave peaks in a different physical place along the cochlear length in the hydropic and non-hydropic ear of the same individual, the frequency coded (and the pitch perceived) might differ between ears. It has been anecdotally reported that many Meniere's patients and individuals with EH have diplacusis; however, this has not been systematically investigated. Here, we report measurements of interaural pitch shifts assayed using binaural pitch-matching across multiple test sessions.
Methods: Subjects were 16 adults with EH (10 unilateral; 6 bilateral) and 14 normal-hearing controls. We developed an adaptive, two-alternative forced choice paradigm for measuring interaural frequency shifts in human ears, modified from Colin et al., (2016). To be certain that subjects understood and could perform the task, we applied an inclusion criteria of reliable performance on a monaural pitch comparison task. Binaural pitch matching was evaluated at low- to mid-frequencies (i.e., LESS THAN 3000 Hz) with tones presented at 30 dB SL in each ear, which ensured audibility and compensated for differences in hearing threshold between groups. We tested for group differences in binaural pitch-matching between the control and EH groups and probed the stability of pitch-matching over a period of 5 months.
Results: Interaural frequency shifts were near zero for normal-hearing controls whereas subjects with unilateral EH showed a positive pitch shift consistent with the hydropic ear matching a higher frequency tone to the non-affected ear. These interaural frequency shifts were significantly different from those of normal control and bilateral EH groups, whereas controls and bilateral EH groups did not differ. While binaural pitch matching was variable in all cases, the greatest variability across test sessions (and among subjects) was observed in those with EH.
Conclusions: Here, we addressed the question of whether a group with EH showed diplacusis (compared to healthy individuals who perceived matched pitch between ears). Findings suggest that binaural pitch matching differed in hydropic ears vs healthy ears, which may be consistent with an altered frequency place map in some individuals with EH. This test could be of value when assessing and monitoring patients with EH. Future work should address other factors that influence performance, such as disease stage (active vs dormant), degree of hearing loss and age.

SU180. Memory Errors Reveal Cross-Cultural Variation in Representations of Environmental Sounds

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Category: Psychoacoustics
Background: Audition is likely shaped by cultural experience, yet little is known about cross-cultural similarities and differences for the perception of sounds other than speech or music. We used memory judgments of sounds as a window onto perceptual similarity, on the grounds that errors in recognition tasks are often driven by the similarity of previously encountered stimuli.
Methods: Participants were presented with sequences of sounds. Half of the sounds repeated at some point during the sequence, and we manipulated the interval between the first and second presentations. For each sound, participants judged whether the sound had previously occurred in the sequence. We conducted three experiments using this task, each with a different set of sounds: scenes composed of environmental sounds, excerpts of music, and auditory textures (environmental sounds with stable statistical properties, such as the sound of rain, insects, traffic etc.). We conducted the experiment with three different participant groups: members of an Indigenous People in Bolivia (Tsimane'), residents of a rural Bolivian town, and an online cohort from industrialized countries (US, UK, Canada).
Results: Recognition performance for all participant groups and stimulus sets decreased with the delay between a stimulus and its repetition, with a similar dependence of performance on delay. However, some sounds were consistently more recognizable than others, and some were more likely to be mistakenly judged to have repeated. To assess whether the mental representational space occupied by each set of sounds was similar across cultures, we measured the cross-cultural consistency of memory performance for individual sounds. Consistency was lower between groups than within groups, indicating cross-cultural differences in representational spaces. In addition, consistency was lower between online and Tsimane' participants than between online and Bolivian town participants, or between Tsimane' and Bolivian town participants. These findings held for all three stimulus sets.
Conclusions: The temporal dynamics of auditory memory appear similar across cultures. However, the fidelity of memory for individual stimuli exhibits considerable cross-cultural variation. At least for the three groups studied here, results were most different between groups whose lifestyles and experiences are likely to be most different. The simplest explanation of the results is that memory errors are determined by the representational similarity of heard sounds, and that these similarity relations differ between cultures. The results also suggest that cross-cultural variation in auditory perception is not limited to music- or speech-related stimuli.

SU181. Optimization Under Ecological Realism Reproduces Signatures of Human Speech Recognition

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Category: Psychoacoustics
Background: Recent advances in machine learning have made real-world perception tasks feasible for computers, in many cases approaching levels of performance similar to those of humans. For instance, models trained to recognize speech in noise reproduce some characteristics of human speech recognition. However, human and model speech behavior have

not been extensively compared, leaving the extent to which such models can account for human speech perception unclear.
Methods: We trained 6 neural network models to recognize words from simulated cochlear input. We then comprehensively evaluated the effect of a large set of speech manipulations on model speech intelligibility, comparing the results to human speech intelligibility in the same conditions. We varied the training data for which the 6 models were optimized, with the goal of illuminating how the "auditory diet" influences human-model similarity. Some training conditions were intended to be more realistic than others. For instance, training data varied in the presence of background noise, reverberation, and the degree of variation in speech characteristics.
Results: Training in conditions intended to approximate the distribution of real-world speech yielded a fairly good overall match to human behavior. Models trained on larger and more varied sets of speech better matched human behavior than models trained on smaller and less varied data sets. The models trained on what we intended to be realistic conditions nonetheless exhibited some discrepancies with humans. We found that including the discrepant conditions in the model training data largely resolved the behavioral discrepancy, demonstrating that the model architecture is capable of mediating human-like behavior even in these conditions, if trained on them.
Conclusions: The results support the idea that the phenotype of human speech recognition can be largely understood as a consequence of having been optimized for the problem of speech recognition in natural conditions. The origins of the human-model discrepancies that remain are unclear at present, but the results suggest that these discrepancies are not due to limitations of the model architecture.

SU182. Cross-Cultural Influences of Beating on Music Perception

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Category: Psychoacoustics
Background: Frequencies that are present concurrently create the phenomenon of beating – amplitude fluctuations over time that are audible to humans if the frequencies excite the same part of the ear. Previous work has found cross-culturally consistent aversive responses to beating along with pronounced cross-cultural variation in preferences for musical intervals. These previous results leave open whether the aversion to beating exerts any cross-culturally consistent influence on the perception of musical intervals. This issue is relevant to whether beating might have plausibly shaped the structure of musical systems.
Methods: We measured pleasantness ratings of musical intervals presented either diotically (both notes to both ears) or dichotically (one note to each ear). Dichotic presentation largely eliminates the perception of beating between notes, as it prevents frequencies from interacting in the ear. We used a set of intervals that included a) small-integer ratio intervals that are considered consonant by Western listeners, b) intervals from the chromatic scale judged to be most dissonant by Western listeners, and c) additional intervals selected to maximize roughness

as measured by a modulation filter bank model. We tested members of a small-scale Amazonian society (Tsimane') as well as residents of the US. The main question was whether pleasantness ratings would be higher for dichotic presentation, whether this would be specific to particular intervals, and whether any such effects would be present cross-culturally.
Results: US listeners showed a preference for dichotic presentation for small intervals that produce high levels of beating and that are canonically dissonant to Western listeners (e.g. the minor second), consistent with a role for beating in their evaluation. However, this effect was overall modest, and largely absent for other dissonant intervals. As a result, the pattern of pleasantness vs. interval for US listeners was qualitatively similar for dichotic and diotic presentation. Tsimane' listeners also showed a preference for dichotic presentation of small intervals, though the effect extended over a wider range of intervals. Moreover, for Tsimane' listeners, the variation in pleasantness vs. interval was eliminated by dichotic presentation.
Conclusions: The results suggest that an aversion to beating is present across cultures that has some effect on the evaluation of musical intervals. In at least one culture, the variation in pleasantness that occurs across musical intervals appears entirely explained by this effect. In Western listeners, by contrast, most of the variation across intervals is independent of beating as it persists with dichotic presentation. The results provide further support for the idea that consonance and dissonance as experienced by Westerners is a culture-specific phenomenon that is largely separable from effects of beating. However, the results also identify beating-related effects that could have exerted an influence on musical systems, for instance biasing them to avoid small intervals.

SU183. Perceptual Anchoring to Auditory Textures in Neurotypical and Neurodivergent Listeners

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Category: Psychoacoustics
Background: Learning is crucial for the development of species, enabling them to acquire behaviors, accumulate knowledge, and refine skills. New knowledge can be acquired automatically by extracting regularities or patterns within the external stimuli or environment, this process is known as statistical learning. One mechanism by which this passive learning happens is by forming a 'perceptual anchor', when a repeated regularity is extracted and stored for later use. Our aim is to characterize statistical learning to auditory textures in NeuroTypical and NeuroDivergent listeners, a potential mechanism underlying the diverse rang of auditory experiences reported by Autistic individuals.
Methods: Here, we modified a white noise anchoring paradigm (Agus et al., 2014) to incorporate synthetic auditory textures (McDermott et al., 2013). Four sound textures recordings were used in this study: wind blowing, crackling fire, insects, and bubbling water. Normal hearers identified if two synthetic auditory textures were identical. Participants encountered three blocks of 80 trials per synthetic texture: 20 'Anchor' (two identical excerpts within and across trials), 20 Repeated (two identical excerpts within the trial but never heard again) and 40 Novel (two different excerpts presented within and across trials).

Results: Sensitivity to the Fixed-Repeated stimulus was always significantly higher than to the Repeated stimuli for all textures individually and when collapsed in NeuroTypical listeners, however a different pattern of learning was observed in NeuroDivergent listeners.
Conclusions: Here we demonstrated that synthetized auditory textures are suitable to assess perceptual anchoring in NeuroTypical and Neurodivergent individuals.

SU184. Texture Streaming in Auditory Scenes

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Category: Psychoacoustics
Background: Sound textures are created by the superposition of many similar acoustic events (e.g., rain falling, birds chirping, or people clapping) and are thought to be represented in the auditory system by statistics that summarize acoustic information over time. Real-world scenes frequently contain multiple concurrent textures (as when birds chatter next to a babbling brook), raising the question of whether listeners can "hear out" (i.e., stream) individual textures. We sought to characterize "texture streaming" by asking whether listeners can accurately estimate the statistics of sound texture sources in the presence of concurrent textures.
Methods: We tested whether listeners could accurately estimate the statistics of sound texture sources in auditory scenes. On each trial, participants heard an auditory scene composed of two textures, a "target" and a "distractor" (synthesized from the statistics of real-world textures). Participants judged whether a subsequently presented probe texture was contained within the scene. The probe's statistics were either the same as those of the target or were drawn from an unrelated texture. The target and distractor were either presented synchronously, or with the target onset delayed relative to the distractor.
Results: Listeners performed above chance in the synchronous condition, demonstrating an ability to stream concurrent textures. However, performance was enhanced when there was an onset difference between the target and distractor. Crucially, this enhancement was largely eliminated when a brief silent gap was inserted before the onset of the target, indicating that the benefit of asynchrony was not due to having prior knowledge of which source to listen to, and is instead suggestive of an "old-plus-new" principle operating in the domain of textures. Participants exhibited consistent patterns of errors, indicating that some combinations of textures were difficult to stream. The statistical similarity of target and distractor was partially predictive of these streaming difficulties. However, a substantial portion of the explainable variance in streaming performance could not be predicted from texture similarity alone, suggesting additional (as yet not understood) principles of perceptual organization.
Conclusions: The experiments demonstrate the phenomenon of texture streaming—a neglected aspect of auditory scene analysis in which listeners can stream concurrent textures in auditory scenes.

SU185. Cross-Culturally Shared Sensitivity to Harmonic Structure Underlies Aspects of Pitch Discrimination

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Category: Psychoacoustics

Background: Pitch is thought to be a building block of music. However, the extent to which pitch perception is shared across cultures is unclear. Evidence from Western participants suggests that pitch perception relies on multiple representations depending on the conditions. One observation supporting this claim is that harmonic tones are easier to discriminate in noise than inharmonic tones despite comparable discrimination in quiet, suggesting that f0 representations are used in noise but not in quiet. We sought to test whether the dependence on these multiple pitch representations is present cross-culturally despite previous demonstrations of differences in some aspects of pitch-related behavior.
Methods: We tested participants from the USA and an indigenous community living in the Bolivian Amazon (the Tsimane'). In Experiment 1, participants detected harmonic or inharmonic tones presented in noise. In Experiment 2 participants heard two-note melodies and reproduced the melody by singing. Tones were either harmonic or inharmonic and were presented in noise or quiet.
Results: In the first experiment, both Tsimane' and US participants showed a detection advantage for harmonic tones of comparable size. In the second experiment, Tsimane exhibited two characteristics of pitch perception previously seen in US listeners: the direction of pitch changes could be reproduced with equal accuracy for harmonic and inharmonic tones in quiet but was better for harmonic than inharmonic tones in noise. However, replicating previous work, Tsimane' vocal reproductions were unrelated to the absolute pitch or chroma of the stimulus notes, differing from the tendency seen in Western participants to match pitch and/or chroma.
Conclusions: The results of Experiment 1 indicate that both groups are sensitive to harmonic structure and use it to detect tones in noise. The results of Experiment 2 demonstrate that both groups use harmonic structure to aid pitch discrimination in noise, and also indicate that the two representations that mediate pitch judgments in US listeners - one reliant on individual frequency components and one reliant on the f0, which inharmonic tones lack - are present across cultures, and relied on in similar conditions. Overall, the results indicate that the basic structure of pitch perception is shared across cultures but, on its own, is not sufficient to induce pitch and chroma matching.

SU186. Measuring the Performance of Hearing Aid Fitting Algorithms on Hearing in Noise

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Category: Psychoacoustics

Background: Though hearing aids remain highly successful in clinical settings, the processing technology within them has room for improvement. Among the common cited problems with hearing aids is their poor performance for speech in noisy environments. Recent studies suggests that clinical audiograms primarily measure the responses of low-threshold auditory nerve (AN) fibers which ignores pathologies of the two other fiber types, namely medium and high threshold

fibers. This lack of sensitivity in measuring the ensemble AN response has been termed synaptopathy, or hidden hearing loss, because it evades standard audiological assessments. This has important consequences for suprathreshold speech tasks that tend to be above threshold, which exacerbates hearing in noisy situations and could be one of the reasons why hearing-aid users have difficulty understanding speech in noisy environments. There are no known noninvasive biometric tests to detect the effects of synaptopathy on hearing difficulties. Current models of the cochlea can generate neurograms, analogous to spectrograms, that are time-frequency representations of the AN response. We leverage these methods to shed light on the effect of hearing loss and synaptopathy on hearing in noise and effects of hearing aid fitting parameters on the responses of Auditory Nerve.
Methods: A phenomenological model of cochlea will be used to simulate AN responses (Bruce 2018, 2023) in the form of a neurogram, using a corpus of speech consisting of vowel-consonant-vowel (VCV) tokens at conversational speech level and overlayed with speech shaped noise at varying signal-to-noise ratio (SNR) for normal hearing, for hearing loss due to outer hair cell (OHC) loss, for hearing loss due to synaptopathy, and for hearing loss due to combined effect of OHC loss and synaptopathy. The neurograms for hearing loss will be compared against the normal hearing neurograms using a Neurogram Similarity Index Measure (NSIM; Hines 2012) to generate simulated performance curves for different types of hearing loss. The effect of Hearing Aid fitting algorithms (NAL2 and CAM2) will then be evaluated by processing the corpus based on prescribed gains for hearing loss and measuring the improvements in the performance based on NSIM. We will also evaluate the performance of hearing aid fitting algorithms by testing them on the neural network-based models of speech recognition. The neurogram output from the normal hearing cochlea will be used to train a speech recognition model on a word recognition task. After optimizing the model, hearing loss will be introduced, and performance will be remeasured.
Results: The benefit of hearing aids algorithm will finally be evaluated and reported as performance improvement of the speech recognition model, after using hearing aid algorithm processed speech stimuli as an input to the model.
Conclusions: In this study we measure the efficacy of hearing aid fitting algorithms for various hearing loss profiles.

SU187. Predicting Speech Perception Through Information Processing Rate in a Mild Traumatic Brain Injury Population

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Category: Psychoacoustics
Background: Cognitive abilities have previously been associated with speech understanding in noise. Many pathologies are known to compromise those areas of cognition, including mild Traumatic Brain Injury (mTBI). Previous work has established that despite having hearing thresholds within normal limits, individuals with mTBI commonly report issues understanding speech in noise; however, the relationship between cognitive and auditory processing abilities in this patient population are currently not well understood. Here we 1) determined the relationship

between information processing rate and speech understanding in noise, and 2) compared these relationships across a group with a history of mTBI and a control group.
Methods: The Auditory Visual Divided Attention Task (AVDAT) was used to assess cognitive abilities. The AVDAT is a list recall task that is used to measure attention and working memory. The AVDAT has 6 conditions where participants are presented with auditory or visual stimuli, or a combination of the two, in the form of listed letters or numbers. On the trials where both modalities of stimuli are played, some trials are cued where participants are informed of which stimuli they will be asked to recall while others are not. Previous work did not find a significant difference in AVDAT results between the mTBI and control groups. The current analysis used results from the AVDAT to assess information processing speed using a measure of throughput (TP), which is the number of correct items within a trial divided by the duration in seconds of the trial (s).
Speech understanding in noise was measured through an iPad task using a virtual spatial array where three utterances were either presented at the same spatial location or different locations. Participants were conditioned to respond to the target speaker and ignore the other two speakers.

Results: Statistical analyses were done to determine if there is a relationship between information processing rate and the ability to understand speech in noise. The sample consisted of 55 adult participants, 28 of them reported a history of mTBI. Linear regression models were used to determine the effects of mTBI on performance for each measure after controlling for age and hearing sensitivity and to identify how performance on these measures explains variability in speech understanding in noise. When comparing task performance across the 2 groups, it was found that TP averages were not significantly different. They ranged from (0.69 s- 1.32 s) for the mTBI group, and (0.69 s – 1.28 s) for the control group. SRM threshold group averages were 6.12dB for the control group and 5.09dB for the mTBI group.
Conclusions: By targeting the cognitive and auditory processes that are involved with everyday hearing abilities, we can better understand the complaints patients report about their hearing and advocate for changes in clinical care.

SU188. Enhancing Auditory Localization and Speech-In-Noise Comprehension Through Augmented-Reality Auditory Training

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Category: Binaural Hearing & Sound Localization
Background: Selective auditory attention and sound localization are crucial for communication in noisy environments. This study introduces a novel Augmented Reality (AR) auditory training system designed to enhance these skills. Our approach implements an audiomotor closed-loop system with 6DoF (six degrees of freedom) audio rendering, where real-time user movements dynamically alter the audio stimuli coupled with the given room acoustics. This approach enables realistic in-situ training that adapts to diverse scenarios, potentially offering more effective rehabilitation than traditional methods.

Methods: Six participants (M=28.6 years) with no hearing impairment underwent AR-based auditory training using a smartphone. The system rendered 6DoF audio based on user position and real-time head tracking via Apple AirPodsPro2 motion sensors. Over four weeks, participants completed eight 50-minute sessions. During each session, the application simultaneously presented two target words in background noise, requiring participants to identify the correct one.This paradigm was designed to train selective attention in realistic, challenging auditory environments.
Pre- and post-training assessments were conducted in a controlled laboratory setting, including:

A sound localization task using a 13-speaker array arranged in a 180-degree arc spaced 15 degrees apart. Participants were tested in front-facing (FF), left-facing (LF), and right-facing (RF) orientations, allowing evaluation of localization improvements for sound sources in all directions.

The Korean Matrix Sentence Test at varying signal-to-noise ratios (SNRs) to assess speech-in-noise comprehension.

Results: The Korean Matrix Sentence Test results demonstrated significant improvements following the training. Sentence recognition improved significantly at both -6 dB SNR with reduced error rates ($p = 0.028$, Cohen's $d = -0.54$) and 0 dB SNR ($p = 0.024$, Cohen's $d = -0.29$) using the Wilcoxon signed-rank test.These results suggest that the training benefits generalized to untrained linguistic stimuli, indicating far transfer of the acquired skills.
Additionally, sound localization performance improved post-training. A repeated measures ANOVA revealed significant main effects of both the training intervention and facing orientation on azimuth error ($p = 0.0163$ and p LESS THAN 1.16e-13, respectively). Pairwise t-tests confirmed a significant reduction in localization errors post-training ($p = 0.017$, Bonferroni-corrected). The largest improvement occurred in the right-facing orientation, with RMSE dropping from 17.0° pre-training to 10.8° post-training—representing a significant reduction in localization errors. Although the overall effect size was small (Cohen's $d = 0.090$, 95% CI [0.017, 0.162]), individual participants showed varying degrees of improvement, with effect sizes ranging up to 0.160.

Conclusions: Our AR-based auditory training system, incorporating 6DoF audio rendering and adapting to the user's acoustic environment, led to significant improvements in both sound localization and speech-in-noise comprehension. These findings support the potential of AR auditory training as an effective intervention for individuals with hearing deficits. Future research should investigate long-term retention of these improvements and explore applications in larger, more diverse populations, including those with hearing impairments.

SU189. The Effect of Chirp vs Click Stimuli on the ABR Binaural Interaction Component and Behavioral Sensitivity to Interaural Time Difference in Humans

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Category: Binaural Hearing & Sound Localization

Background: The auditory brainstem response (ABR) is commonly used to estimate hearing threshold and investigate the integrity of the neural auditory pathway. A binaural interaction component (BIC) can also be obtained by subtracting a binaurally evoked ABR from the sum of monaural left and right ear ABRs. Previous research has shown that the BIC amplitude can be modulated by interaural time differences (ITDs) and has been proposed as a biomarker of binaural hearing ability. Traditionally, transient click stimuli are used to evoke an ABR; however, chirp stimuli are widely recommended to compensate for the cochlear traveling wave and enhance wave V. A common challenge encountered during ABR measurements is the presence of myogenic artifact which can obscure small components such as the BIC. The enhancement provided by chirp stimuli was shown in one animal-model study to improve BIC detection and reliability, but to date this has not been systematically examined in humans.
Methods: We are conducting a study using healthy young adult subjects (n = 6; aged 21-29 years old) with normal hearing bilaterally through the extended high frequency range. Measurements include monaural and binaural ABRs, with calculation of the BIC DN1 component, for three different stimuli; 1) 100 μsec clicks, 2) level independent CE Chirps, and 3) Level Specific (LS) Chirps. Meaurements were made at four different intensities ranging from 65 to 40 dB nHL. A subset of the subjects also completed behavioral testing measuring ITD discrimination thresholds for the same three stimuli but at four intensity levels ranging from 60 to 10 dB nHL, plus the stimuli high-pass filtered at 2000 Hz to eliminate low-frequency ITD cues.
Results: Preliminary results indicate that, compared to traditional click stimuli, chirp stimuli tend to elicit larger wave V amplitudes for both monaural and binaural waveforms. Additionally, when a replicable response was present (test-retest), a larger BIC DN1 amplitude was typically observed for chirp responses, particularly at lower intensities. Additional ABR characteristics including latencies, area under the curve, peak identification rates, and individual subject variability will be explored. Preliminary results from the behavioral ITD testing reveal that many subjects have lower ITD thresholds for chirps than clicks, mainly at lower stimulus levels.
Conclusions: Use of chirp stimuli rather than clicks may provide an enhancement to both the ABR wave V and BIC DN1, improving overall signal-to-noise ratio and reliability. Moreover, the improved sensitivity to ITDs with chirps supports the hypothesis that BIC DN1 arises from binaural brainstem nuclei that are important for binaural hearing.

SU190. Emergence of Interaural Time Difference Tuning in a Neural Network Trained for Sound Classification

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Category: Binaural Hearing & Sound Localization

Background: Interaural time difference (ITD) is an essential auditory cue for sound localization, detection, and classification. Numerous neurons in the auditory system are tuned to ITD, playing pivotal roles in these functions (Joris and Yin, Trends Neurosci, 2007; Grothe et al., Physiol Rev, 2010). While neuronal ITD tuning has been extensively studied in the context of sound

localization, its role in tasks like sound detection and classification remains less understood. Our previous study explored ITD tuning in a neural network (NN) trained for sound detection and classification without explicitly involving sound localization tasks (Koumura, Terashima, and Furukawa, 2019, ARO). Our findings revealed that ITD tuning can emerge in NNs, even without the localization task. However, the previous study utilized real-world binaural recordings to construct the model, making it difficult to control both the position and category of sound sources. Controlling those factors is critical for distinguishing between the impact of localization and classification tasks on emergent ITD tunings. In this study, we used controlled virtual binaural sounds, enabling us to compare models trained under different conditions.
Methods: We used an NN designed for everyday sound classification as a model of the auditory system (Tokozume, et al., ICLR, 2018). The input was two-channel sound waveforms, and the output represented the likelihood of 200 sound categories. The model's architecture included convolutional layers, batch normalization, pooling layers, and fully connected layers.
The training data comprised everyday sounds (Fonseca, et al., IEEE/ACM Trans Audio Speech Lang Process, 2022), spatialized using head-related transfer functions (Watanabe, et al., Acoust Sci Tech, 2014). We trained the model under three conditions: random sound positions, the same position for all sounds, and all sounds positioned in front of the listener.

We conducted an analysis simulating single-unit recordings in neurophysiology. The stimulus was a 2-second consisting of two tone-bursts (0.5 s each). The response of each element was measured by averaging the activity over time after stimulus input.

Results: ITD tuning was observed in a significant proportion of neurons, especially in the middle layers of the network. We quantified ITD tuning using two metrics: best ITD and most discriminable ITD. The models trained under different conditions exhibited varying distributions of ITD tuning. The number of units exhibiting small best ITD (≦ 0.57 ms) were slightly larger in the fixed and the front conditions than in the random position condition. Especially, the model trained with front-positioned sounds appeared to have slightly more units tuned to front-facing sounds.
Conclusions: Our findings suggest that ITD tuning can emerge in NNs trained for sound classification, not just localization. The training conditions seemed to have a slight impact on the emerging ITD tuning, but their significance is unclear and requires further investigation.

SU191. Deep Neural Network Models of Human Sound Localization Indicate Which Aspects of Localization Are Mediated by Explicit Binaural Processing

Mathias Dietz*[1], Mohammad Dehghani-Habibabadi[1], Mark Saddler[2], Josh McDermott[3]

[1]*University of Oldenburg,* [2]*Technical University of Denmark (DTU),* [3]*MIT*

Category: Binaural Hearing & Sound Localization
Background: Deep neural networks trained to localize sounds using simulated binaural auditory nerve input have emerged as promising new models of human sound localization. Despite never being fit to human data, these networks reproduce patterns of human behavior in several classic psychoacoustic experiments, suggesting they learn to use similar cues as humans do. However, relative to prior models, these networks do not explicitly simulate known binaural circuitry and may therefore neglect implementation constraints that shape human sound localization. Here, we

critically examine cases where deep neural networks and humans exhibit discrepant sensitivity to localization cues, raising the possibility that incorporating explicit binaural circuitry may lead to better models in the future.
Methods: The deep neural network model of Francl and McDermott (2022) was used as an artificial test subject in five previously published psychoacoustic studies and one electrophysiologic study. The studies were selected as those thought to be diagnostic of mammalian binaural circuitry.
Results: The model replicated several response characteristics of normal-hearing human listeners, such as ITD- and ILD-based lateralization. However, some discrepancies were evident for experiments believed to be diagnostic of binaural circuitry. For instance, the model did not lateralize artificially enlarged ITDs that can be reliably lateralized by humans. And the models localization of 2 and 3 kHz pure tones matches their true location, whereas humans have a systematic center bias when localizing such tones.
Conclusions: The discrepancies between human and model localization are consistent with the idea that partially hard-wired subcortical circuits for binaural processing place constraints on human sound localization. In the absence of these explicit circuits a model optimized for localization appears to rely on cues that are not identical to those employed by humans. The architecture-dependent ability to generate some human-like response characteristics offers a window to study prerequisites of the neural circuitry as well as the potential and limitations of the cortex to decode sound localization information from binaural signals.

SU192. To Glimpse or Not to Glimpse: Behavioral and Neuroimaging Evidence for Binaural Glimpsing

Jörg Encke*[1], Hamish Innes-Brown[2], Heivet Hernandez-Perez[1], David McAlpine[1]

[1]*Macquarie University,* [2]*Eriksholm Research Centre*

Category: Binaural Hearing & Sound Localization
Background: In everyday life, we frequently encounter complex acoustic environments filled with echoes and reverberations. To focus on a single speaker within a crowd, we must separate their voice from the mix of concurrent sources and their reflections. This superposition of sounds from various directions creates fluctuations in binaural cues, making them unreliable for spatial perception. This unreliability can be quantified using interaural coherence (IAC). It has been proposed that the brain employs a "glimpsing" strategy to address this challenge. By focusing on binaural cues during rising segments of the sound envelope, the influence of delayed reflections might be minimized. Additionally, the brain could monitor IAC and extract sound location information only when IAC - and thus cue reliability - is high.
Methods: To investigate the extent of binaural glimpsing, we designed a stimulus that allowed for systematic modulation of IAC, both temporally and spectrally. Brief segments of high stimulus coherence were embedded within different phases of a modulation cycle to assess whether the brain utilizes binaural glimpsing strategies beyond the previously reported onset weighting for sound localization. To explore the underlying mechanisms, we employed Functional Near-Infrared Spectroscopy (fNIRS) to record hemodynamic responses from the Planum Temporale, a brain region known to be modulated by changes in IAC.

Results: In the behavioural experiment, we utilized ITD thresholds as a metric of binaural performance. Employing the stimulus with modulated IAC, we observed significantly lower ITD thresholds when a brief segment of high IAC was positioned in the early phases of the modulation cycle compared to the peak or later phases. Our fNIRS results, while still preliminary, replicated previous findings demonstrating elevated hemodynamic activity for stimuli with low IAC compared to those with high IAC. Interestingly, we found comparable activity for stimuli with brief sections of high coherence during both the rising and falling segments of a modulation cycle, despite ITD thresholds for these two stimuli differing by an order of magnitude.
Conclusions: Our results indicate that binaural cues primarily contribute to source localization during the stimulus onset. Binaural cues presented in later epochs of a modulated sound waveform appear to be largely disregarded, even when IAC is high and broadband. This suggests that IAC glimpsing beyond the onset may not play a significant role in sound localization. Our preliminary fNIRS findings further imply that the extraction of these onset cues occurs within the cortex, rather than at the brainstem level as previously suggested.

SU193. Evaluating the Effectiveness of Short Interpulse Interval (SIPI) Stimulation in Multichannel Cochlear Implants

Yibo Fan*[1], Demi Wu[1], Rene Gifford[2]

[1]*Vanderbilt University,* [2]*Vanderbilt University Medical Center*

Category: Binaural Hearing & Sound Localization
Background: Continuous Interleaved Sampling (CIS) improved cochlear implant (CI) outcomes by encoding speech with rapid, non-overlapping pulses across multiple electrode channels. However, CIS fails to preserve temporal fine structure and precise interaural timing information, limiting auditory perception in complex listening environments and reducing sound localization abilities. Very low pulse rates have been shown to enhance sensitivity to interaural time delays (ITDs) by introducing non-spectral pitch cues (i.e. rate-based pitch) for CI patients, but high pulse rates are needed for better sound quality and speech understanding. To overcome this speech-rate dilemma, Srinivasan et al. (2018) proposed inserting additional short interpulse intervals (SIPIs) into a high-rate pulse train, adding a layer of low-rate stimulation. This SIPI method showed promising results in single-electrode systems. We extended this concept to operate on a multielectrode system, delivering both low- and high-rate pulses on all active electrodes. To validate its effectiveness, we compared participants' ability to utilize bilateral hearing by assessing their performance in sound localization and speech recognition tasks using both CIS and SIPI stimulation strategies.
Methods: Our testing comprises two components: a left-right discrimination task and a Binaural Intelligibility Level Difference (BILD) task using spondees. In the left-right discrimination task, we use narrow-band noise centered at 250 Hz and 1000 Hz as stimuli, with interaural time differences of 125, 250, 375, or 500 μs imposed on one ear. Participants are asked to determine the direction from which the signal originates, and we compare their correct response rates under both CIS and SIPI stimulation strategies. In the BILD task, spondees serve as the signal while broadband noise is used for masking; the signal and background noise are either synchronized across both ears (N_0S_0) or a $\pm\pi$ interaural phase difference is imposed on the signal in one ear

($N_0S\pi$). We employ a one-up two-down adaptive protocol to determine the signal-to-noise ratio (SNR) at which participants achieve a 70.7% correct recognition rate. Each participant completes two trials under each condition (N_0S_0 and $N_0S\pi$) using both CIS and SIPI. We then average the results and calculate the additional gain participants obtain when switching from N_0S_0 to $N_0S\pi$, comparing these outcomes between CIS and SIPI.
Results: Data collection is ongoing, with plans to include at least 10 participants. Data will be analyzed using a within-subject repeated-measures design. We aim to determine the effectiveness of the SIPI method in enhancing binaural cue sensitivity for ITDs and the resultant interaural phase-related release from masking.
Conclusions: Deploying the SIPI method on a multichannel system can provide valuable insights into whether this stimulation strategy can be implemented in clinical devices to help patients achieve better performance. We will discuss outcomes as relevant to both stimulation strategy and potential for clinical application in current and future CI systems.

SU194. A Transient Memory Lapse in Humans Less Than One Hour After Training on a Sound-Localization Task

Allison A. May[1], Hannah R. Rostollan[1], Beverly A. Wright*[1]

[1]*Northwestern University*

Category: Binaural Hearing & Sound Localization
Background: During learning, memories are transformed to a stable state through a post-training process of consolidation. Work in non-human species indicates that this process involves multiple phases with distinct time courses and molecular requirements. This work also indicates that the transitions between memory phases are commonly associated with transient memory lapses. Such lapses have been reported only rarely in humans and never for perceptual learning. However, we recently observed a transient memory lapse in humans after training on interaural-level-difference (ILD) discrimination at 4 kHz. Performance worsened within a testing session 1-3 h after training, but not before or after that time point, resembling a lapse pattern documented in non-human species. Here we provide additional, preliminary, evidence for transient memory lapses in ILD discrimination, this time at 0.5 kHz.
Methods: We trained normal-hearing young adults (n=4-16/group) for 300 trials on ILD discrimination at 0.5 kHz--either (nominally) solely or immediately following equal training on ITD discrimination at 0.5 kHz—and tested different subgroups on ILD discrimination at 0.5 kHz either 30 minutes or 24 hours after training. Note that for this preliminary examination, the 24-h subgroup who were nominally trained only on ILD discrimination at 0.5 kHz were also trained on ILD discrimination at 4 kHz immediately thereafter. Though not ideal, we have reasons to think that the additional training did not affect the outcome.
Results: When training was on ILD discrimination alone: (1) during training, performance improved (2) 30’ after training, performance initially was better than ever, but then worsened, returning to naïve values, and (3) 24 h after training, performance returned to near the best value achieved 30’ after training and remained consistent. When ITD discrimination was trained immediately before ILD discrimination: (1) during training, performance on ILD discrimination initially was notably better than in naïve listeners, but then worsened, (2) 30’ after training,

performance remained consistently near the worst values from the previous session, and (3) 24 h after training, performance returned to the best values observed earlier and remained consistent. **Conclusions:** The results provide additional, preliminary, evidence of transient memory lapses in human perceptual learning. The overall lapse pattern for ILD discrimination at 0.5 kHz, here, resembled our previous observations in humans for ILD discrimination at 4 kHz. However, the lapse occurred LESS THAN 1 h after training at 0.5 kHz, but 1-3 h after training at 4 kHz, suggesting that the lapse kinetics can be stimulus specific for the same task. In addition, training on ITD discrimination at 0.5 kHz aided performance on ILD discrimination at 0.5 kHz, documenting generalization across localization cues at the same frequency. In sum, it appears that the consolidation of perceptual learning involves transient memory lapses that demarcate separate memory phases.

SU195. Direct Connectivity Between the Inferior Colliculus and the Lateral Vestibular Nucleus in the Integration of Auditory and Vestibular Systems

Sunghee Kang*[1], Youngrae Ji[1], Gunsoo Kim[1]

[1]*Korea Brain Research Institute*

Category: Binaural Hearing & Sound Localization
Background: The vestibular system plays a crucial role in balance, posture, and head movement. For the body to maintain stability, the vestibular system needs to operate in conjunction with other sensory systems such as vision and proprioception. However, when this delicate balance is disrupted, neurological disorders can occur, leading to symptoms affecting both hearing (auditory) and balance (vestibular). Although these two systems share similar sensory mechanisms, their interactions in the brain remain unclear. There is no research on the ascending pathway, so here hypothesized that the vestibular nucleus (VN) interacts with the interior colliculus (IC) which integrates sound signals and reacts to sound.
Methods: 8-week-old male C57BL6/J mice were used in this study. We utilized CTB-Alexa568 or AAV administration. Mice were under ketamine/xylazine anesthesia. A headpost was implanted prior to recording. To investigate the neural physiological recording of the IC, two small cranial windows were made over the IC and the LVN for neural recording and optogenetic stimulation, respectively. The neural recording was made using a 6-channel tungsten electrode array (~5MΩ, FHC). We conducted sound response in the LNV. All mice were placed in a sound booth, and the speaker was located approximately 6 inches far from one side of the mouse's ear. Sound exposure began with 1 hour silencing time, then 70 dB of sound (4,8,16 and 32 kHz) was played for 2 hours. Subsequently, whole brains were harvested to assess c-fos expression.
Results: When CTB-Alexa568 and AAVreg were injected into the IC, labeling was specifically observed in the lateral vestibular nucleus (LVN), but not in other parts of the VN, indicating direct neuronal connectivity between them. Anterograde virus tracing into the LVN was primarily revealed in the ventral medial region of the IC. We identified areas sending input signals to the LVN using the AAVreg, which showed labeling in the lobe IX of the cerebellum, and cochlea nucleus. Additionally, we analyzed neuronal activity in the IC by providing optogenetic stimulation to the LVN during recording. The results indicated a strong modulation in the firing rate of IC neurons with short latency. To determine whether the LVN responds to sound c-fos expression was detected following sound stimulation at 4, 8, and 16 kHz.

Conclusions: This study is the first to suggest a neuronal connection between the IC and the LVN. And IC might receive the motor-related input from the LVN through the lobe IX of the cerebellum. Based on the c-fos expression from the sound exposure experiment, the LVN may influence not only balance control but also auditory sound processing. These findings provide new insights into the integration of auditory and vestibular functions, potentially enhancing our understanding of neurological disorders that involve both hearing and balance symptoms.

SU196. Pcp Auto Count: An Imagej Plug-In Developed Using Ai (Actual Intelligence) for Automated Cell Counting and Measurement of Planar Cell Polarity

Kendra Stansak[1], Luke Baum[1], Sumana Ghosh[1], Punam Thapa[1], Brad Walters*[1]

[1]*University of Mississippi Medical Center*

Category: Other

Background: Numerous investigators working in the fields of hearing and balance rely on micrographic images and the quantification of cell numbers and cell orientations to uncover various aspects of development, aging, function, and pathological processes. These studies typically require multiple investigators to ensure blinding, and often consume large amounts of time and effort to complete. To facilitate cell counting and the measuring of planar cell polarity, we have created an ImageJ plugin with a graphical user interface that works well with a number of staining procedures and sample types including phalloidin labeled stereocilia and β-Spectrin immunolabeled cochlear and vestibular hair cells. The plug-in also offers some rudimentary analyses such as the calculation of descriptive circular statistics and a function for graphing rose/windmill diagrams.

Methods: An ImageJ plug-in was coded to analyze binary images and identify cells or “chunks” of white pixels that contain “caves” of infiltrated black pixels. Angles of cell orientation are then measured by generating vectors connecting the centers of mass of each chunk and its cave. Cochlea (P4) and utricles (E17.5) from wildtype mice were immunostained for β-Spectrin and/or labeled with fluorescently conjugated phalloidin. Samples were then imaged on a confocal microscope and made binary in ImageJ. For E17.5 utricle images, an investigator manually measured hair cell numbers and polarity. A separate investigator ran the PCP Auto Count (PCPA) plug-in on binary images created from the same sampling boxes and measurements were compared. For P4 cochlear images, two investigators independently preprocessed the images then ran PCPA. Angle measurements from both investigators were evaluated by a third investigator who assigned each cell a score from 1-5 reflecting angle measurement accuracy, where 1 = perfect measurement, 2 = LESS THAN 10° incorrect, 3 = 11-40° incorrect, 4 = 41-90° incorrect, and 5 = 91-180°. PCPA was also tested against a variety of images copied from publications examining PCP in various tissues and across various species.

Results: PCP Auto Count achieved GREATER THAN 99% accuracy in counts of P4 cochlear hair cells. For sample boxes from E17.5 utricles PCPA and manual angle measures had a concordance correlation coefficient of 0.999, and Bland-Altman limits of agreement (95% CI) ranged from -7.8 to +8.8 degrees with minimal bias (0.48 degrees). PCPA angle measurements from mouse cochlea samples using beta-spectrin labeling had GREATER THAN 98% accuracy, though measures using phalloidin staining were slightly less accurate, returning largely similar

mean angle measures, but with slightly increased variability. Analyses of murine radial glia, ependymal cells, and Drosophila ommatidia demonstrated capability of PCPA across various tissues and species as well as the ability to detect the effects of known PCP altering mutations.
Conclusions: These results have been published at https://doi.org/10.3389/fcell.2024.1394031 and the plug-in can be imported from https://sites.imagej.net/PCP-Auto-Count/

SU197. Computer Vision Based Audiogram Symbol Detection

Ruoyu Yang*[1], Dana Mae Salvador[2], Carl Ehrett[1], Peter Dixon[2]

[1]*Clemson University,* [2]*Medical University of South Carolina*

Category: Other
Background: A principal limitation to the use of real-world data stored in the electronic health record (EHR) for hearing health services research is the inability to definitively identify hearing loss and quantify its severity. In this work, a contour-based computer vision (CV) method has been developed to extract structured data from scanned, hand-drawn audiograms stored as media files in the EHR of a tertiary academic health network.
Methods: The pipeline can be divided into three steps: (1) Image cropping to segment the pure tone threshold plot, (2) Pattern detection: Image preprocessing methods, including grayscale conversion and contrast enhancement, are applied to the cropped image to facilitate better feature extraction during the subsequent pattern contour identification. Once the image is preprocessed, the system detects all relevant pattern contours within the audiogram. The identification of these contours allows for the calculation of their center points, which represent the key data points necessary for further analysis. (3) Pattern coordinate calibration: the algorithm translates the detected pattern center points from the pixel coordinate system to the audiogram coordinate system based on the accurate axis label detection algorithm. This calibration is essential for diagnostic purposes, as it aligns the detected points with the audiogram's axis labels, thereby contextualizing the data within the standard hearing sensitivity framework.
Results: To validate the effectiveness of this CV-based audiogram pattern detection algorithm, the mean absolute error (MAE) is applied to compare the difference between ground truth and CV-generated symbol x and y coordinate values. Tests on 30 audiograms yielded MAEs of 136 and 1.3 for the x (frequency) and y (threshold) coordinates, respectively, within value ranges of 16,000 (x) and 130 (y). The results demonstrate that the coordinate values for most patterns matched well with ground truth values.
Conclusions: In future work, we aim to integrate deep learning algorithms, such as convolutional neural networks (CNNs), to enable pattern classification tasks, which can further optimize and expand the capabilities of the system. Further, a pipeline will be developed for real-time abstraction of tabular data from audiogram image files within the EHR. By leveraging the power of computer vision and deep learning, this integration process will facilitate automated language summaries of audiograms, real-time comparison with prior audiogram results, and a host of hearing health services use applications.

Mini-Podium 1: Otitis Media: Imaging, Immunity and Innovative Therapy

Moderators: Arwa Kurabi and Peter Santa Maria

3:00 p.m. - 4:00 p.m.

Ocean Ballroom 9 - 12

A 3D and Explainable Artificial Intelligence Model for Evaluation of Chronic Otitis Media Based on Temporal Bone Computed Tomography: Model Development, Validation, and Clinical Application

Binjun Chen*[1], Fanglu Chi[2], Yike Li[3], Dongdong Ren[2]

[1]*Eye and ENT Hospital, Fudan Univeristy,* [2]*ENT Institute,Eye and ENT Hospital, Fudan University,* [3]*Vanderbilt University Medical Center*

Background: Temporal bone computed tomography (CT) helps diagnose chronic otitis media (COM). However, its interpretation requires training and expertise. Artificial intelligence (AI) can help clinicians evaluate COM through CT scans, but existing models lack transparency and may not fully leverage multidimensional diagnostic information.

Methods: Temporal bone CT scans were retrospectively obtained from patients operated for COM between December 2015 and July 2021 at 2 independent institutes. A region of interest encompassing the middle ear was automatically segmented, and 3D CNNs were subsequently trained to identify pathological ears and cholesteatoma. An ablation study was performed to refine model architecture. Benchmark tests were conducted against a baseline 2D model and 7 clinical experts. Model performance was measured through cross-validation and external validation. Heat maps, generated using Gradient-Weighted Class Activation Mapping, were used to highlight critical decision-making regions. Finally, the AI system was assessed with a prospective cohort to aid clinicians in preoperative COM assessment.

Results: Internal and external data sets contained 1661 and 108 patients (3153 and 211 eligible ears), respectively. The 3D model exhibited decent performance with mean areas under the receiver operating characteristic curves of 0.96 (SD 0.01) and 0.93 (SD 0.01), and mean accuracies of 0.878 (SD 0.017) and 0.843 (SD 0.015), respectively, for detecting pathological ears on the 2 data sets. Similar outcomes were observed for cholesteatoma identification (mean area under the receiver operating characteristic curve 0.85, SD 0.03 and 0.83, SD 0.05; mean accuracies 0.783, SD 0.04 and 0.813, SD 0.033, respectively). The proposed 3D model achieved a commendable balance between performance and network size relative to alternative models. It significantly outperformed the 2D approach in detecting COM ($P \leq .05$) and exhibited a substantial gain in identifying cholesteatoma
(P LESS THAN .001). The model also demonstrated superior diagnostic capabilities over resident fellows and the attending otologist (P LESS THAN .05), rivaling all senior clinicians in both tasks. The generated heat maps properly highlighted the middle ear and mastoid regions, aligning with human knowledge in interpreting temporal bone CT. The resulting AI system achieved an accuracy of 81.8% in generating preoperative diagnoses for 121 patients and contributed to clinical decision-making in 90.1% cases.

Conclusions: We present a 3D CNN model trained to detect pathological changes and identify cholesteatoma via temporal bone CT scans. In both tasks, this model significantly outperforms the baseline 2D approach, achieving levels comparable with or surpassing those of human

experts. The model also exhibits decent generalizability and enhanced comprehensibility. This AI system facilitates automatic COM assessment and shows promising viability in real-world clinical settings. These findings underscore AI's potential as a valuable aid for clinicians in COM evaluation.

A Multispectral Approach to Reducing Recurrence in Cholesteatoma Surgery: A Pilot Feasibility Study

Roy Park*[1], Mark Nyaeme[1], Daniel Penaranda[1], Iram Ahmad[1], Tulio Valdez[1]

[1]*Stanford*

Background: Cholesteatoma is a benign, aggressive disease of the middle ear that the can be associated with multiple co-morbidities, requiring surgery for removal. Recurrence rates following cholesteatoma surgery can range up to 50%, and incomplete removal of cholesteatoma intraoperatively will result in recurrence. The current gold standard for identifying residual nests of cholesteatoma relies on white light in the visible spectrum, which can be difficult to distinguish cholesteatoma from surrounding blood, nerves, and bone. Recent research with in-vitro cholesteatoma samples have demonstrated the feasibility of utilizing autoflourescence properties to successfully distinguish between cholesteatoma and mucosa. We present the results of the first pilot study using autoflourescence to successfully identify cholestestoma intraoperatively. Similarly, we report the findings of a multispectral wavelength approach to cholesteatoma identification via short wave infrared wavelengths (SWIR, 1000 nm+) to distinguish cholesteatoma from surrounding bone and mucosa.
Methods: We developed a custom macroscopic imaging system with an articulating arm capable of measuring autoflourescence using bandpassed 470 nm LEDs as excitation sources. Emission signals were captured between 500 and 550 nm for autoflourescence at an exposure time of 50 ms. For the multispectral analysis of tissues in the SWIR, an InGaAs sensor based camera (CRED-2, First Light Vision) was utilized along with wavelenghts at 1050, 1200, 1300, and 1550 to analyze cholesteatoma, mucosa, and bone samples intraoperatively.
Results: A total of 4 patients were enrolled in the pilot study for autoflourescence imaging. The custom imaging system was able to successfully identify cholesteatoma compared with visible light. The flourescence ratio of cholesteatoma from surrounding bone and mucosa was 4.5 to 1 (cholesteatoma to surrounding tissues). Contrastingly, the intensity ratio of cholesteatoma to surrounding tissues under visible broadband white light was only 1.2. On a multispectral analysis of tissues in the SWIR, cholesteatoma was seen to have significant reflective properties at 1050 nm, 1200 nm, and 1300 nm compared to mucosa, bone, and blood. This allowed successful identification of cholesteatoma at alternative wavelengths that could increase the specificity and sensitivity of current methods.
Conclusions: In our intraoperative pilot study, cholesteatoma was successfully identified from surrounding bone and mucosa via autoflourescence and was vastly superior to white light identification. Similarly, several SWIR wavelengths that could visualize cholesteatoma from surrounding structures were identified. Combining these wavelengths in a multispectral guided approach will increase the sensitivity and specificity of cholesteatoma identification compared to the current gold standard.

Innate Immune Gene Expression and Regulation by Epithelial Cell Types during Otitis Media

Allen F. Ryan*[1], Arwa Kurabi[1], Nathan Zemkle[1]

[1]*University of California, San Diego*

Background: The epithelial cells lining the middle ear (ME) cavity form the first line of defense against infection during otitis media (OM). Mediated by innate immune receptors, the epithelium marshals a robust response to bacteria that includes hyperplasia, the secretion of mucus and antimicrobial molecules, and the recruitment of leukocytes. The purpose of this project was to assess the contributions of the various ME epithelial cell types as reflected in the expression of innate immune receptor, adaptor and effector genes by individual cells. In addition, the regulation of genes by changes inaccessible DNA was also assessed.
Methods: . OM was induced in mice by the transbullar injection of nontypeable Haemophilus influenzae (NTHi). The contents of the ME bulla were harvested before and at various times after infedtion. Single-cell RNA-seq was performed, as was single-nuclear RNA-Seq/ATAC-Sec.
Results: Major categories of innate immune genes expressed by all epithelial cells in the resting ME encoded receptors, chemotactic factors, antimicrobial peptides and mucins. Expression was dramatically increased for receptors, chemokines and cytokines after infection, while antimicrobial and mucin expression remained relatively constant. Only Tlr2 was highly expressed in the resting ME, while Tlr2, Tlr3 and Tlr4 were expressed after infection. However, Tlr2 and Tlr3 expression waslimited to secretory cells. The set of chemokines expressed were distinct in non-secretory cells as compared to secretory, basal and ciliated cells. As expected, secretory cells expressed more mucin genes than non-secretory, but non-secretory cells expressed more Defb1. Innate immune effector genes upregulated in epithelial cells included Hbegf (mucosal hyperplasia), Fut2 (mucus assembly) and Muc5b. ATAC, focused on the region adjacent to the expressed sequence, showed NTHi-induced changes in DNA accessible regions for HBegf and Fut2, but not for Muc5b.
Conclusions: Innate immunity is distributed across all epithelial cell types, with different cells peforming different functions. Epithelial cell types can be distinguished by differences in innate immune gene expression, but there are more similarities than differences. Accessible DNA changes in innate immune genes during OM can reveal transcriptional control for epithelial cells.

Exploiting Bacterial Nutritional Dependence: A Novel Therapy to Prevent Middle Ear Infections

Brianna Atto*[1], Caitlyn Granland[2], Jack Pepper[2], Stephen Tristram[1], Robyn Marsh[3], Ruth Thornton[2], Lea-Ann Kirkham[2]

[1]*School of Health Sciences, University of Tasmania,* [2]*Wesfarmers Centre of Vaccines and Infectious Diseases, The Kids Research Institute Australia, Perth, WA, Australia,* [3]*Child and Maternal Health Division, Menzies School of Health Research, Charles Darwin University, Darwin, NT, Australia*

Background: Middle ear infection (otitis media; OM) is an important disease in early childhood- it is one of the most common reasons for healthcare visits and antibiotic prescription, and the leading cause of conductive hearing loss(1). Globally, there are ~709 million cases every year, with Australian Indigenous populations experiencing the highest rates and severity of OM(2). The bacterium nontypeable Haemophilus influenzae (NTHi) accounts for most cases, and OM caused by this bacterium is associated with higher disease severity, antibiotic treatment failure, recurrence, persistence and need for repeat surgery, compared to other OM pathogens(3, 4). There are currently no effective vaccines, and antibiotics have limited efficacy in clearing NTHi or preventing re-infection.
Our laboratory has discovered and characterised a novel protein (RL-2020) produced by a healthy throat bacterium which specifically inhibits the growth of NTHi through starvation of the critical nutrient, haem. We have previously demonstrated that RL-2020-producing strains or purified RL-2020 alone is protective against NTHi infection in respiratory cell culture models. We therefore aimed to determine if live RL-2020-producing bacteria (probiotic), or RL-2020 alone (protein) could prevent NTHi-associated OM in mice.

Methods: A world-first influenza virus-driven OM ascension model (5) using BALB-cJ mice was used to mimic in vivo infection whereby OM is preceded by exposure to a respiratory virus. The probiotic (or ST-2020 knockout, or saline), or protein (or saline) was delivered to mice intranasally (n=18/group), followed by intranasal MEM71 Influenza virus the next day, and intranasal NTHi challenge three days later. At 48 and 96 hours post NTHi challenge, mice were culled and nasal washes, middle ear tissue and lung tissue were collected for NTHi colony counts and cytokine analysis.
Results: Mice pre-treated with the probiotic had significantly lower densities of NTHi in the nose (log10 0.96- 3.34 CFU/mL, p LESS THAN 0.0002) and none (0/18) developed OM, compared with the RL-2020 knockout (IQR, log10 4.20-5.20 CFU/mL; 12/18 OM) or saline controls (IQR, log10 3.4- 4.74 CFU/mL; 15/18 OM) at 48- and 96-hours post NTHi challenge. Conversely, the protein therapy significantly reduced NTHi loads in the nose and middle ear at 96-hours post NTHi challenge only, with 8/18 developing OM, compared to 18/18 in the placebo group. Mice receiving bacterial preparations (probiotic or hpl- knockout) had lower pro-inflammatory cytokine concentrations in the nose and middle ear, compared to the protein or saline groups.
Conclusions: The probiotic demonstrates exceptional clinical utility in preventing OM by reducing NTHi nasal colonization (the first step of infection) through a combination of ST-2020 and immune-modulating mechanisms. The protein may have a role in reducing infection/colonization duration but further work on dosing is required to determine effectiveness in preventing OM.

Special Session 2: Making History: Celebrating Black Scientists in Otolaryngology

Chair: Jeffrey Cheng*, Massachusetts Eye and Ear / Harvard Medical School*
Co-chair: Melissa McGovern*, University of Pittsburgh School of Medicine*

3:00 p.m. - 5:00 p.m.

Ocean Ballroom 1 - 4

Making History: Celebrating Black Scientists in Otolaryngology

Melanie Barzik[1], Jeffrey Cheng[2], Melissa McGovern[3], Michele Insanally[3], Ilkem Sevgili[4], Ambroise Wonkam[5], Brandie Verdone[6], Nikolas Francis[7], Andrea McQuate[8]

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Making History: Celebrating Black Scientists in Otolaryngology

Tejbeer Kaur[1], Michele Insanally[2]

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Making History: Celebrating Black Scientists in Otolaryngology

Ilkem Sevgili[1]

[1]*Harvard Medical School*

Visuo-Vestibular Interactions During Gaze Control in Mice: Implications for Successful Navigation

Brandie Verdone[1]

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Individual Abstract: Animals rely on visual and nonvisual cues to form a spatial representation of their environment, a necessity for successful navigation. Monitoring spatial cues allows for rapid updating of an animal's orientation by estimating the linear and angular displacement of the head. The mouse uses heading to traverse a terrain in order to track prey and evade predators; combined with their lateral eye placement and binocular overlap, the mouse has frontal, lateral, and overhead surveillance capabilities during navigation. The vestibulo-ocular reflex (VOR) ensures stable gaze (defined as the combined position of the head-in-space and eye-in-orbit) during navigation by eliciting eye movements counter to head movements. This is not unlike strategies evoked in primates. However, a prevailing view is that mice rely on the VOR to actively redirect their gaze towards a target, in contrast to active eye movement strategies employed by foveate vertebrate species (i.e. primates). Using a novel active behavioral paradigm combined with head and eye position tracking, we suggest that mice can in fact actively redirect their gaze using mechanisms beyond vestibular reflexes, in line with strategies employed across foveate and afoveate vertebrates. Further, we employ neuronal recording strategies to understand the activity of brainstem nuclei known to relay vestibular information to relevant networks. Specifically, head-eye tracking while recording from the nucleus prepositus hypoglossi (NPH) in mice, a region thought to convey vestibular-relating heading information, sheds insight into the

role of the NPH in conveying eye-movement information. This is significant, as the NPH is a known oculomotor integrator essential for voluntary (active saccades) and involuntary (reflexive) eye movements across various species, including primates. These studies provide insight into the visuo-vestibular dynamics of mouse gaze strategies, furthering our knowledge of gaze interpretation in the rodent brain and its convergence with primate strategies.

Investigating the Neurophysiology of Listening

Nikolas Francis[1]

[1]*University of Maryland*

Individual Abstract: As we celebrate Black History Month, the Francis lab strives toward creating a supportive research environment where lab members embrace racial diversity and the inclusion of underrepresented minorities in science. Our lab culture is informed by my experience as a Black and Hispanic American scientist, and our research aims to understand how humans and other animals listen to sound. While "hearing" pertains to our acoustic sensitivity, it remains unclear how the central nervous system (CNS) enables "listening," i.e., how we perceive, remember, and attend to sound. To investigate the neurophysiology of listening, my research over the past 20 years has examined how auditory task performance affects CNS activity in humans, ferrets, and mice.

My graduate research with Dr. John J. Guinan Jr. in the Speech and Hearing Bioscience and Technology (SHBT) program at MIT/Havard Medical School used otoacoustic emissions to study medial olivocochlear (MOC) efferent control of human cochlear responses to sound. MOC activity is thought to help us hear in noisy environments and enable attentional control of cochlear amplification. After receiving my Ph.D., I continued to study the neurophysiology of listening as a postdoctoral researcher at the University of Maryland (UMD), first working as a fellow in the Center for Comparative and Evolutionary Biology of Hearing with Drs. Shihab Shamma and Jonathan Fritz, and subsequently completing my postdoctoral work with Dr. Patrick Kanold. We used 2-photon imaging and multi-channel electrophysiology in the auditory and prefrontal cortices of awake-behaving animals to study the neural basis of auditory attention and decision-making. Our experiments described how neuronal networks encode auditory and task-relevant information.

Today, the Francis lab at UMD investigates the neural mechanisms underlying pitch perception, sensory-guided decision-making, and predictive coding of sensory events in mice. We are also studying how psychedelic drugs affect the neural coding of sound in mouse auditory cortex. Our lab's first peer-reviewed publication described how psilocybin treatment affects intrinsic versus stimulus-driven cortical activity. Going forward, research in the Francis lab will combine auditory task performance in mice with neural imaging, electrophysiology, optogenetics, and pharmacology to clarify how cortical micro-circuits produce auditory perception and cognition.

The Spaghetti and Bean Journey: How I Began Studying Mitochondria in Zebrafish Mechanosensory Hair Cells

Andrea McQuate[1]

[1]*University of New Mexico*

Individual Abstract: I come from a prestigious legacy of scientists and scholars who prevailed under conditions of unimaginable adversity. It is an honor to acknowledge the great minds that have come before me. Yet, I am glad that I found my way to research on my own terms, in a meandering route that involved a passion for writing fiction. My recent assistant professorship at the University of New Mexico serves as both gratitude for the past and a force towards a better future in the biomedical sciences for all walks of life, as I strive to carry that great legacy forward. In my lab, we study the relationship between mechanosensory hair cells and their mitochondria. There are over 30 mutations in mitochondrial genes associated with hearing loss, and mitochondria are implicated in multiple routes of hair cell death, including noise overexposure and aging. However, little is known about the basic aspects of hair cell mitochondrial biology. Using the zebrafish lateral line as a model for inner ear hair cells, and a combination of serial block-face scanning electron microscopy, confocal microscopy, and electrophysiology, we seek to determine how mitochondria form specific morphologies to influence hair cell physiology, and to provide unique insights into “mitochondrial deafness.” We are also committed to creating an inclusive and equitable space where all individuals can thrive.

Africa is the Next Frontier for Hearing Loss Novel Genes Discovery

Ambroise Wonkam[1]

[1]*Johns Hopkins University School of Medicine*

Individual Abstract: Hearing impairment (HI) is the most common sensory disability, affecting individuals in both high-income countries (about one per thousand live births) and sub-Saharan African countries (up to six per thousand). Early detection of HI in children is critical for improving language acquisition, learning, psychosocial adjustment, and parenting. Extending early detection to include molecular diagnosis can inform genetic counseling, clinical guidance, and therapeutic interventions. In Africa, about 30 to 50 out of every 100 cases of childhood HI are believed to have genetic origins. However, there is a significant knowledge gap in the genomics of childhood HI in 41 out of 54 African countries, largely due to a lack of genomic workforce and funding opportunities.

Among genetic cases of childhood HI, approximately 80% are non-syndromic HI (NSHI). Variants in the GJB2 gene, which are major contributors to NSHI in European and Asian children, are infrequent in most sub-Saharan African populations, indicating that other genomic markers for HI remain to be identified. Notable exceptions include a GJB2 pathogenic variant (p.Arg143Trp) found in one-fourth of familial NSHI cases in Ghana, and another GJB2 variant (p.Arg32Cys) in Senegal. The Ghanaian variant, appearing in a single ancestor about ten thousand years ago (thus named the "Ghanaian founder variant"), has also been reported in NSHI cases in Senegal and Mali. This variant is predominantly found among African and Latin American alleles in the All of Us database, along with the Senegalese variant, which likely migrated regionally within West Africa and trans-continentally during the transatlantic slave trade.

To date, around 120 genes have been associated with NSHI. We expanded this list by analyzing whole-exome sequencing (WES) data from 51 multiplex Ghanaian families without pathogenic GJB2 variants. This analysis revealed variants in 20 known genes (40 out of 51 families, 78%) and identified seven novel genes (14%; INPP4B, CCDC141, MYO19, DNAH11, POTEI, SOX9, and PAX8). Similar trends were observed in WES analyses of families from Cameroon, Mali, South Africa, Senegal, and Rwanda, suggesting that Africa is the next frontier for discovering novel HI genes.

Investing in genomics research on HI in African populations will 1) increase the number of new HI genes discovered and advance our understanding of HI pathobiology, 2) improve equity in genomic diagnosis, and 3) ultimately promote equity in the implementation of genomic medicine for all populations in the US, Africa, and globally.

Podium 6: Decoding Speech Perception: Insights From Neural, Behavioral, and Technological Perspectives

Moderators: Joseph Luetkehans and Rose Rizzi

3:00 p.m. - 5:00 p.m.

Ocean Ballroom 5 - 8

Leveraging AI to Improve Speech Perception With Hearing Devices: Multiple Microphones and Speaker Selection Enhance Performance in Realistic Noisy Situations

Tobias Goehring*[1], Iordanis Thoidis[2], Clement Gaultier[3]

[1]*Cambridge Hearing Group, University of Cambridge,* [2]*School of Electrical and Computer Engineering, Aristotle University of Thessaloniki,* [3]*Center for Research and Innovation on Human Audiology, Institut de l'Audition*

Background: Speech perception is a major challenge for people with hearing devices in noisy environments with several competing speakers, background noise and reverberation. Assistive hearing devices such as hearing aids and cochlear implants often fail to restore speech intelligibility. AI approaches based on deep learning have shown large potential to overcome these limitations and are now being introduced for commercial devices. However, they suffer from limitations, such as the generalisation to realistic acoustic situations and allowing the user to choose a speaker of interest. We developed and evaluated novel AI approaches to leverage multiple microphones in noisy-reverberant scenarios and to select the voice of a target speaker in ambiguous situations with multiple competing speakers.

Methods: Several deep learning algorithms based on recurrent neural networks were developed that used either one or multiple microphones to enhance speech intelligibility in realistic noisy-reverberant situations. We further developed a speaker-informed algorithm to select a specific target speaker out of a mixture of competing speakers. The algorithms were trained to generalise

across a variety of acoustic conditions, and tested on unseen, held-out data during evaluation. The algorithms were first compared using objective computational metrics. Double-blind speech recognition experiments were then conducted with several groups of listeners, including normal-hearing listeners (N=19), hearing-impaired listeners (N=12, PTA average of 47 dB HL), normal-hearing listeners using cochlear implant simulations (N=15) and listeners using cochlear implants (N=12, experienced unilateral users, Advanced Bionics).
Results: For the evaluation of the deep-learning algorithms with access to one or multiple microphones (two unilateral, six bilateral), there were large and statistically significant improvements in speech recognition by 7.4 and 10.3 dB SRT for the multi-microphone algorithms, respectively. These improvements were significantly larger than the improvements obtained with the single-microphone algorithm. For the speaker-informed algorithm there were statistically significant improvements of 17% and 31% in speech perception for people without and with hearing loss, respectively. These improvements were statistically significant also when comparing to an uninformed baseline algorithm in ambiguous situations with multiple speakers. All algorithms showed superior performance in objective metrics over the unprocessed noisy speech while introducing only a short delay of less than 5 ms for the processing.
Conclusions: Deep learning algorithms provided large and significant improvements for speech intelligibility in difficult noisy situations with reverberation or multiple competing speakers. These findings extend previous studies that used deep learning and show the benefits that users of hearing devices, including hearing aids and cochlear implants, could receive in the future. The deep learning algorithms fulfilled the requirements for short processing latency and generalised successfully towards unseen scenarios with different speakers, acoustics and languages. These results show that the limitations of previous deep learning algorithms can be overcome to help with speech perception in challenging situations.

Cochlear Implant Users Improve Speech-on-Speech Perception With Piano Training

Eleanor Harding*[1], Etienne Gaudrain[2], Robert Harris[3], Barbara Tillmann[4], Bert Maat[5], Steven de Rooij[6], Rolien Free[5], Deniz Başkent[5]

[1]*University of Groningen,* [2]*Lyon Neuroscience Research Center, CNRS UMR5292,* [3]*Prince Claus Conservatory, Hanze University of Applied Sciences,* [4]*Laboratory for Research on Learning and Development (LEAD), CNRS UMR5022,* [5]*University of Groningen, University Medical Center Groningen,* [6]*NHL Stenden University of Applied Sciences*

Background: Speech-on-speech perception is especially challenging for cochlear-implant (CI) users. According to neuroscientific literature, speech and music domains have functional overlap in auditory and motor networks. This suggests musical training could benefit speech-on-speech perception, supported by some (but not all) musician/non-musician studies. However, previous musical training approaches with this group reported mixed results and small effect sizes. In particular, neural activity in dorsal-stream networks may benefit speech outcomes for cochlear implant users. We therefore employed a novel piano training method that was designed with the intention to engage dorsal-stream networks by the type of learning and practice techniques. We hypothesized that cochlear implant users would improve speech-on-speech perception after the piano training.

Methods: Twenty-four cochlear implant users were randomized to three groups: piano training (n=8), active control training (n=7) and a no-training control (n= 9). The piano training was designed to stimulate dorsal auditory-motor networks via improvisation exercises and emphasis on learning finger movements instead of learning to read music. The active control training was Minecraft gaming lessons, designed to be as comparable as possible to piano training in features such as lesson duration, interactions with an instructor and student-led goal setting. Each training lasted six months. The no-training control participated in tests only. The primary outcome was a speech-on-speech perception test, the Child-friendly Coordinate Measure Response, which measures perception of target sentences with number and color keywords presented in a single-talker gibberish masker. Multiple secondary outcomes were collected as part of the larger CIMUGAME study and not reported here.
Results: We found that six months of training improved speech-on-speech perception in CI users only for the piano training group, and a Bayesian sequential analysis confirmed that this effect was strong. The training effect was not yet developed after three months of training, and had receded at three-month follow-up. Speech-on-speech was anecdotally improved in the Minecraft group and did not change from baseline in the no-training control group.
Conclusions: We conclude that neuroscience-informed methods of musical training can improve speech-on-speech perception in CI users in at least six months, and continued training is ideal. Moreover, participant feedback indicated that the piano group enjoyed their training more than the Minecraft group, thus motivated engagement with the training may influence outcomes.

Auditory-Motor Entrainment and Listening Experience Shape the Perceptual Learning of Concurrent Speech

Jessica MacLean*[1], Jack Stirn[1], Gavin Bidelman[1]

[1]*Indiana University*

Background: Plasticity from auditory experiences shapes the brain's encoding and perception of sound. Though prior work has shown that neural entrainment (i.e., brain-to-acoustic synchronization) aids speech perception, how long- and short-term plasticity influence entrainment to concurrent speech has not been investigated. Here, we explored neural entrainment mechanisms and interplay between short- and long-term neuroplasticity for rapid auditory perceptual learning of concurrent speech sounds in young, normal-hearing musicians and nonmusicians.
Methods: Participants (n = 27) were separated into musician (n=13) and nonmusician (n=14) groups based on the extent of their formal music training (musicians: GREATER THAN 10 years, nonmusicians: LESS THAN 5 years). Participants learned to identify double-vowel mixtures (/a/ + /e/, /i/ + /e/, /i/ + /a/) during ~45 min training sessions recorded simultaneously with high-density EEG. We examined the degree to which brain responses entrained to the speech-stimulus train (~9 Hz) to investigate whether entrainment to speech just prior to making a behavioral decision predicted task performance. Source and directed functional connectivity analyses of the EEG probed whether behavior was driven by group differences in coupling between auditory and motor cortices.
Results: While both groups showed rapid perceptual learning in accuracy and reaction time with speech training, musicians showed faster behavioral decisions than nonmusicians overall.

Interestingly, listeners' neural entrainment strength prior to target speech mixtures predicted their behavioral identification performance; stronger neural synchronization was observed preceding incorrect compared to correct trial responses. We also found stark hemispheric biases in auditory-motor coupling during speech entrainment, with greater auditory to motor connectivity in the right hemisphere for musicians.
Conclusions: Our findings confirm stronger neuroacoustic synchronization and auditory-motor coupling during speech processing in musicians. Stronger neural entrainment to rapid stimulus trains preceding incorrect behavioral responses supports the notion that alpha-band (~10 Hz) arousal/suppression in brain activity is an important modulator of trial-by-trial success in perceptual processing.

Age-Related Dual-Task Cost of Speech Perception in Quiet and Noise While Walking

Yossi Bugannim*[1], Alon Kalron[1], Liat Kishon-Rabin[1]

[1]*Tel Aviv University*

Background: Everyday tasks often require dual tasking (DT), such as conversing while walking, demanding the integration of cognitive, auditory processing, and motor skills. Older adults (OA) often struggle with such tasks due to age-related declines in these capacities, leading to Motor-Cognitive Interference (MCI). Few studies have examined the connection between speech perception using sentence recognition and walking, and those that did mostly relied on treadmill assessments, limiting ecological validity of gait speed. This study addresses this gap by simulating real-life DT situations, evaluating walking along a predefined path while recognizing words in sentences in quiet (SiQ) and noisy (SiN) conditions using an adaptive test. This method allows for an ecological assessment of how different auditory conditions impact gait and cognitive load.
Methods: A total of 24 healthy adults participated in the study: 12 OA (mean age: 78.9 ± 9.4 years) and 12 younger adults (YA, mean age: 34.9 ± 2.4 years), all within normal hearing ranges for their age. OA had MOCA scores GREATER THAN 23.
Participants completed a series of five tests integrating SiQ and SiN, alongside motor tasks that included walking. These tasks were organized into three single tasks (ST)—SiQ and SiN performed while seated and walking—and two DT tasks, which involved walking while concurrently performing SiQ or SiN. Speech perception was evaluated using the Hebrew Matrix Test (HEBMatrix). Gait metrics were recorded using the APDM Mobility Lab system. Performance was analyzed by comparing raw data and calculating Dual Task Costs (DTCs), expressed as [(DT−ST)/ST] ×100 to represent performance changes. Additionally, all participants completed cognitive assessments, including evaluations of visual attention, working memory, processing speed, and linguistic fluency.

Results: (1) Speech perception showed that YA consistently outperformed OA in both SiQ and SiN. In DT scenarios, while walking, both groups exhibited a decline in performance, with YA demonstrating a 5.5 dBSNR advantage over OA in achieving 50%-word recognition; (2) DTCs increased during walking, with greater DTCs for OA with no significant differences between the groups; (3) OA experienced a reduction in all gait metrics while engaging in SiQ, with a further

decline observed during SiN, and significantly differences between the groups; (4) Lower cognitive scores correlated strongly with decreased gait metrics and poorer speech perception. **Conclusions:** The findings highlight the challenges OA face in DT scenarios, where significant cognitive resources are allocated to maintain motor stability. The cognitive-related motor interference observed in OA highlight the need for targeted habilitation programs.

Attentional Dynamics Drive Narrative Lingering Under Effortful Listening Conditions

Ryan Panela*[1], Björn Herrmann[2], Alexander Barnett[3]

[1]*University of Toronto,* [2]*Rotman Research Institute,* [3]*McGill University*

Background: Investigations into speech-comprehension difficulties often focus on intelligibility of short, disconnected sentences, potentially limiting generalization to real-life listening. Novel approaches to understanding naturalistic speech listening are critical for insight into impaired speech processing and its downstream effects on other cognitive processes. Specifically, recent memory research has demonstrated that experiences linger in our minds, spontaneously returning to thought even after their conclusion. This phenomenon, driven by attention, is likely to persist when situational meaning is extracted rather than low-level semantics; however, this has not been investigated in audition under challenging listening conditions. This research investigates the effects of background noise on psychological lingering of spoken stories.
Methods: Participants (N=26, 18-38 years) listened to three 5-minute stories overlaid with twelve-talker babble in three conditions, ranging from easy to difficult speech intelligibility: clear, +4 dB SNR, and -2 dB SNR. Before and after listening, participants performed a free association task, where they freely typed words for three minutes. Importantly, instructions did not prompt participants to type words related to the narrative. Lingering was assessed using AI-based semantic similarity analytics, by quantifying the extent to which narrative themes and direct narrative words were expressed in participants' pre- and post-story free associations. Speech intelligibility for sentences extracted from other narratives was also assessed as the proportion of correctly heard words.
Results: Speech intelligibility decreased with SNR, as expected. Narrative lingering was observed across noise conditions, with post-story free associations showing an increased semantic similarity compared to pre-story. Across noise conditions, semantic similarity was greater for direct narrative words than thematic words. For the direct words, more challenging listening conditions resulted in a decrease in semantic similarity, while thematic words were more prominent in the -2 dB SNR condition compared to the easier conditions. Moderated by noise condition, semantic similarity logarithmically decreased across successive free association words. Clear speech yielded the greatest semantic similarity at the beginning of the post-story free association, while +4 dB SNR and -2 dB SNR conditions showed a faster decline over time.
Conclusions: Direct narrative words were more prominently represented in free associations than thematic words, indicating that language dictated during listening is more resilient in memory; however, reduced intelligibility caused fewer retained direct narrative words, but a higher reliance on thematic content. This suggests a shift in cognitive focus from precise language to broader themes with additional cognitive load. These findings suggest that background noise not only disrupts immediate comprehension but also the persistence of specific

narrative information in short-term memory, with clearer speech facilitating maintained lingering. These results reveal a link between background noise and auditory memory as additional cognitive load and reduced intelligibility introduced with more challenging noise conditions, may disrupt narrative lingering, leading to faster decay in psychological representations.

Human-Like Feature Attention Emerges in Task-Optimized Models of the Cocktail Party Problem

Ian Griffith*[1], Preston Hess[2], Josh McDermott[2]

[1]*Harvard University,* [2]*Massachusetts Institute of Technology*

Background: Attention enables communication in settings with multiple talkers, allowing us to select sources of interest based on their features. Decades of research have left two gaps in our understanding of feature-based attention. First, humans succeed at attentional selection in some conditions but fail in others, for reasons that remain unclear. Second, neurophysiology experiments implicate multiplicative gains in selective attention, but it remains unclear whether such gains are sufficient to account for real-world attention-driven behavior. To address these gaps, we optimized an artificial neural network with stimulus-computable feature-based gains for the task of recognizing a cued talker's speech, using binaural audio input (a "cocktail party" setting).
Methods: We optimized a deep neural network to report words spoken by a cued talker in a multi-source mixture. Audio was spatialized within simulated reverberant rooms using human head-related transfer functions. Attentional gain was implemented as learnable logistic functions operating on the time-averaged model activations of a cued talker. Gains could be high for features of the cue, and low for uncued features, as determined by parameters optimized to maximize correct recognition. Task performance was measured by word recognition accuracy as a function of target-distractor ratio (SNR) and target-distractor spatial proximity.
Results: The model successfully learned to use both spatial and vocal timbre cues to solve the word recognition task. In the presence of competing talkers the model correctly reported the words of the cued talker and ignored the distractor talker(s). Similar to humans, the model showed higher accuracy with single-talker distractors than with multi-talker distractors. The model's internal representations revealed that attentional selection occurred only at later model stages.
Conclusions: We provide a framework to quantitatively model feature-based auditory attention by optimizing a deep neural network to perform an attentional word recognition task. The model provides hypotheses for how attention might be expected to modulate neural responses at different stages of the auditory system, and can help understand the conditions in which attentional selection is intrinsically difficult.

The Influence of Semantic Context on the Intelligibility Benefit From Speech Glimpses in Younger and Older Adults

Priya Pandey*[1], Björn Herrmann[1]

[1]*Rotman Research Institute*

Background: Speech is often masked by background sound that fluctuates over time. Fluctuations in masker intensity can reveal glimpses of speech that support speech intelligibility, but older adults have frequently been shown to benefit less from speech glimpses than younger adults when listening to sentences. Recent work, however, suggests that older adults may leverage speech glimpses as much, or more, when listening to naturalistic stories. Naturalistic stories are both rich in semantic context and are engaging/motivating. It is unclear which of these two factors is leveraged by older adults to benefit from speech glimpses. The current study directly investigated whether semantic context helps older adults benefit from speech glimpses released by fluctuating (modulated) maskers compared to stationary (unmodulated) maskers more than younger adults.
Methods: In two experiments, we either reduced or extended semantic information of sentence stimuli in modulated and unmodulated speech maskers for younger and older adults. In Experiment 1, participants (36 younger, 33 older) listened to a total of 128 semantically meaningful and semantically non-meaningful sentences in either modulated or unmodulated 12-talker babble maskers at varying signal-to-noise ratios (SNRs: -11, - 8.33, -5.67, -3, -0.33, +2.33, +5 dB, clear). In Experiment 2, context sentences were generated that either provided or did not provide additional context for the 128 target sentences in babble (39 younger, 37 older adults) in modulated and unmodulated babble maskers at different SNRs (-13, - 10, -7, -4, -1, +2, +5 dB, clear). In each of the two experiments, participants were asked to type the target sentences into a text box, and speech-reception-thresholds (the SNR at which 50% of the words were correctly reported) were calculated from word-report data to assess speech intelligibility.
Results: In both experiments, we found that semantic context improves speech intelligibility for younger and older adults, such that conditions with more semantic context elicited greater intelligibility than conditions with lower context (Experiment 1: meaningful vs non-meaningful sentences; Experiment 2: additional vs non-additional speech context). Both age groups also exhibit better speech intelligibility for a modulated (fluctuating) than an unmodulated (stationary) masker. However, this benefit from the speech glimpses was reduced in older compared to younger adults. Critically, although semantic context amplified the benefit gained from the speech glimpses in both experiments, there was no indication that this amplification was larger in older adults. If anything, younger adults benefitted more.
Conclusions: Our results suggest that the deficit in the speech-glimpsing benefit in older adults generalizes to situations in which extended speech context is available. That previous research found a greater benefit in older than younger adults during story listening may suggest that other factors, such as thematic knowledge, motivation, or cognition, may amplify the benefit from speech glimpses for older adults under naturalistic listening conditions.

Uncovering Genetic Comorbidities Related to Speech in Noise Deficits Using Polygenic Risk Score From Two Independent Cohorts

Srividya Grama Bhagavan*[1], Valerie Ingalls[1], Ishan Bhatt[1]

[1]*The University of Iowa*

Background: Speech-in-noise (SIN) deficits refer to difficulties understanding speech against background noise. A recent genome-wide association study (GWAS) revealed polygenic

architecture underlying SIN deficits in individuals with self-reported normal hearing. The objective of the present study was to investigate the genetic comorbidities of SIN deficits. We employed a polygenic risk score (PRS)-based association analysis to identify genetic comorbidities of SIN deficits across the health phenome. PRS can quantify the risk of complex health traits based on the genetic predisposition to disease-associated variants.
Methods: The PRS-based association analysis of SIN deficits was conducted on the UK Biobank cohort, followed by a replication analysis using clinical measures of SIN deficits in a cohort of healthy young adults with normal audiograms. The UK Biobank sample included 279,911 participants with 58,847 cases reporting SIN deficits with self-reported normal hearing in quiet and 221,067 controls without SIN deficits and reporting normal hearing in quiet. The replication sample included 300 healthy young adults (18-37 years) with self-reported normal hearing. Self-reported SIN deficits were assessed by the Speech, Spatial and Qualities of Hearing Scale (SSQ12). QuickSIN and Dichotic Digit Test (DDT) were performed to evaluate SIN processing. DNA samples from the saliva were subjected to low-pass whole genome sequencing. PRS calculation was performed using a custom PRS calculator. Around 2600 PRS models were derived from an open-access Polygenic Risk Score catalog. A logistic regression model was used to identify the PRS predictors associated with SIN deficits in the UK Biobank cohort. A linear mixed model was utilized to identify PRS predictors of SSQ12, QuickSIN, and DDT in the replication cohort.
Results: The regression analysis identified PRS predictors across the health spectrum associated with SIN deficits in the UK Biobank. PRS of sensory traits, such as acquired hearing loss and tinnitus, revealed significant associations with SIN deficits. PRS of neuropsychiatric conditions, including schizophrenia, major depression disorder, self-reported depression, anxiety, and risk-taking tendency, revealed robust associations with SIN deficits. Several PRS across the health spectrum, including autism spectrum disorder, alcohol consumption, body mass index, cholesterol, and lung cancer revealed significant associations with SIN deficits. PRS associated with SIN deficits were enriched (overexpressed) in several trait categories, including neuropsychiatric, mental health, and endocrine/metabolic. The replication analysis using SSQ12, QuickSIN, and DDT revealed complementary results.
Conclusions: The present study identified genetic comorbidities associated with SIN deficits in individuals with self-reported normal hearing in two independent cohorts. The results indicate that genetic predisposition to certain health traits can explain intersubject variability in SIN deficits. The results are consistent with the polygenic inheritance of SIN deficits. We posit that efficient communication of the genetic risk of SIN deficits at younger ages can help prevent or delay the onset of clinical representation of SIN deficits.

Mini-Podium 2: Etiologies and Novel Treatments of Inner Ear Disorders

Moderators: Jose Antonio Lopez-Escamez and Jarnail Singh

4:15 p.m. - 5:15 p.m.

Ocean Ballroom 9 - 12

Vanadium, Uranium, and Silver Metal Levels Are Associated with Poorer Auditory Test Performance Among Children in a Nicaraguan Mining Community.

Marissa Kachadoorian*[1], Torri Lee[1], Jessica Fitzgerald[2], Michaela Geffert[2], Adrian Fuente[3], Catherine Rieke[2], Anastasiya Kobrina[2], Odile Clavier[4], Jiang Gui[1], Jie Zhou[1], Siting Li[1], Margaret Karagas[1], Brian Jackson[5], Karen Mojica[6], Christopher Niemczak[7], Jay Buckey[7], James Saunders[7]

[1]*Geisel School of Medicine,* [2]*Dartmouth-Hitchcock,* [3]*University of Montreal,* [4]*Creare LLC,* [5]*Dartmouth College,* [6]*Vivian Pellas Hospital, Medical Director, Mayflower Medical Outreach, Managua, Nicaragua,* [7]*Dartmouth-Hitchcock, Geisel School of Medicine*

Background: Several heavy metals, particularly mercury and lead are known to be ototoxic and affect both the peripheral and central auditory system. To examine which metals may also affect either central or peripheral auditory function, we measured 21 metals in the toenails of 201 children living in artisanal mining communities in Nicaragua.
Methods: 201 children (120 M, 81 F, mean age 12 years) performed a battery of central and peripheral auditory tests of which included pure tone audiometry (PTA) and the dichotic digits test (DDT). Toenail samples were collected and were analyzed for 21 metals using mass spectrometry. The relationship between peripheral or central hearing test results and metal levels was examined using elastic net regression analysis. To assess the strength of association, elastic net regression was also performed 100 times using bootstrapping with replacement. The number of times an individual predictor was selected was expressed as a percentage. Regressions were run with the individual auditory tests as outcomes and the metal, age, and interaction of age and metal level as predictors.
Results: DDT had the strongest relationship to metal levels. Elastic net regression showed vanadium (V), uranium (U), iron (Fe), tin (Sb), mercury (Hg) and lead (Pb) were all predictors of performance on DDT. Bootstrapping analysis showed that V and U were most consistently associated with DDT performance (86% and 95% of the time respectively). Linear regression analysis showed that higher V and U levels were associated with worse DDT performance with no age interaction (both p LESS THAN 0.001). For audiometry, only silver (Ag) was associated with poor audiometry performance. Linear regression results for Ag showed worsening PTA with higher Ag levels (p=0.002) with no age interaction.
Conclusions: Although many metals are known to have effects on the central and peripheral auditory system, V, U, and Ag are not often associated with poor performance on these tests. The results from this large-scale study in Nicaraguan artisanal mining communities suggests these metals may affect auditory pathways. More investigation of their effects is needed.

Machine Learning Model for Predicting Acute Hearing Loss Episodes in Patients With SLC26A4 Variants

Pei-Hsuan Lin*[1], Yu-Jen Wu[2], Ta-Wei Yang[2], Yu-Ting Chiang[3], Yu-Xhin Lu[4], Tien-Chen Liu[4], Chuan-Jen Hsu[5], Cheng-Fu Chou[2], Chen-Chi Wu[6]

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Background: Pathogenic variants in SLC26A4 are a major cause of hereditary hearing impairment, resulting in both syndromic (Pendred syndrome) and non-syndromic (DFNB4) hearing loss. These patients often present with an enlarged vestibular aqueduct (EVA) and incomplete cochlear partition type II (Mondini dysplasia). Hearing patterns are typically progressive and fluctuating, and some patients may experience recurrent episodes of acute hearing loss. Predicting these episodes remains a challenge due to the heterogeneous nature of hearing loss progression. The aim of this study is to develop a machine learning model to predict such episodes in patients with SLC26A4 variants.
Methods: Patients diagnosed with EVA and/or carrying pathogenic SLC26A4 variants were included. Demographics, genotypes, vestibular aqueduct size, presence of Mondini dysplasia, and serial audiograms were analyzed. A total of 231 Taiwanese patients with 1,827 serial audiograms were reviewed. Hearing thresholds at six frequencies (0.25K, 0.5K, 1K, 2K, 4K, and 8K) from both ears were analyzed separately. Patients with fewer than four audiograms were excluded. An episode of acute hearing loss was defined as a hearing threshold decline ≥ 15 dB at two consecutive frequencies or a decline ≥ 30 dB of the three-frequency average between two audiograms within six months. We used three consecutive audiograms to predict the occurrence of acute hearing loss within the following six months. After preprocessing, data analysis consists of two parts: (1) statistical feature extraction using ElasticNet model for regression; (2) time series feature extraction using autoencoders to capture acute hearing loss episodes. Instead of using the raw numerical data of hearing level, we extracted the "shape" feature of hearing trends by categorizing them into five groups. Fivefold cross-validation was performed to evaluate the model performance.
Results: A total of 125 patients with 3,267 hearing threshold data points were included in the analysis. Approximately 82% (102/125) of these patients experienced acute hearing loss, with 195 episodes in the left ear and 186 in the right ear. The average number of acute hearing loss episodes per individual were 1.5±1.8, with an average age of occurrence at 8.1±8.7 years. In our dataset, sex, genotype, vestibular aqueduct size, and the presence of Mondini dysplasia were not associated with acute hearing loss. Our model using ElasticNet with Categorical Boosting achieved good predictive performance with an area under the curve (AUC) of 0.766.
Conclusions: Our machine learning model successfully predicts acute episodes of hearing loss in patients with SLC26A4 variants based on the "shape" of their hearing trends. This predictive capability enables timely intervention and closer monitoring of patients at risk for hearing decline, potentially leading to improved patient outcomes.

SPI-1005 Improves Auditory and Vestibular Deficits in Meniere's Disease in a Multi-Center Phase 3 Randomized Placebo-Controlled Trial (STOPMD-3)

E Emily Harruff[1], Jacqueline Nguyen[1], G Michael Wall[1], Shaun Nguyen[2], Paul Lambert[2], Jonathan Kil*[1]

[1]*Sound Pharmaceuticals, Inc.,* [2]*MUSC*

Background: Meniere's disease (MD) causes episodic vertigo or dizziness, fluctuating low-frequency hearing loss, and intermittent or constant tinnitus, and is thought to be due to a swelling of the inner ear. Adults are typically 40-65 years of age at diagnosis and can progress to severe to profound hearing loss and intractable tinnitus. The majority are medically managed with low salt diets, diuretics, and corticosteroids. No FDA-approved therapies exist. SPI-1005 capsules, a novel orally dosed investigational anti-inflammatory tested in Phase 1b (N=39) and Phase 2b (N=126) randomized clinical trials (RCTs), improved low-frequency hearing loss, speech discrimination, and tinnitus loudness after 21 or 28-days of treatment, respectively. We now report blinded results from a Phase 3 RCT (STOPMD-3) and unblinded results from the Open Label Extension (OLE) involving adult participants with definite MD (N=221).
Methods: Adults (18-75 years) were consented and screened for eligibility at 11 US sites (NCT04677972). Eligible participants were randomized to either SPI-1005, 400 mg, or placebo, BID for 28 days. Pure-tone audiometry (PTA), words-in-noise (WIN), tinnitus-functional-index (TFI) including VAS-loudness (TL), vertigo-symptoms-scale (VSS), dizziness-handicap-inventory (DHI), and VAS-aural fullness (AFS) were assessed at baseline, 28, 56, and 84-days. Compliant participants entered OLE and received SPI-1005, 400 mg, BID, for up to 12-months and were reassessed every 3-months or until study exit. Primary efficacy endpoints were improvements in low-frequency PTA and WIN from baseline using responder analyses: ≥10 dB improvement at 250, 500, or 1000 Hz; and ≥4-word improvement in WIN score. Secondary endpoints were based on improvements in TFI, TL, VSS, DHI, and AFS scores from baseline. Safety was determined using H and Ps, MedDRA coding for adverse events, hematology (CBC), and serology (Chem-20).
Results: Of the 254 participants screened, 221 (mean age 55.7; 101 females/120 males) were randomized with 212 completing the RCT and 201 entering OLE. During RCT, participants showed improvements in low-frequency PTA (35.5%, 39.4%, and 46.9%) and WIN scores (24.1%, 35.7%, and 35.5%) at 28, 56, and 84-days, respectively. Improvements or reductions in TFI (51.1 to 43.7), TL (6.6 to 5.7), VSS (13.9 to 10.4), DHI (39.1 to 32.9), and AFS (6.5 to 5.6) scores were also observed. During OLE, low-frequency PTA and WIN responder rates improved to 51.5% and 38.4%, and reductions in TFI, TL, VSS, DHI, and AFS scores improved to 34.9, 4.8, 7.5, 26.1, and 4.4, respectively.
Conclusions: In this Phase 3 trial, SPI-1005 was safe for up to 12-months of treatment. Continued improvements in low-frequency hearing loss, speech discrimination, TFI, TL, VSS, DHI, AFS were observed through OLE. Safety and efficacy between active and placebo groups will be unblinded by the meeting. To our knowledge, STOPMD-3 is the longest continuous treatment trial involving an investigational new drug ever completed for a hearing loss or tinnitus indication.

Phex Gene Dosage Effect as a Likely Trigger for Meniere Disease in Patients with X-Linked Hypophosphatemia

Paula Robles-Bolivar*[1], Divya Chari[2], David Bächinger[3], Arpan Bose[4], Kimberly Ramirez[5], Eva Liu[5], Steven Rauch[6], Andreas Eckhard[2]

[1]*Massachusetts General Hospital, Harvard Medical School,* [2]*Massachusetts Eye and Ear Infirmary,* [3]*University Hospital Zurich,* [4]*University of Massachusetts Chan Medical School,* [5]*Brigham and Women's Hospital,* [6]*Harvard Medical School, Massachusetts Eye and Ear*

Background: Meniere's Disease (MD) is a chronic inner ear disorder characterized by spontaneous vertigo, fluctuating sensorineural hearing loss, and tinnitus; with considerable variability in onset, progression, and severity among patients. Its etiology remains unknown and is likely the result of a complex interplay between predisposing and triggering factors. Recent research strategies aimed at understanding its pathophysiology have focused on grouping patients with identical or highly similar clinical, radiological, and pathological characteristics. A distinct clinical and radiologically defined subset of MD is defined by an underdeveloped (hypoplastic) endolymphatic sac, along with a clinical phenotype of male predominance, bilateral disease, and a significant family history of MD. Interestingly, there was a surprising high prevalence of X-linked hypophosphatemia (XLH) in the subgroup, a rare disorder of phosphate metabolism caused by mutations in the PHEX gene in X chromosome. Therefore, we here hypothesize a potential genetic link between this hypoplastic endotype of MD and XLH.

Methods: XLH patients were prospectively recruited from two tertiary neurotological centers. MD-related symptoms were evaluated through medical history, pure tone audiometry, video head impulse, and caloric testing. MD endotype was evaluated using high resolution temporal bone computed tomography (CT) imaging. Whole genome sequencing from blood samples targeted rare coding and splice-site genetic variants, prioritizing variants in PHEX gene and those in genes from pathways previously associated with phosphate regulation and audiovestibular phenotypes.

Results: Among 34 XLH patients (Zurich = 10, Boston = 24), none of the 26 females exhibited MD or vestibular symptoms. Six of the eight males presented with bilateral MD, characterized by vestibular aqueduct hypoplasia. One male had bilateral hypoplasia of the vestibular aqueducts on radiologic imaging without MD symptoms and was of a comparatively young age, while another displayed mosaicism in PHEX. No specific PHEX variants correlated with the MD endotype in males.

Conclusions: A male-specific distribution of MD among XLH patients was observed, suggesting a PHEX “gene dosage effect" as the cause of MD in XLH patients, potentially linked to phosphate metabolism dysregulation. This is the first genetic association of MD with a distinct patient subgroup, opening new paths for genetic diagnostics and therapeutics.

Inner Ear Courses

6:30 p.m. - 7:30 p.m.

Ocean Ballroom 1 - 4

Biology of the Inner Ear

Katie Kindt*, NIH/NIDCD*

Alain Dabdoub*, Sunnybrook Research Institute/University of Toronto*
Daniel Tollin*, University of Colorado School of Medicine*

JAX Course

Matthew Kelley*, NIH/NIDCD*

NIDCD EARssentials Course

Elyssa Monzack*, NIDCD*
Melanie Barzik*, National Institutes of Health*

Monday, February 24, 2025

Symposium 3: Inner Ear Immunity: Unraveling the Immune Dynamics in Hearing

Chair: Cathy Yea Won Sung*, National Institute on Deafness and Other Communication Disorders, National Institutes of Health*

8:00 a.m. - 10:00 a.m.

Ocean Ballroom 1 - 4

Symposium Description: The inner ear, traditionally viewed as an immune-privileged site, has recently been recognized for its intricate and active immune responses. Understanding the role of immune cells and immune mechanisms within the inner ear is crucial not only for addressing various otologic diseases but also for developing targeted therapies that can modulate these responses. This symposiu`m, titled "Inner Ear Immunity: Unraveling the Immune Dynamics in Hearing," aims to bring together leading experts in the field to explore the latest research and advancements in inner ear immunology.

This symposium aims to advance our understanding of inner ear immunity and its clinical implications. The symposium will cover a broad spectrum of topics, including the identification and roles of resident and infiltrating immune cells in the inner ear, the impact of inflammation on inner ear health, and the interplay between the immune system and common inner ear pathologies (e.g., noise-induced hearing loss, ototoxic drug-induced hearing loss, age-related hearing loss, genetic hearing loss, virus/bacterial infection-induced hearing loss, or otitis media).

Attendees will gain insights into how the inner ear's immune system can be both a protector and a contributor to pathology and how this knowledge can be harnessed to improve diagnostic and treatment strategies to mitigate hearing loss by modulating the immune system. This includes the potential for (targeted) delivery of anti-inflammatory agents, the use of immune modulators, and gene therapy techniques. By highlighting the latest advancements, we aim to share how the immune system influences hearing and how it can be leveraged to develop treatments for inner ear diseases, ultimately improving patient outcomes and quality of life.

Immune Mediated Sensory Hearing Loss in Chronic Suppurative Otitis Media

Peter Santa Maria[1]

[1]*University of Pittsburgh*

Individual Abstract: We will present the immune implications of how sensory hearing loss (SHL) is caused by chronic suppurative otitis media (CSOM): severe chronic middle ear infections. CSOM is a neglected disease that afflicts 330 million people worldwide and is the most common cause of permanent hearing loss among children in the developing world. It is characterized by a chronically discharging infected middle ear, and there is currently no effective cure. We show how CSOM leads to macrophage activation and specifically, the NLRP3

inflammasome which causes sensory hearing loss in our animal model. The NLRP3 inflammasome serves as an intracellular immune sensor expressed in monocytes, macrophages and dendritic cells. Its activation mediates caspase-1 activity, leading to IL-1b and IL-18 secretion. The activation of the NLRP3 inflammasome has been linked to several inflammatory and autoinflammatory diseases. The protein levels of NLRP3, IL-1b and IL-18 were significantly elevated in CSOM cochleae compared to control cochleae, confirming the activation of the NLRP3 inflammasome. In addition, mRNA levels of NLRP3 inflammasome factors, including NLRP3, PYCard and Caspase1, were downregulated when macrophages were depleted in CSOM, verifying cochlear macrophages as the origin of the NLRP3 inflammasome activation. We demonstrated that outer hair cells are protected in CSOM when macrophages were depleted and further, in the NLRP3 inflammasome knockout condition (NLRP3-/-). Finally, we revealed that inhibiting NLRP3 activation and inflammasome formation with blocking ATP hydrolysis or using a recombinant human interleukin-1 (IL-1) receptor antagonist protected OHC loss in CSOM.

Harnessing Macrophages for the Treatment of Noise-Induced Hidden Hearing Loss

Tejbeer Kaur[1]

[1]*Rutgers University, Robert Wood Johnson School of Medicine, Rutgers Brain Health Institute*

Individual Abstract: Noise-induced hidden hearing loss is due to damage and loss of the ribbon synapses between sensory inner hair cells and auditory nerve fibers in the cochlea that is not readily diagnosed by threshold audiograms, the standard clinical examination for hearing loss. The consequence of synaptic loss is deficits in hearing acuity, leading to difficulty in speech recognition and listening in noisy environments. It is now well established that there is some degree of natural synaptic repair following noise trauma. Deciphering the mechanisms regulating synaptic degeneration and repair might inform interventions to preserve and/or restore the loss of synapses and hearing. The talk will focus on non-sensory immune cells, macrophages and their interactions with the noise-damaged ribbon synapses and how such interactions influence synaptic degeneration and repair. It will also delineate novel immunotherapy strategy to harness macrophages to regenerate lost ribbon synapses and hearing in animal model of noise-induced hidden hearing loss.

Single-Cell, Spatial, and Fate-Mapping Analyses Uncover Niche Dependent Diversity of Cochlear Myeloid Cells

Bahareh Ajami[1]

[1]*Oregon Health and Sciences University*

Individual Abstract: Recent advances in fate mapping and single-cell technologies have revealed how the dynamics and function of tissue-resident macrophages are shaped by their environment. However, macrophages in sensory organs such as cochlea where the central nervous system and peripheral nervous system meet remain understudied. The cochlea—long thought to be immune-privileged—is a sensory organ in which the presence of macrophages has been reported. Yet the core gene signature, ontogeny, dynamics, spatial distribution, and functions of these macrophages remain poorly defined. Here, by combining single-cell transcriptomics, fate mapping, and parabiosis experiments, we identified several types of immune cells—monocytes, dendritic cells, and three macrophage subpopulations—in the mature

mouse cochlea. The three macrophage subsets had distinct transcriptomic profiles that closely related to microglial cells, epineurial or endoneurial sciatic nerve macrophages, while cochlear monocytes and dendritic cells transcriptionally resembled spleen monocytes and dendritic cells, respectively. Further analyses of their gene signatures suggested that the macrophage subsets had different functions, including angiogenesis and synaptic pruning. In addition, a spatially resolved analysis of the three macrophage subsets revealed distinct topography across cochlear compartments. Using fate mapping and parabiosis models, we further demonstrated that cochlear macrophages were partially derived from yolk sac progenitors during development. In adulthood, most cochlear macrophages were classified as long-term resident, except in the spiral lamina and the spiral limbus, where macrophages were partially and slowly replaced by monocyte-derived macrophages. Finally, we showed that macrophage morphology and density changed during aging. Our findings highlight cochlea as a unique microenvironment with an unappreciated diversity of macrophages in terms of gene expression, ontogeny, dynamics, and function, suggesting a previously unknown role for the immune system in the cochlea.

Single-Nucleus RNA Sequencing Reveals Transcriptional Markers of Congenital CMV Infection in the Mouse Cochlea

Daniel Romano[1]

[1]*Washington University School of Medicine*

Individual Abstract: Congenital cytomegalovirus (cCMV) infection is the single leading cause of congenital, non-genetic hearing loss in the United States. The underlying pathophysiologic mechanism of cCMV-related hearing loss has until now remained unsettled. A mouse model of cCMV has recently been developed which recapitulates many characteristics of the human cCMV phenotype, including elevated hearing thresholds in ~60% of mice. An early study using the cCMV mouse model showed that spiral ganglion neuron counts are modestly reduced in cCMV infected mice and inversely correlate with hearing thresholds. However, several questions remain including: 1) the mechanism of spiral ganglion neuronal loss in cCMV-related hearing loss, and 2) whether other cellular and molecular mechanisms contribute to pathogenesis. Therefore, we sought to employ single-nucleus RNA sequencing (snRNA-seq) to elucidate cell-specific gene regulatory networks and intra- and inter-cellular signaling pathways involved in cCMV-related hearing loss. C57BL/6 mice were injected with intraperitoneal murine cytomegalovirus (mCMV) within 12 hours of birth. Cochleae were harvested at multiple early postnatal dates, and the cochlear membranous labyrinth was dissected and segregated into modiolus (along with organ of Corti) and lateral wall tissues for separate analysis. Single nuclei were isolated from pooled tissues from n = 10 mice, and snRNA-seq was performed with the 10x Genomics Chromium and Illumina NovaSeq Platforms. snRNA-seq data analysis was performed in the R software environment. Pre-processed snRNA-seq datasets for the cCMV mouse model and control animals were merged together, and differential gene expression, functional enrichment, and cell-cell signaling analyses were performed. snRNA-seq analysis revealed significant changes in cell-specific gene expression and cell-cell signaling in the early postnatal mCMV-infected mouse cochlea. Our results provide insight into the transcriptional underpinnings of cCMV-related hearing loss, and carry implications for other inflammatory and infectious conditions of the inner ear.

Assessment of AAV-Mediated Innate and Adaptive Immunity in the Mammalian Inner Ear

Yasuko Ishibashi[1]

[1]*National Institute on Deafness and Other Communication Disorders, National Institutes of Health*

Individual Abstract: Background: Adeno-associated virus (AAV) is a safe and effective viral vector that has been widely used in gene therapy studies. However, it has been shown that the host immune response to AAV may affect its potency, efficacy, and persistence. In the mammalian inner ear, multiple studies have shown that gene therapy can be effective at improving the auditory and vestibular functions in various mouse models of hereditary hearing loss. Some of these studies have led to the initiation of clinical trials in hereditary hearing loss patients. Therefore, it's critical to comprehensively evaluate the immune responses triggered by AAV in the mammalian inner ear to ensure the safety of AAV-mediated gene therapy applications. In this study, we examined the innate and adaptive immune responses triggered by AAV-mediated gene delivery into the adult mouse inner ear.

Methods: We injected AAV2.7m8-GFP, AAV2-GFP or a viral vehicle solution (5% glycerol in PBS) into 8- to 10-week-old C57BL/6J mice through the posterior semicircular canal (PSC) approach. Cochleae were dissected on postoperative day (POD)3 and POD28 and immunohistochemically stained with macrophage marker Iba1 and T-cell marker CD3. The number of macrophages and T-cells were quantified using confocal microscope images. Multi-spot cytokine and chemokine assays were used to quantify immune activation in serum and perilymph samples. The presence of neutralizing antibodies against AAVs was assessed using neutralizing antibody assay. ELISpot test was performed to assess IFNɣ secretion from splenic cells mediated by antigen-specific T-cells against AAVs.

Results: The number of macrophages was significantly increased in AAV2.7m8-injected and vehicle-injected mice compared to non-surgery mice on POD3 and in AAV2.7m8-GFP-injected, AAV2-GFP-injected, and vehicle-injected mice on POD28. The number of T-cells in injected mice was not significantly increased compared to non-surgery mice. IL-6 in perilymph samples from AAV2.7m8, AAV2, and vehicle-injected mice was increased on POD3 compared to non-surgery animals, but IL-6 level was not increased in serum samples from these animals. Neutralizing antibodies were detected in AAV2.7m8-injected and AAV2-injected mice on POD28. Most animals that underwent either AAV2.7m8 or AAV2 injections did not show an increase in capsid specific IFNɣ secretion.

Conclusions: After AAV-mediated inner ear gene delivery, the innate immune responses are mainly triggered by surgical trauma. Neutralizing antibodies against AAVs, which are involved with adaptive immune responses, are detected in the mouse serum. Future studies on repeated and biaural AAV-mediated inner ear gene delivery would provide additional insights into the immune responses in the mammalian inner ear.

Role of the Immune System in the Development of Endolymphatic Hydrops and Hearing Instability in Humans

Samuel Adadey[1]

[1]*National Institute on Deafness and Other Communication Disorders*

Individual Abstract: The pathogenesis of hearing instability (HI) disorders is not well understood, complicating diagnosis and treatment of hearing loss despite indications that the immune system may play a role. To explore the immune system's contribution to the development of endolymphatic hydrops (EH) and HI in affected patients, we performed longitudinal deep phenotyping of HI patients under an NIDCD clinical protocol at the NIH Clinical Center. Patients with HI disorders were recruited and grouped based on MRI-confirmed EH. Plasma and peripheral blood mononuclear cells (PBMCs) were isolated from blood samples for cytokine and proteomic analyses, while PBMCs were further examined using a 40-fluorescent marker panel full spectrum flow cytometry (FSFC).
Our cytokine analysis revealed elevated proinflammatory cytokines in some HI patients, implicating inflammation. Notably, increased Th17 cytokine levels were observed in EH-HI patients, suggesting an autoimmune component. FSFC profiles of monocytes, natural killer cells, and CD8+ T-cells showed significant differences between hydrops and non-hydrops patients. Proteomic analysis identified potential protein biomarkers for HI disorders.

Preliminary findings indicate that HI patients have distinct immune profiles, suggesting new diagnostic and therapeutic targets. Ongoing recruitment and longitudinal assessments, incorporating FSFC, scRNA-Seq, and proteomic profiling, aim to further substantiate the immune system's role in these complex disorders.

Organ of Corti Macrophages: A Distinct Group of Cochlear Macrophages With Potential Roles in Cochlear Development and Supporting Cell Degeneration

Mengxiao Ye[1]

[1]*University at Buffalo*

Individual Abstract: Macrophages are the primary immune cells in the cochlea, playing a critical role in maintaining cochlear homeostasis and orchestrating inflammatory responses to pathogenesis. These immune cells are found in various parts of the cochlea. However, their presence in the organ of Corti (OC), the key structure for hearing, remains less clear. Here, we report on our observations of macrophages in the OC, focusing on the conditions that allow their presence and their potential functions in normal cochlear development and pathogenesis. In mice, we observed the presence of OC macrophages at birth. Interestingly, these macrophages underwent apoptosis as the OC gradually completed its maturation during postnatal development, suggesting a potential role for macrophages in the postnatal maturation of the OC. In the mature cochlea, the OC is devoid of macrophages. To investigate OC macrophages under pathological conditions, we utilized an ototoxicity model induced by cyclodextrin, a cyclic oligosaccharide compound that caused the rapid onset of OHC death at a high dose. This cochlear pathogenesis led to a significant infiltration of monocytes into the cochlea. However, despite the massive loss of OHCs during the acute phase of cochlear pathogenesis, the OC remained devoid of macrophages. This observation suggests that a unique local environment or regulatory mechanism within the organ of Corti prevents macrophage entry in response to sensory cell damage. In contrast, we observed macrophages within the OC in the chronic phase,

examined six and nine weeks after cyclodextrin administration. These cells appeared only in the cochlear regions where there was a complete loss of OHCs but supporting cell pathogenesis continued. Importantly, their location was adjacent to areas with missing supporting cells. These OC macrophages displayed a strong galectin-3 expression, indicating their activation. They also expressed the phagocytic protein CD68, indicating their active involvement in the formation of supporting cell lesions. To investigate whether a similar pattern of change occurs in other etiologies of chronic pathologies within the cochlea, we examined the aging cochleae of C57BL/6J mice. As expected, macrophages were found only in the regions where there was a complete loss of OHCs but supporting cell pathogenesis continued. Together, our findings reveal both the presence and dynamic activity of macrophages in the OC under both normal and pathological conditions, highlighting their potential roles in cochlear development and supporting cell pathogenesis.

Podium 7: Cochlear Mechanics: Models, Experiments, and Problems

Moderators: Wei Dong and Paul Secchia

8:00 a.m. - 10:00 a.m.

Ocean Ballroom 5 - 8

3D Finite Element Modeling of Human Cochlear Responses to Air and Bone Conductions for Blast Overpressure and Acoustic Wave Transmission

John Bradshaw[1], Marcus Brown[1], Alexander Bien[2], Mirembe Mulimba[1], Yijie Jiang*[1], Rong Gan[1]

[1]*University of Oklahoma,* [2]*University of Oklahoma Health Sciences Center*

Background: Hearing loss due to exposure to blasts is one of the most prevalent disabilities among veterans. Blast-induced injury to the inner ear, especially the organ of Corti (OC) within the cochlea, is challenging to examine experimentally. Recently, the multiscale 3D finite element (FE) model of the human ear to simulate the blast and acoustic transmissions via air conduction (AC) through the ear canal into the OC has been reported from our group. This model included a macroscale entire ear model and a microscale OC model at the cochlear middle turn with fully fluid-structure coupled simulations. However, the effect of blast and acoustic transmission via bone conduction (BC) and the responses of the OC at different basilar membrane (BM) locations are not well studied. This paper reports the most recent study on development of a microscale OC model at the BM basal turn and the investigation of biomechanical responses of the OC or hair cells to both AC and BC conductions of the blast and acoustic waves.
Methods: The fluid-structure coupled OC model at the BM basal turn includes the BM, outer hair cells (OHCs), reticular lamina (RL), hair bundles (HBs), tectorial membrane (TcM), and supporting cells, as well as the endolymph fluid. Hyperelastic and viscoelastic properties to enable nonlinear mechanical responses were assigned to the OHCs and TcM. Blast overpressure and acoustic pressure were applied at the entrance of the ear canal as AC; the dynamic vibration along the ear canal bony wall was applied as BC. The BM displacement and cochlear pressure calculated from the macroscale model was applied as the input to the microscale model.

Results: The maximum displacements and strains in the OHCs, HBs, TcM, and RL were derived from the OC model. The maximum strains were concentrated where the HBs connect to the RL and TcM. The data obtained from the OC model at basal turn was compared with that from the model of middle turn to predict the possible injuries. Comparisons between models with AC, BC, and both AC and BC inputs were performed to demonstrate the biomechanical responses induced by different pathways.
Conclusions: The microscale OC model reported here has expanded the multiscale model of the human ear into both AC and BC hearing pathways. The results have demonstrated how the OC or hair calls' response to blast overpressure and acoustic wave transmission via AC and BC conductions vary along the BM from the base to middle turn. The OC model will be further improved with an increased number of hair cell rows and the bone conduction through the cochlear bone wall.

Broadband Nonlinearity in Vibrations of the Mouse Cochlear Apex

James Dewey*[1]

[1]*University of Southern California*

Background: Waves traveling along the cochlea are nonlinearly amplified by outer hair cell (OHC)-generated forces. While the motion of the basilar membrane (BM) at a given location is amplified only near the characteristic frequency (CF) of that site, the motion of the OHCs themselves has recently been shown to be actively enhanced and nonlinear over a much broader range of frequencies. How these nonlinear motions influence the surrounding structures is not yet well understood. Here, the frequency extent and level dependence of active, nonlinear motions measured from within and around the OHC region were characterized in the mouse cochlear apex.
Methods: Optical coherence tomography was used to measure sound-evoked vibrations from the apical cochlear turn in anesthetized, adult CBA/CaJ mice. Responses to single- or two-tone stimuli varied in frequency and level were obtained from a location tuned to a CF of ~9 kHz. Of particular interest were responses far below the CF, where BM motion is largely linear.
Results: Broadband nonlinearity was present in the motions observed throughout the OHC region and adjacent structures, including at the reticular lamina, the nearby supporting cells, and the overlying tectorial membrane (TM). For stimulus frequencies as low as 1 kHz, responses of these structures exhibited compressive growth as long as the stimulus pressure was raised to sufficiently high levels. Responses to low-frequency tones could also be suppressed by a second tone and were almost always reduced postmortem. For the TM, the influence of broadband OHC activity varied along its radial dimension. While the motion at the lateral tip of the TM exhibited broadband nonlinearity, the motion near the spiral limbus more closely resembled that of the BM. Phenomena like expansive growth and non-monotonic suppression were also observed in low-frequency TM responses and appeared to result from interference between active and passive components of motion.
Conclusions: In the mouse cochlear apex, motions of the structures directly connected to the OHCs exhibit nonlinearity at frequencies three or more octaves below the CF. Since this low-frequency nonlinearity was observed at both the reticular lamina and TM, it may also be present in the mechanical stimulus to the inner hair cells. Whether nonlinear OHC forces are similarly

coupled to the organ of Corti structures in more basal, high-frequency locations of the mouse cochlea remains to be determined.

Determining Parameters for an Active Radial-Slice Model Using Lumped Element Modeling and Simulation-Based Inference

Julius Kraut*[1], Daniel Cardosi[2], Sunil Puria[2]

[1]*Massachusetts Eye and Ear,* [2]*Harvard Medical School*

Background: A finite element slice model representation for the active cochlea generated significant gain of basilar membrane (BM) and reticular lamina (RL) motion (Kraut and Puria, 2024 Zenodo). However, the model included an unphysiologically fast adaptation time constant for the outer hair cell (OHC) bundle mechanics. OHCs generate forces due to electromotility, which increases motion of the organ of Corti (OoC) when coupled with the correct phase. Previously, the idea that adaptation introduced a phase lead that compensates for lags along the feedback path was hypothesized (Kraut and Puria, 2024 Zenodo). Finite-element models are computationally expensive, resulting in long solution times that make it challenging to determine new parameter sets. Lumped element models (LEM) of the OoC are much simpler (e.g., Allen, 1980) and allow for the use of sequential estimation techniques like simulation-based inference (SBI) to determine parameters. The goal of this study was to determine a plausible parameter set, including a realistic adaptation time constant, using an SBI-tuned LEM to further tune the detailed slice model.
Methods: We used a finite element model of a 20-μm longitudinal radial slice representing the middle turn of the gerbil cochlea with characteristic frequency of ~2.5 kHz and three rows of active OHCs. A LEM (Gálvez and Elliott, 2013, U of Southampton, ISVR Technical Memorandum) was modified to incorporate OHC activity per this slice model. With fixed OHC parameters, the OoC parameters for the LEM were determined via SBI (Greenberg et al., 2019) to match the slice model's BM and RL displacements. Then SBI was used to find a range of OHC parameters to better match experimental measurements, including longer hair bundle adaptation time constants.
Results: The LEM was able to approximately reproduce the passive and active BM and RL motion of the detailed slice model. However, the best frequency (BF) was slightly too low and the phase decreased too rapidly. Small adjustments to OHC parameters in the detailed slice allowed for good fits of experimental BM motion at BF (Meenderink et al., 2022). The gain at low frequencies was lacking, however, leading to a peaky response. The adaptation time constant remained too fast, indicating that other changes to the active OHC model may be required.
Conclusions: LEMs and SBI are a fast way to test different parameter sets compared to working with the slice model directly. Small changes to OHC parameters did not slow down adaptation sufficiently in the slice. Rabbitt et al. (2019) proposed a membrane capacitance with both real and imaginary values, while Frank et al. (1999) suggested higher speeds of prestin molecules than what is currently used. Both would allow for an improved, slower adaptation time constant.
[Work supported by R01 DC07910 from the NIDCD of NIH.]

Inner Ear Fluids Imbalance in Meniere's Disease Between Blood and Cerebrospinal Fluid

Michael Burcon*[1]

[1]*Meniere's Disease Research Institute*

Background: The human skull is a semi-closed hydraulic system securing the brain, blood and cerebrospinal fluid (CSF). After eliminating a brain tumor with an unremarkable MRI resulting in a diagnosis of Meniere's disease (MD), if one of the two fluid capacities is raised, the other must be lowered. Based on one thousand consecutive MD patients studied (all diagnosed by otolaryngologists), this paper hypothesizes whether a substantial percentage have normal pressure hydrocephalus caused by cerebellar tonsillar ectopia, the result of whiplash/concussion trauma. The CranioCervical Junction (CCJ), is the most complex area of the spine and is extremely vulnerable to injuries to its stabilizing ligaments, creating a potential "choke point." Over time, idiopathic high intracranial pressure can admit too much CSF into the inner ear, via the internal auditory canal or cochlear aqueduct, putting excess pressure on Reissner's membrane, stimulating over production of endolymphatic fluid, further contributing to the dramatic lowering of blood flow into that ear. Based on this theory, the effectiveness of Cervical Specific Chiropractic 10 Step Protocol was tested for controlling vertigo and improving hearing in these patients by manually realigning the openings in head and neck in the CCJ, helping to release trapped CSF.

Methods: Detailed case histories were taken with an emphasis on head and neck injuries prior to onset of Meniere's symptoms. Patterns were established utilizing spinal thermography and detailed relative leg length testing, determining when and where to adjust. Patients were only adjusted when in pattern. Upper cervical specific chiropractic adjustment listings were obtained by Blair x-ray analysis, lower cervical listings by Pierce Results x-ray analysis. Cerebellar tonsils were imaged utilizing upright MRI.

Results: One thousand MD patients were followed for a minimum of two years, checked a minimum of twelve times. On a scale of zero to ten, using a patient questionnaire first recorded pre-treatment compared to two years later, vertigo, aural fullness and nausea improved by ninety percent or more in 97% percent of cases. Audiogram tests demonstrated hearing improved by sixty percent or more in 30% of cases. Significant improvements in tinnitus were 29%. Negative finding of increase in severity or frequency of headaches was 3%.

Ninety patients' blood flow to and from the posterior brain was tested on 90 patients pre and post treatments with ultrasound. Improvement average was forty percent. Although only one patient's CSF flow exiting the skull was tested by cine upright MRI, she improved by sixty percent.

Conclusions: Intracranial hypertension may represent a shared pathogenetic step explaining the large epidemiological comorbidity between migraine and vestibular symptoms, conceptualized as vestibular migraine. Traumas to the head and neck set the stage for significant problems an average of fifteen years later. Realigning atlas and axis with the foramen magnum can improve ischemia and hydrocephalus.

Internal Motion of the Organ of Corti in the Absence of Traveling Waves

Francesco Gianoli*[1], Rodrigo Alonso[2], Brian Fabella[1], A. Jim Hudspeth[3]

[1]*The Rockefeller University,* [2]*HHMI/The Rockefeller University*

Background: The organ of Corti is central to cochlear mechanics, housing the sensory hair cells that power the active amplification process within its complex structure. However, the precise cellular interactions behind its internal motion and how its patterns of vibration enable amplification remain unclear. This is difficult to unravel in vivo due to both narrow viewing angles through the oval window and the interference of traveling waves, which obscure local dynamics. Our recent advancements in in vitro cochlear preparations now offer the ability to study these active mechanisms under controlled conditions, enabling a clearer distinction between local and global processes.
Methods: We employed a novel in vitro preparation of segments of the gerbil's cochlea, which preserves the active process by mimicking the tissue's physiological conditions. Using Optical Coherence Tomography imaging techniques coupled with sound stimulation, we analyzed the internal motion of the organ of Corti in response to stimuli and in the absence of traveling waves.
Results: We observed complex, localized vibration patterns within the organ of Corti during sound stimulation. These internal motions provide compelling evidence that the qualitative nonlinear features of cochlear amplification do not depend on traveling waves. The segment's ability to amplify stimuli nonlinearly, without the presence of traveling waves, highlights the local nature of these amplification processes, revealing a fundamental aspect of cochlear mechanics that has previously been difficult to disentangle from traveling waves.
Conclusions: Our study provides new insights into the internal motion within the organ of Corti, revealing previously uncharacteristic mechanical interactions that underpin cochlear amplification. These findings open new avenues for understanding the micromechanics of hearing.

On the Growth of the Wave Vector of the Cochlear Traveling Waves, and its Dependence on the Relative Phase of Reticular Lamina and Basilar Membrane

Renata Sisto*[1], Arturo Moleti[2]

[1]*INAIL Research,* [2]*University of Rome Tor Vergata*

Background: In the overturned amplification mechanism (Altoè, 2022) the OHC active force mechanism would amplify the motion of the RL more than that of the BM.
While there is still debate about whether the active force mechanically acts directly on the BM, one should consider the contribution to the TW due to the volume deformation of the OoC, dependent of the motion of both OoC lower and upper parts.

We show how to keep into account the contributions of RL and BM to the development of the TW. To make the mechanism most effective the two oscillators should move approximately in phase. In a two-mass system dominated by an internal active force, which does not accelerate the center of mass, the RL and the BM would move approximately in phase opposition in the peak

region. To explain this apparent dyscrasia, hydrodynamic phenomena are necessary, associated with the sharp growth of the wavevector in the short-wave region.

Methods: A WKB solution was computed for a 2DOF transmission line cochlear model, with the RL and BM acting as two coupled oscillators. The OHCs active force acts as an elastic internal force between the two masses. A low pass filter mechanism mimics the build-up of the transmembrane potential causing the OHC elongation, responsible for the amplification in the peak region. Due to low-pass filtering, the phase of the active force is rotated by about 90 degrees, with an antidamping effect. The model also includes the most important hydrodynamic phenomena, the fluid focusing and the stabilization due to the viscosity. The contributions of the RL and BM motion were both considered in the calculation of the TW wave vector.
Results: The simulations confirm that the maximum contribution to the wave vector is obtained when the two masses move in phase, and that the BM gain is drastically reduced if the two masses move in counterphase. This is observed for reasonable values of the ratio between the oscillation amplitude of the RL and the BM. If the amplification was only related to the active force, we should observe that they move with large gain in phase opposition at the peak. Our simulations shows that the wavevector growth and the related fluid focusing permit to observe a large BM and RL gain also if the two oscillators move quite in phase.
Conclusions: The RL and the BM may both contribute coherently to the traveling wave in the peak region. As the RL moves with larger oscillations than the BM, it is the RL contributing mainly to the wavevector in the maximum activity region. In the region in which the two motions are of comparable size, to make the hydrodynamic amplification maximally efficient the oscillators should move approximately in phase.

Low Frequency Tuning in the Apical Turn of the Cochlea in Aged Mice

Takeru Ota*[1], Kazuya Ono[1], Hiroki Takeda[1], Hiroshi Hibino[1]

[1]*Osaka University*

Background: In human, age-related hearing loss (ARHL) is characterized by hearing threshold elevation that begins from high-pitch regions and gradually extends to low-pitch range. In this irreversible disease, histological and molecular biological changes of sensory hair cells and other cellular and tissue elements in the cochlea have been shown by numerous experiments; nevertheless, the pathophysiological architecture remains poorly understood. C57BL/6J mice are the most frequently used for studies on ARHL. In this work, we analyzed morphology and sound-evoked vibrations of cochlear sensory epithelium in live mice by optical coherence tomography (OCT). We focused on the apical turn, because in this low-pitch region hair-cell damage is not as severe as that in high-pitch region in aged animals. To our knowledge, functional change of mouse sensory epithelium during aging has not yet been examined by OCT.
Methods: All experimental protocols involving mice were approved by the Animal Care and Use Committee of Osaka University Graduate School of Medicine (Reference Number: 02-083-028). Each mouse (male) was anesthetized adequately with an intraperitoneal injection of the combination anesthetic (0.3 mg/kg of medetomidine, 4.0 mg/kg of midazolam, and 5.0 mg/kg of butorphanol). A tracheotomy was conducted for maintenance of spontaneous breathing. A fenestra was surgically opened on the ventrolateral site of the bulla to provide the light beam derived from a swept-source OCT system for the sensory epithelium in the cochlear apical turn.

The epithelial vibrations were measured with acoustic stimuli at different frequencies (3.6−14.4 kHz; 40−80 dB SPL). Thresholds of auditory brainstem responses (ABR) were determined before and after the in vivo vibration measurement. Student t-tests were used for statistical analysis.
Results: The best frequency (BF) measured at the basilar membrane region of sensory epithelium in mice at 6−7 months of age was 6.8 ±1.4 kHz (mean ± S.D.; n = 7), which was significantly lower than the value, 9.4 ±0.9 kHz, obtained in mice at 1−3 months of age (n = 6) (p = 0.006).This BF change is comparable to ~15% position shift along longitudinal axis of the cochlea. Note that thickness and width of the sensory epithelium in the apical turn and ABR threshold at 4 k and 8 kHz were similar between the two groups, although significant hearing loss at 32 kHz and 46 kHz were already detected in older mice.
Conclusions: These observations indicate that frequency tuning in the apical turn is altered in half a year after birth in C57BL/6J mice, although the hearing level remains intact. This element may partially contribute to change of hearing quality during aging in human.

Revisiting Key Hypotheses in Cochlear Micromechanics Regarding Traveling Wave Amplification and Longitudinal Vibrations

George Samaras[1], Julien Meaud*[1]

[1]*Georgia Institute of Technology*

Background: Recent vibratory data of the organ of Corti (OoC) challenges the classical view of cochlear amplification centered around the basilar membrane (BM). Not only have substantial differences been observed in the transverse vibrations of the outer hair cells (OHCs) and Deiters' cells (DCs) compared to those of the BM, but also a large longitudinal vibratory component has been identified experimentally at the OHC-DC junction. Altogether, these recent experiments require a revision of theories about cochlear micromechanics.
Methods: In this work, we build on a previously developed, physiologically based model of the gerbil cochlea that includes a detailed micromechanical model of the OoC. The previous micromechanics only allowed for rigid DCs, did not include the phalangeal processes (PhPs), nor any longitudinal vibration. In this work, we expand the model in several ways: 1.) we allow for DC compliance, 2.) we include the longitudinal tilt of the OHCs and PhPs, 3.) we model the 3D vibrations of the OHC-DC junction including its longitudinal component. This is realized by using a lumped-element representation of the OoC in which structures are modeled as discrete elements with bending and axial stiffnesses. The impact of these changes is assessed by comparing model predictions to experiments and by characterizing power flow within the organ of Corti in the context of TW amplification. We also evaluate how longitudinal motion influences the observed motion along a specific direction in a way that mimics how OCT measures displacement in the OCT beam direction.
Results: We demonstrate that the inclusion of DC compliance limits power delivery from the bottom of the OHCs to the BM through the DCs. We find that model predictions are inconsistent with measurements unless the OHCs deliver power to BM-fluid TWs through an alternative pathway via the pillar cells (PCs). This model is in good agreement with a series of experimental results: 1.) Extended frequency range of nonlinearity at the OHC-DC junction, 2.) phase lag of the OHC-DC junction relative to the BM, 3.) large tonic displacement on the OHC-DC junction.

Using realistic anatomical and stiffness and parameters, we find that the longitudinal tilt of the OHCs and PHPs give rise to longitudinal motion that is larger than the transverse motion at the OHC-DC junction. When the projected direction is varied, the phase of the OHC-DC junction relative to the BM varies in the same way as observed in recent OCT measurements.
Conclusions: Our model with compliant DCs and power delivered through the PCs make predictions consistent with experiments. With the longitudinal tilt of the OHCs and PhPs, the vibrations of the OHC-DC junction are predicted to be primarily longitudinal, which is also in line with recent experiments.

Podium 8: Hair Cell Regeneration in Fish and Mice

Moderators: Brandon Cox and Charles Morgan

8:00 a.m. - 10:00 a.m.

Ocean Ballroom 9 - 12

Cross-Species Meta-Analysis Identifies Shared and Unique Gene Expression Differentiating Hair Cells From Supporting Cells

Lisa Goodrich*[1], Mahashweta Basu[2], Nesrine Benkafadar[3], Amanda Ciani Berlingeri[4], Ivan Cruz[4], Emilia Luca[5], Jeremy Sandler[6], Seth Ament[7], John Brigande[7], Alain Dabdoub[7], Albert Edge[7], Ksenia Gnedeva[7], Andrew Groves[7], Stefan Heller[7], Ronna Hertzano[7], Tatjana Piotrowski[7], David Raible[7], Yehoash Raphael[7], Jennifer Stone[7], Litao Tao[7], Mark Warchol[7]

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Background: Hair cells exhibit a variety of morphologies and functions and differ in the capacity to regenerate from the surrounding supporting cells across the animal kingdom. To understand the molecular differences that influence how hair cells are generated during development and after damage, we carried out a cross-species, cross-organ meta-analysis of gene expression in 29 populations of hair cells from developing and mature zebrafish, chicken, mouse, and human sensory organs.
Methods: For each hair cell population, we identified genes enriched by comparison to the aggregate population of supporting cells in the same dataset. Since gene expression was measured using a variety of approaches, the data were harmonized by calculating the area under the receiver operator curve (AUC), a machine learning metric for how well a gene's expression can distinguish between supporting cells and hair cells. This allowed us to directly compare the degree of gene enrichment between different hair cell datasets.
Results: 4964 genes were enriched in at least one hair cell population based on their AUC scores. Unsupervised hierarchical clustering identified 22 groups of genes with highly correlated expression patterns. Twelve clusters segregated by species, including one for mouse inner hair cells and one for mouse outer hair cells. Four clusters showed similarities across two species,

including one enriched for hair cells associated with hearing in mouse and chicken. Additionally, four clusters contained genes enriched in most hair cell types, regardless of species, organ, or age. Several of these pan-hair cell-enriched genes encoded proteins known to be required for mechanotransduction. To survey more broadly, we used machine learning to compare gene expression in each hair cell population to a curated set of known hair cell-enriched genes and identify those that are consistently enriched in hair cells compared to supporting cells. This list of 884 hair cell-enriched (HCE) genes included many known deafness genes and represented gene ontology terms such as “sensory perception of sound”, “stereocilium”, and “pre-synapse”. Enriched expression in hair cells was confirmed by in situ hybridization in fish, chicken, and mouse sensory organs. In addition, we confirmed that varying proportions of HCE genes are expressed in immature hair cells prior to the onset of mechanotransduction, in newly regenerated chicken hair cells, in hair cells produced from neonatal mouse supporting cells in organoids, and in hair cell-like cells generated by expression of Gfi1, Atoh1, and Pou4f3 in fibroblasts.
Conclusions: Our analysis uncovered a rich landscape of gene expression that drives similarities and differences among hair cells. The HCE gene list offers a new tool for assessing and dissecting mechanisms of regeneration across systems. Further, the complete set of harmonized data can be further mined on gEAR to identify candidate genes associated with a specific hair cell type or for promoting regeneration.

Multimodal Analysis of Gene Regulatory Networks Driving Zebrafish Inner Ear Regeneration

Erin Jimenez*[1]

[1]*Johns Hopkins University*

Background: Damage to mammalian inner ear hair cells is irreversible, leading to permanent hearing loss or vestibular defects. This lack of regenerative capacity sets mammals apart from most other vertebrates, which can continually produce new hair cells throughout their lifetimes or can regenerate them in response to trauma. The zebrafish is a non-mammalian vertebrate that has the remarkable ability to completely regenerate hair cells in the inner ear. Previously, gene regulatory networks controlling hair cell regeneration were reconstructed by integrating transcriptomic and epigenomic data at single-cell resolution. We identified unique, cell-specific transcription factor (TF) motif patterns in the chromatin that opened specifically during regeneration. We correlated this emergent enhancer activity with differential gene expression to identify key gene regulatory networks driving regeneration. We observed a pattern of overlapping Sox- and Six-family transcription factor (TF) gene expression and binding motifs was detected, suggesting a combinatorial program of TFs driving regeneration and cell identity. Analysis of single-cell transcriptomic data suggested supporting cells within the inner ear changed cell identity to a “progenitor” cell population that could differentiate into hair cells or potentially revert into supporting cells.
Methods: We investigated if Sox- and Six- transcription factors and putative regeneration-responsive regulatory regions containing Sox- and/or Six- binding motifs were necessary in zebrafish hair cell regeneration by systematically testing function through gene knockouts and mass mutagenizing putative enhancer regulatory regions using CRISPR/Cas9 guided mutagenesis. We screened for hair cell regeneration defects using the lateral line as a proxy for

large scale testing of regeneration phenotypes. Finally, we used fluorescent in situ hybridization to gain spatial information and determine the expression pattern of sox and six genes in the inner ear.
Results: We targeted the sox2, sox21a, sox4b, sox10, six1a, six1b, six2a, six2b, six4b genes and assessed hair cell regeneration phenotypes in "crispants". In comparison to controls, non-injected 7-day old fish that undergone regeneration, we found a significant hair cell regeneration defect in larval lateral line for the sox2, sox21a, sox4b, six4b, six2a, and six2b crispants. Sox10, six1a, six1b crispants were lethal. Spatially resolving the subcellular localization of all Sox and Six TFs detected in our scRNA-seq dataset indicates that the Sox TFs are mostly enriched in the supporting cells, while Six TFs are enriched in hair cells during regeneration. Implementation of MIC-Drop to target 40 loci containing Sox and/or Six transcription binding motifs indicates that 7% of the regeneration enhancers tested are essential for hair cell regeneration.
Conclusions: By combining knockout strategies, phenotypic analysis, and transcription factor gene expression, we find that hair cell regeneration is driven by a combinatorial "code" of TFs that initiate regeneration and instruct hair cell differentiation.

Accessibility of Developmental Enhancers Maintains Competency for Hair Cell Regeneration

Tuo Shi[1], Xizi Wang[1], Yeeun Kim[1], Juan Llamas[1], Gage Crump[1], Ksenia Gnedeva*[1]

[1]*University of Southern California, Keck School of Medicine*

Background: Unlike non-mammalian vertebrates, adult mammals cannot replenish lost hair cells, resulting in permanent hearing loss. This deficiency stems, in large part, from the failure of mature supporting cells to activate hair cell gene program even in response to Atoh1, a transcriptional master regulator necessary and sufficient for induction of mechanosensory hair cells during development. We recently demonstrated that competence to respond to Atoh1 is established in the prosensory progenitors between E12.0 and 13.5. The transition to the competent state is rapid and is associated with extensive remodeling of the epigenetic landscape.
Methods: We utilized single cell and bulk RNA and ATAC sequencing analyses to investigate gene expression and chromatin accessibility changes during inner ear development. We used Tol2 transgenesis to test the ability of identified putative enhancers to drive fluorescent reporter expression, when combined with a minimal E1b promoter. In mouse the same elements were similarly tested for their ability to drive reporter activity when delivered to the inner ear in vivo via AAVs.
Results: We identified a group of putative regulatory elements established in E13.5 and maintained in the neonatal supporting cells capable of transdifferentiation. Focusing on Atoh1 locus itself, we show that these enhancers are silenced in the mammalian inner ear postnatally, correlating with loss of regenerative capacity. Strikingly, however, the same elements are maintained in the adult supporting cells of the zebrafish inner ear, capable of regeneration. Deletion of these enhancers in zebrafish severely impaired atoh1a expression and HC formation during development and regeneration.
Conclusions: Our findings demonstrate the pivotal role of epigenetic changes in establishing and maintaining competence for sensory receptor differentiation in the inner ear. We hope that

identifying the molecular program that controls competency-related enhancers may uncover a path for mammalian hair cell regeneration.

Exploring Ger-Derived Organoids as a Model for Cochlear Regeneration: Insights From a Single-Cell RNA Sequencing Study

Marie Kubota*[1], Julia M. Abitbol[2], Paul K. Lee[2], Sonia Bustos Barocio[2], Taha A. Jan[3], Alan G. Cheng[2], Stefan Heller[2]

[1]*Stanford University School of Medicine,* [2]*Vanderbilt University Medical Center*

Background: The induction of hair cell regeneration is efficient in neonatal mice. Cochlear non-sensory cells, primarily greater epithelial ridge (GER; Kolliker's organ) cells in the organ of Corti, serve as progenitors and give rise to new hair cell-like cells in vitro and in vivo. We previously demonstrated that GER cells have robust proliferative ability in vitro, forming otic organoids that contain cochlear sensory epithelium-like patches composed of new hair cell- and supporting cell-like cells. In this study, we aim to 1) analyze growing GER-derived organoids using single-cell RNA sequencing to identify genes and pathways that drive the regenerative proliferation of GER cells, and 2) analyze the in vivo relevance of the organoid model to explore the organoid system as a screening platform for cochlear hair cell regeneration in adult mammals.
Methods: GER cells were collected at GREATER THAN 90% purity from postnatal day 2 cochlear duct cells of Fgfr3-tdTomato/Sox2-GFP transgenic mice using fluorescence-activated cell sorting (FACS). GER cells were cultured in a media conducive to organoid growth, harvested at serial proliferation phases, and analyzed with single cell-RNA sequencing. Computational analysis combined with a modulation of specific pathways using small molecules and adeno-associated virus (AAV)-mediated overexpression of putative key genes in GER-derived organoids revealed essential gene expression patterns associated with GER cell proliferation. An in vivo mouse cochlear damage/regeneration model was used to investigate the significance of the proliferation process identified in GER-organoids.
Results: We identified putative cell growth-promoting effectors in GER-derived organoids, including galectins, Myc, and a set of transcription factors. Small molecule screening and AAV-mediated overexpression of genes revealed that galectin-1, galectin-3, and Myc are essential drivers of otic organoid cell growth. The upregulation of these genes was observed in an in vivo mouse damage model during the regenerative process. The in vivo effect of the pathway modulation, as well as the correlation to the transcriptomic changes in GER-derived organoids is explored here.
Conclusions: Our single-cell transcriptomic analysis of GER-derived organoids revealed dynamic gene expression changes during organoid growth. We identified effector molecules that activate GER cell proliferation. We are in the process of validating our findings in an in vivo mouse cochlear damage model to examine the potential of employing the organoid system as a testing platform for regenerating cochlear hair cells in adult mice.

Spatial and Transcriptomic Determinants of Regenerated Hair Cell and Supporting Cell Fates

Sara Billings*[1], Lingjun Zhang[1], Roshni Parulekar-Martins1[1], Andrew Groves[2], Alan G. Cheng[1]

[1]*Stanford University,* [2]*Baylor College of Medicine*

Background: The mammalian cochlea consists of sensory and non-sensory cells that are precisely arranged spatially: there are gradients of gene expression over the apex-base and medial-lateral axes. As the mammalian cochlea is non-regenerative, numerous groups have successfully applied reprogramming approaches to induce hair cell regeneration. The gene expression of regenerated cells and whether and how they form gradients are largely unknown. Emerging technologies such as Slide-Seq (CurioSeeker) combines the sensitivity of traditional single cell RNA sequencing methods with barcoded bead technology that captures RNA directly from the tissues on a slide, thereby preserving spatial information. Here, we generated a transgenic mouse model (Glast-Cre; Rosa-Atoh1) with ectopic hair cells and supporting cells in the greater epithelial ridge (GER) and applied CurioSeeker to probe their gene expression.
Methods: Glast-Cre; Rosa-Atoh1 and control littermates were administered tamoxifen and examined from P4-21 as sections and whole mounts. Tissues were fixed and immunostained or processed for RNA scope in situ hybridization. Separately, tissues were frozen, sectioned and processed for CurioSeeker. Subsequent sequencing of the barcodes and captured mRNA allows identification of the original location of each transcript. These data were computationally analyzed and validated histologically.
Results: In the control cochlea, central and medial GER lacks Sox2 expression and the whole GER atrophies between P7-10. In the Glast-Cre; Rosa-Atoh1 mice, robust formation of ectopic Myo7a+ hair cells and Sox2+ supporting cells was noted in the GER in each turn of the P7 cochlea. Both medially and laterally positioned hair cells appear innervated and express markers of inner or outer hair cells over time. Interestingly, inner hair cell-like cells are surrounded by inner phalangeal cell-like cells (Fabp7+) and outer hair cell-like cells by Deiters' and pillar cell-like cells (Fgfr3+), suggesting a medial-lateral gradient. In both control and Glast-Cre; Rosa-Atoh1 cochlea, CurioSeeker confirms that mRNA was captured and present in the expected sensory and non-sensory cell types shown histologically. We will share additional computational analyses and validation of maturation of sensory and non-sensory cell subtypes.
Conclusions: Ectopic hair cells and supporting cells form spatial gradients in postnatal mouse cochlea. Their spatial identities and expression can be captured using CurioSeeker with histologic validation.

The Notch Ligand Jagged1 Plays a Dual Role in Cochlear Hair Cell Regeneration

Angelika Doetzlhofer*[1], Xiao-Jun Li[2], Charles Morgan[1], Lin Li[2], Elena Chrysostomou[3]

[1]*Johns Hopkins University School of Medicine,* [2]*Xi'an Jiaotong University,* [3]*Salk Institute for Biological Studies*

Background: Hair cells (HCs) within the inner ear cochlea are highly specialized mechano-sensory cells that enable us to detect sound. In humans and other mammals, HC loss is permanent and a leading cause of deafness. Recent studies in newborn mice revealed that cochlear supporting cells (SCs) have the capacity to form HCs, and that inhibition of Notch

signaling using gamma-secretase inhibitors (GSI) dramatically increases the otherwise low rate of SC-to-HC conversion. It has been proposed that in the absence of HCs, the SC-specific Notch ligand Jagged1 (JAG1) mediates the HC-repressive role of Notch signaling.
Methods: We investigated the role of JAG1 and its potential Notch receptors (Notch1-3) in HC regeneration using cochlear organoid and explant culture models. To manipulate gene function, we used mouse genetic tools, lentiviral strategies, small molecule inhibitors and peptides. JAG1-regulated genes/pathways were identified by bulk RNA sequencing and changes in PI3K-Akt-mTOR signaling strength was accessed using immuno blots.
Results: We find that acute ablation of Jag1 mildly increasing the rate of HC-formation in early postnatal cochlear organoid and explants culture. However, loss of Jag1 also diminishes the ability of cochlear SCs cells to re-enter the cell cycle and proliferate, which we find is linked to a reduction in the expression of progenitor and metabolic genes, and an attenuation of PI3K-Akt-mTOR signaling. Furthermore, we demonstrate that Notch2 function is critical for maintaining mitotic capacity of cochlear SCs and show that Notch1 and Notch2 are required to sustain PI3K-Akt mTOR signaling in cochlear SCs. Moreover, we show that transient stimulation of JAG1/Notch signaling prior to administration of HC-fate inducing cues (GSK3 inhibitor and GSI treatment) enhances SC-to-HC conversion in an mTOR-dependent manner.
Conclusions: Our findings indicate that Notch ligand JAG1 has opposing functions in cochlear HC regeneration: On one hand JAG1 antagonizes HC fate induction in SCs, and on the other JAG1 is required to preserve the "progenitor-like characteristics" of SCs, a feature we previously found to be critical for cochlear HC regeneration. Moreover, our findings suggest that while high levels of Notch signaling limit HC fate induction, a baseline level of JAG1/Notch1/2 signaling is required to maintain the mitotic and HC-regenerative potential of cochlear SCs.

Reprogramming Supporting Cells With Small Molecules for Cochlear and Vestibular Hair Cell Regeneration

Hanae Lahlou*[1], Hong Zhu[2], Wu Zhou[2], Albert S. B. Edge[3]

[1]*Harvard Medical School,* [2]*University of Mississippi Medical Center,* [3]*Massachusetts Eye and Ear Infirmary/Harvard Medical School*

Background: We previously showed that a drug cocktail consisting of a GSK-3β inhibitor to activate Wnt signaling and an HDAC inhibitor to modify the openness of chromatin increased proliferation of supporting cells and differentiation of cochlear and vestibular hair cells in organoids made from newborn Lgr5-expressing cochlear progenitor cells. Here we asked if this cocktail was effective in the regeneration of hair cells in the adult vestibular system.
Methods: To investigate the impact on vestibular hair cell regeneration, we employed adult Pou4f3-DTR mice, expressing the diphtheria toxin (DT) receptor in hair cells for targeted ablation, as well as Plp1-CreER crossed to floxed-STOP-tdTomato reporter mice for tracing of cell lineage. Utricles from in vivo DT-ablated WT and Pou4f3DTR/+ adult mice were cultured in the presence of a GSK-3β inhibitor (CHIR), an HDAC inhibitor (VPA), or their combination (CHV). We also compared spontaneous vs drug-induced regeneration between treated and untreated (contralateral) ears after in vivo local drug delivery via the posterior semicircular canal.
Results: CHV treatment significantly increased MYO7A+ hair cells compared to CHIR and VPA alone. Lineage tracing revealed both type I (MYO7A+SOX2-) and type II

(MYO7A+SOX2+) hair cells derived from Plp1+ supporting cells. In vivo, CHV treatment resulted in regeneration of 58% of the normal number of hair cells after 2 months as compared to 32% in the DT-treated ear without drug treatment. Functional improvements were confirmed by vestibuloocular reflex and vestibular afferent recordings.
Conclusions: Our findings provide further knowledge of the molecular mechanisms of vestibular hair cell regeneration, highlighting the potential clinical application of this drug combination for treating balance disorders related to loss of hair cells.

Treatment of Vestibular Dysfunction Through Hair Cell Regeneration by Dual Aav-Mediated Crispr Activation

Chenxi Jin*[1], Zhengyi Chen[2], Yong Tao[3], Hao Wu[4]

[1]*Shanghai Jiao Tong University,* [2]*Mass Eye and Ear Infirmary/Harvard Medical School,* [3]*Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine,* [4]*Shanghai Ninth People's Hospital, Ear Institute, Shanghai Jiao Tong University School of Medicine; Shanghai Key Laboratory of Translational Medicine on Ear and Nose Diseases*

Background: The vestibular system is important for maintaining balance. Vestibular hair cells (vHCs) death is the major cause of vestibular dysfunction affecting a large portion of the elderly population. vHC regeneration is a viable approach to restore vestibular function. In mammals, spontaneous vHC is limited. Viral-mediated Atoh1 overexpression has been extensively used to regenerate vHCs by transdifferentaiton of supporting cells. However, the vHC regeneration efficiency is low and the clear evidence of the restoration of vestibular function by the approach is lacking. Here, we used dCas9-CRISPR mediated activation (CRISPRa) to modulate Atoh1 expression to a level similar to the Atoh1 endogenous level to promote efficient vHC regeneration and restore vestibular functions.
Methods: Neonatal and adult dCas9-SPH transgenic (dCas9 TG) mice and WT mice were used. To activate Atoh1 using CRISPRa, cre-gRNAs were delivered to the inner ear of dCas9 TG mice, and dual AAVs carrying the CRISPRa were injected to WT mice. Intraperitoneal injection of IDPN was used to cause bilateral vestibulopathy, with IDPN injected 1 day before AAV injection in adult mice or 2 weeks after AAV in neonatal mice. Mouse utricles were harvested for RT-PCR analysis and quantification for vHCs. vHCs were studied by stereocilia morphology and neural connections using confocal and scanning electron microscopy. The vestibular balance functions were evaluated by HC electrophysiological functional test (VsEP, VEMP and VOR) and behavioral tests (balance beam, rotarod and open field).
Results: Compared to the damaged group, CRISPRa-mediated Atoh1 expression increased the number of vHCs, which had characteristic vestibular stereocilia morphology and neuron connections. Type I and type II vHCs were efficiently regenerated and were viable long-term. The vestibular electrophysiological functions and balance functions were significantly restored in dCas9 transgenic and WT adult mice for 6 months after damage.
Conclusions: CRISPRa activates Atoh1 in a manner similar to the endogenous Atoh1 expression, resulting in robust regeneration of mature vHCs with neuronal connections. vHC regeneration restores vestibular functions long-term in mice with bilateral vestibulopathy. The study lays a foundation for developing therapies for balance disorders by vHC regeneration in humans.

Symposium 4: Hair-Cell Evolution: Insights From New Model Organisms, Comparative Studies, and Molecular Analyses

Chair: Marcos Sotomayor*, University of Chicago*

10:30 a.m. - 12:30 p.m.

Ocean Ballroom 1 - 4

Symposium Description: The sensory epithelium of the inner ear, found in all extant lineages of vertebrates, is older than the vertebrate lineage itself. Having originated as a small patch of cells in our aquatic ancestor, it has been subjected to over 500 million years of evolution and natural selection, resulting in the complex and finely tuned inner ear of modern vertebrates. Inner ear adaptations are as diverse as the species from which they are found, and such unique anatomical variations have been well studied. However, the evolutionary details of the molecular machinery that allows for this diversity are less well known. This symposium will focus on new model organisms and comparative studies providing insight into hair-cell evolution as well as on the molecular evolution of the machinery that enables sensory perception and sound amplification by hair cells. The symposium will start with an introduction to hair-cell mechanotransduction and tip link evolution (Sotomayor, 15 min), followed by presentations on the molecular evolution of prestin (Araya-Secchi, 15 min) and the identity of the gating spring in Drosophila (Göpfert 30 min). The second half of the symposium will be dedicated to new model organisms and the evolution of mechanosensitive proteins (Fuller, 30 min and Perozo, 30 min).
Eduardo Perozo*, University of Chicago*

Molecular Evolution of Tip-Link Proteins

Emily Scheib[1]

[1]*The Rockefeller University*

Individual Abstract: Vertebrate hair cells have evolved over 500 million years to function as exquisitely precise and robust mechanosensors that transform mechanical stimuli into electrochemical signals for brain processing. Critical for this process of mechanotransduction is the tip link, a fine protein filament conveying force to gate ion channels and trigger sensory perception. In various mammals, cadherin-23 and protocadherin-15 interact to form tip links. Crystal structures of mouse protein fragments have shown that this interaction is mediated by the protein tips, which engage in an antiparallel “handshake complex” essential for hair-cell mechanotransduction that has been validated in vitro and in vivo. Structural studies have also shown that the mammalian protocadherin-15 protein forms parallel homodimers involving two subdomains, and that cadherin-23 features a membrane adjacent domain that might be mechanically weak. Here we report evolutionary analyses, biophysical experiments, and structures of non-mammalian tip-link proteins that suggest specific adaptations and general mechanisms of tip link function throughout vertebrates.

Structural Insights Into the Evolution of Mammalian Prestin From Anion Transporter to Area Motor

Raul Araya-Secchi[1]

[1]*Computational Biophysics Group; Facultad de Ingenieria, Arquitectura y Diseño. Universidad San Sebastian*

Individual Abstract: The mammalian cochlea shows remarkable sensitivity and frequency selectivity, primarily owing to the mechanical amplification of sound by Outer Hair Cells (OHCs) through electromotility (EM). This process is driven by prestin, a membrane protein from the SLC26A family that acts as a voltage-dependent area-motor. Prestin serves this role in mammals, whereas in non-mammals, it functions as an anion transporter without significant piezoelectric activity. Previous studies have identified changes in the transmembrane domain (TMD) that could explain the transition from transporter to area-motor; however, they have not been examined in the context of its structure and dynamics. Additionally, the IVS in the STAS domain, a highly variable region of the SLC26A family, has been largely overlooked. In this study, we investigated the structural and functional evolution of prestin using phylogenetic analysis, ancestral sequence reconstruction (ASR), and structural modeling. We identified key amino acid substitutions that differentiated mammalian prestin from non-mammalian ancestors and analyzed these changes in a structural context. Our approach highlights known functional areas and focuses on unexplored regions, particularly the IVS of the STAS domain. Our results indicate that early substitutions in the TMD, which occurred during the transition from amphibians to amniotes, reduced ion transport capability and increased voltage sensitivity crucial for EM by altering protein interactions and lipid interactions. Intermediate and late substitutions further optimized this function, enhancing the frequency selectivity that is essential for mammalian hearing. In placental mammals, a unique patch of negatively charged residues in the IVS region of the STAS domain appears to interact with the intracellular cavity of the adjacent monomer, potentially affecting voltage response and EM. These findings suggest that sequence and structural changes in prestin have been finely tuned throughout evolution to meet the auditory needs of mammals.

The Nompc Gating Spring

Thomas Effertz[1]

[1]*University of Goettingen*

Individual Abstract: The transient receptor potential (TRP) channel NOMPC is a mechanosensory transduction channel implicated in Drosophila touch sensation, proprioception, and hearing. Compared to other TRPs, NOMPC bears an exceptionally long amino-terminal ankyrin repeat (AR) domain that, consisting of 29 ARs, assumes a helical structure reminiscent of a coil spring. Accordingly, the NOMPC AR domain seems deemed to function as a gating spring. Consistent with such function, the NOMPC AR domain (i) tethers the channel intracellularly to microtubules, (ii) conveys force to the gate of the channel, and is (iii) essential for NOMPC mechano-gating. Moreover, NOMPC gating introduces a nonlinear gating compliance in the fly's auditory mechanics, signaling that NOMPC gating involves a gating spring.

To qualify as the gating spring, softening the NOMPC AR domain through domain duplication should reduce the propensity of the channel to open spontaneously and necessitate twice larger displacements to force open the channel. Most importantly, duplicating the AR domain should alter the NOMPC-gating compliance by halving the gating stiffness, especially as stacks of AR repeats show Hookean behavior and act as nanosprings.

We now have performed these tests, revealing that the AR domain is not the NOMPC gating spring. Tandem duplication of this domain affected neither NOMPC gating in vitro, NOMPC function in vivo, and the intrinsic stiffness of the NOMPC gating spring. Molecular dynamics simulations reveals that the ankyrin domain is flexibly hinged on the gate by a short linker element. When we tandem duplicated this linker, the channel opened spontaneously less often in vitro and its open probability was shifted to twice larger stimulus pressures. In vivo, twice larger displacements were required to activate NOMPC, and the gating spring stiffness was reduced by 50%. This halving of the gating spring stiffness was specific; channel number, gating swing, and single channel conductance all remained unchanged. Intriguingly, the duplicated linker functionally reverted into a single one when half of it was stabilized by crosslinking. Judging from our in vivo mechanical measurements, the linker governs native gating spring mechanics and, accordingly qualifies for being the gating spring.

Implications concerning gating springs in general, and the hair cell gating spring in particular, will be discussed.

Molecular Characterization of Tunicate Coronal Organ Mechanosensory Cells

Gwynna Fuller[1]

[1]*Baylor College of Medicine*

Individual Abstract: The vertebrate inner ear is a complex sensory organ that detects balance and sound information using specialized mechanosensory cells called hair cells. Hair cells possess an apical bundle made of actin-rich stereovilli and a single kinocilium that extend from the cell body. In response to mechanical stimuli, hair cells depolarize and release neurotransmitters to the afferent neurons that innervate them. While the development and function of vertebrate hair cells are well studied, the evolutionary origin of this cell type in chordates remains unknown. Tunicates, the closest living relatives to vertebrates, are sessile filter-feeders as adults and use the coronal organ, a row of mechanosensory “hair cell-like cells”, to detect particle and water flow through their oral siphons. We have begun characterizing these mechanosensory cells using single-nucleus RNA-seq (snRNA-seq) and hybridization chain reaction (HCR). From our snRNA-seq data, we have identified a putative hair cell-like cell population which expresses homologs of vertebrate hair cell-associated genes, including human deafness genes like Otof and Cdh23. Additionally, these cells express genes associated with vertebrate hair cell mechanotransduction and synaptic activity. Our preliminary results indicate that the tunicate coronal organ sensory cells are molecularly homologous to vertebrate hair cells.

Our future experiments will focus on functional and developmental testing to support this conclusion.

Evolutionary Approaches to Cellular and Molecular Mechanotransduction

Eduardo Perozo[1]

[1]*University of Chicago*

Individual Abstract: Mechanotransduction, the process by which cells convert mechanical cues into electrical or chemical signals, plays a fundamental role in a wide range of biological processes. As in other sensory systems, the molecular underpinnings of mechanotransduction represent evolutionary solutions to explicit physicochemical problems faced by living organisms: How to sense voltage across the membrane; how to detect odorants mixtures at low concentration; how to detect single photons of multiple wavelengths. However, mechanical transduction displays a broad array of molecular solutions to the problem of sensing force in biological systems. This fact highlights the many biophysical principles by which force can be transduced in biological systems. Here, I will provide an overview of the different cellular and molecular solutions that have evolved to detect force in sensory systems, their putative evolutionary relations, and evaluate alternative approaches to address challenging questions surrounding mechanosensitivity.

Podium 9: Auditory Cortex: Human and Animal Studies

Moderators: George Ordiway and Steven Eliades

10:30 a.m. - 12:30 p.m.

Ocean Ballroom 5 - 8

Large-Scale Recordings of Human Single Neuron Activity During Auditory Working Memory

Joel Berger*[1], Alexander Billig[2], Phillip Gander[3], Sukhbinder Kumar[3], Christopher Kovach[3], Ariane Rhone[3], Christopher GARCIA[3], Hiroto Kawasaki[3], Matthew Howard[3], Timothy Griffiths[4]

[1]*University of Iowa,* [2]*UCL Ear Institute,* [3]*University of Iowa Hospitals and Clinics,* [4]*Biosciences Institute, Newcastle University*

Background: In order to perceive the world around us and communicate effectively, our brains have to maintain elements of auditory scenes in memory. Recent intracranial work employing visual paradigms has begun to elucidate the specific neural mechanisms involved in these processes at the single neuron level, highlighting contributions of structures such as the hippocampus and cingulate cortex. However, it is not yet clear whether the same mechanisms are involved in the processing of acoustic stimuli.

Methods: Here, we report human intracranial recordings of a large number of single neurons (n = 1269), recorded from various brain structures while participants performed an auditory working memory task. We intentionally used non-verbal stimuli to isolate memory-specific processes and avoid potential confounds of including semantic information. Participants were required to keep in mind a target tone on each trial and then – following a delay period – adjust a repeated tone to match the frequency of the target. Single neurons were isolated offline using an automated procedure with manual curation.
Results: We found neurons within the hippocampus, insula and cingulate cortex for which firing rates were modulated at various phases of the working memory task, including throughout the delay period and during active tone adjustment. Often, these neurons showed suppression rather than increased activity, though there was heterogeneity in response types across the population. Across the entire neuronal population, state space analyses demonstrated that the different task phases were clearly separable.
Conclusions: Overall, these data implicate the hippocampus, insula and cingulate cortex in auditory working memory.

An Eligibility Trace for Synaptic Plasticity in the Auditory Cortex

Brendan Williams*[1], Tanya Danaphongse[2], Seth Hays[3], Crystal T. Engineer[3]

[1]*University of Texas at Dallas,* [2]*Texas Biomedical Device Center,* [3]*The University of Texas at Dallas, Texas Biomedical Device Center*

Background: A fundamental concern in neuroscience and neural rehabilitation is how the brain adapts and changes its representation of sensory information. The representation of sensory information is sensitive to experience, changing with sensory organ loss (e.g. hair cell damage) or extensive training (e.g. musicians), but changes can also be directly induced with paired neuromodulator release. Stimulation of the vagus nerve drives activity in the nucleus basalis/ambiguous, ventral tegmental area, locus coeruleus, and dorsal raphe nucleus - causing widespread cortical activation and the corelease of acetylcholine, dopamine, norepinephrine, and serotonin. Pairing vagus nerve stimulation with a 9kHz tone leads to an increase in the representation of 9kHz in the auditory cortex. This makes vagus nerve stimulation a unique tool for studying cortical plasticity, since it can directly evoke it. Previous studies have shown, ex-vivo, a time window after synaptic activity where a synapse is marked and eligible for plasticity. Here we ask a similar question in-vivo, whether neuromodulator release (driven by VNS) needs to be precisely coupled with synaptic activity to lead to plasticity.
Methods: Adult male and female sprague dawley rats were implanted with cuff electrodes around the left cervical vagus nerve at postnatal day 90. After recovery, rats underwent 20 days of vagus nerve stimulation paired with a 9kHz tone. Across six groups, we varied the timing of VNS relative to the tone - before, during, or after. 24 hours after the last tone pairing, rats were anesthetized, and multi-unit extracellular recordings were taken from the primary auditory cortex. To compare the degree of cortical plasticity that has resulted from each pairing paradigm, we calculated the percent change in the representation of each frequency octave from experimentally naïve animals.
Results: At the time of submission, this data collection is still in progress.

Conclusions: Since we know that concurrently paired VNS leads to cortical plasticity, we hypothesize that the degree of plasticity evoked by VNS will taper off as neuromodulator release is dissociated from synaptic activity. These findings will directly inform the clinical application of vagus nerve stimulation which currently relies heavily on the precise timing of VNS for motor and auditory rehabilitation. Furthermore, determining the precise timing of neuromodulator release necessary for inducing cortical plasticity may provide evidence for a novel molecular mechanism behind eligibility traces.

Human Auditory Cortex Integrates Information in Speech and Music Using Similar Timescales

Zehua Kcriss Li*[1], Thomas Wychowski[1], Webster H. Pilcher[1], Samuel Norman-Haignere[1]

[1]*University of Rochester Medical Center*

Background: Music and speech play a central role in human hearing, and there is evidence that the human auditory cortex has specialized neural mechanisms for processing these two important sound categories. One longstanding hypothesis is that the auditory cortex integrates across distinct timescales in speech and music with longer integration windows for music vs. speech. However, no studies have directly estimated integration windows for speech and music in the human auditory cortex, in part due to the challenge of measuring integration windows from nonlinear systems like the brain using coarse, non-invasive neuroimaging methods.
Methods: To overcome this challenge, we separately measured integration windows for speech and music using spatiotemporally precise intracranial recordings from human neurosurgical patients. Integration windows were measured using a recently developed method (the temporal context invariance or TCI paradigm) that is effective in nonlinear systems. The TCI method measures the smallest time window within which stimuli can alter a neural response and outside of which stimuli have little effect. Integration windows were measured throughout primary and non-primary auditory cortex in both hemispheres.
Results: We replicate prior data showing that integration windows increase substantially as one ascends the cortical hierarchy from primary to non-primary regions bilaterally. In contrast, we find that integration windows for speech and music are closely matched throughout primary and non-primary auditory cortex in both the left and right hemispheres.
Conclusions: These findings suggest that neural integration windows do not change substantially with the category of sound and thus that information in music and speech is integrated using similar temporal windows.

Dynamic Role of Perineuronal Nets in Modulating Auditory Neural Plasticity During Perceptual Learning

Jessica Winne*[1], Rebecca Schrader[1], Melissa Caras[1]

[1]*University of Maryland*

Background: Perceptual training (a regimen in which a stimulus feature is gradually made more difficult to detect or discriminate) can improve a listener's ability to detect weak acoustic stimuli

or discriminate highly similar sounds. This process shapes the acquisition of speech, language, and musicianship, transforming a perceptual novice into an expert. Although prior work has revealed a critical link between cortical plasticity and the emergence of expertise, we still do not understand how perceptual expertise is consolidated. Perineuronal nets (PNNs) are extracellular matrix structures that envelop parvalbumin-positive (PV+) interneurons and regulate synapse formation and stabilization. We hypothesize that during perceptual training, transient reductions in PNN expression facilitate reorganizations of the auditory cortical network that give rise to perceptual expertise and that restoration of PNN expression promotes network stability and the consolidation of expert performance.
Methods: We performed two experiments to test our hypothesis. In the first experiment, we trained Mongolian gerbils on a perceptual training paradigm designed to elicit expert amplitude modulation (AM) depth detection and used immunohistochemistry to assess PNN expression in the primary auditory cortex (A1) at various training time points. Animals that trained with only a single highly-salient AM stimulus (and therefore should not become AM detection experts) served as controls. In the second experiment, we infused chondroitinase ABC (chABC) into A1 to enzymatically degrade PNNs before and during the training. Control animals received infusions of either saline or penicillinase.
Results: Experiment 1 revealed that training significantly reduced PNN expression in A1. This reduction was evident four hours after training as a significant decrease in overall PNN staining intensity and as a decrease in the proportion of PV+ cells surrounded by PNNs. PNN expression was renormalized within 24 hours but degraded again after each subsequent training session. Over eight days, this cycle of degradation and renormalization gradually disappeared, such that PNN expression stabilized as expert AM depth detection thresholds emerged, and PNN expression levels predicted final perceptual thresholds. In contrast, this degradation/renormalization cycle continued in animals that trained with only a highly salient AM stimulus. These results appear to be specific to A1, as training had no significant effect on PNN expression in the somatosensory cortex. In Experiment 2, we found that animals treated with chABC prior to training learned the basic task as quickly and as well as controls. However, over eight days of training, the perceptual thresholds of chABC-treated animals were significantly slower to improve than those of control animals, suggesting that chronic PNN instability interferes with day-to-day skill consolidation.
Conclusions: These findings support the hypothesis that auditory training degrades PNNs, facilitating synaptic remodeling and learning, and that PNN reconstitution is necessary to stabilize newly formed connections and consolidate newly acquired auditory expertise.

Listening to the Room: Disrupting Activity of Dorsolateral Prefrontal Cortex Impairs Learning of Room Acoustics

Heivet Hernandez Perez*[1], Jessica Monaghan[2], Jason Mikiel-Hunter[1], James Traer[4], Paul Sowman[1], David McAlpine[1]

[1]*Macquarie University,* [2]*National Acoustic Laboratories,* [3]*The University of Iowa*

Background: Navigating complex sensory environments is critical to survival, and brain mechanisms have evolved to cope with the wide range of surroundings. In noisy spaces listeners place more emphasis on early-arriving sound energy, nevertheless, reverberant energy is highly

informative about those spaces per se, and human listeners show improved speech understanding when re-encountering known reverberant environments.
Methods: We assessed the ability of listeners to perceive speech (Coordinate Response Measure corpus) in noisy and reverberant rooms. We mimicked the acoustic characteristics of real rooms using loudspeakers positioned within an anechoic chamber. Listeners were also exposed to repetitive transcranial stimulation (rTMS) to disrupt the dorsolateral prefrontal cortex activity (DLFPC), a region believed to play a role in statistical learning.
Results: Our data suggest listeners rapidly adapt to statistical characteristics of an acoustic environment to improve speech understanding. This ability is impaired when rTMS is applied bilaterally to the DLPFC. The data demonstrate that speech understanding in noise is best when exposed to a room with reverberant characteristics common to human-built environments.
Conclusions: Our findings provide evidence for a reverberation "sweet spot" and the presence of brain mechanisms that might have evolved to cope with the acoustic characteristics of listening environments encountered every day.

Instinct Versus Insight: Neural Competition Between Prefrontal and Auditory Cortex Constrains Sound Strategy Learning

Kai Lu*[1], Kelvin Wong[1], Chengcheng Yang[1], Lin Zhou[1], Yike Shi[1], Maya Costello[1], Robert Liu[1]

[1]*Emory University*

Background: In nature, animals can learn the adaptive function of new sounds, allowing them to alter their innate behaviors to better adjust to their environment. For instance, female mice are naturally motivated to search for and retrieve pups, instinctively relying on a default spatial strategy of returning to the location where they last found pups. However, they can also learn to use a new strategy relying on pup-associated sound cues to search more efficiently for pups. Learning such a sound strategy is thought to just involve reinforcing new associations and flexibly switching to use a better stimulus-action rule, but we discover that it also requires overcoming a dynamic competition against the default strategy, which actively impedes learning the new strategy.
Methods: Naïve virgin mice were trained in a T-maze to use an amplitude-modulated (5Hz) band-pass (30~50kHz) noise to search for and be rewarded with pups to retrieve (Dunlap et al, 2020). All mice initially followed a spatial "win-stay" of returning to where they last received a pup and then learned to use the sound to locate pups within 3 to 8 days. We chemogenetically inactivated the auditory cortex (ACx; Gi + CNO N = 15; Gi + Saline N = 15; EGFP + CNO N = 8) and the medial prefrontal cortex (mPFC; Gi + CNO N = 12; Gi + Saline N = 8; EGFP + CNO N = 8) during training to test their roles in sound strategy learning. We also used a model fitting approach to investigate the underlying processes during learning and the effect of chemogenetic manipulation on learning. Finally, we recorded single units from ACx (6 mice, 829 single-units, 450 multi-units) and mPFC (6 mice, 519 single-units, 447 multi-units) in freely moving animals across 8 days of training to search for the neural correlates of the default and new strategies and their changes in learning.
Results: Chemogenetic inhibition of the ACx slowed learning (p LESS THAN 0.001), as anticipated, but silencing mPFC unexpectedly accelerated learning (p LESS THAN 0.001). A

phenomenological model simulating strategy competition explained decisions better than simply reinforcing sound associations. In vivo electrophysiology in freely moving mice revealed neural correlates of the model's strategy weights in the ACx responses to sounds, which were increasingly prognostic of correct sound-cued outcomes – even on the first day of training (p LESS THAN 0.001). Meanwhile, mPFC encoded the last pup location, but this decayed as the spatial-memory strategy declined (p LESS THAN 0.001).
Conclusions: Our findings expand the concept of cognitive flexibility to include studying how a new modality for informative cues can come to prevail in decision-making and demonstrate opposing roles for prefrontal and auditory cortex in the competition between strategies in a naturalistic behavior.

Neural Decoding of Continuous Speech for Different Acoustic Features: Effects of Intelligibility and Spectral Degradation

Alexis D. MacIntyre[1], Robert P. Carlyon[1], Matthew H. Davis[1], Tobias Goehring*[2]

[1]*Cambridge Hearing Group, MRC Cognition and Brain Sciences Unit, University of Cambridge,*
[2]*Cambridge Hearing Group, University of Cambridge*

Background: During speech listening, patterns of neural activity become temporally coupled to stimulus features, which can be "decoded" from electroencephalography (EEG). These features can include acoustic properties— typically, the amplitude envelope—as well as linguistic information, such as phonemic transcription or word surprisal. Thus far, speech envelope decoding has received substantial attention as a neural correlate of auditory speech processing with the potential for clinical applications. However, an emerging consensus suggests envelope decoding primarily reflects auditory, and not speech-specific, processing. Here, we explore whether other acoustic features can be neurally decoded, and if their decoding accuracy reflects speech-specific processing.
Methods: We investigate speech acoustic feature decoding in typically-hearing listeners (N=38, 18-35 years old) who underwent continuous EEG recording during a story listening task. We disentangle auditory from speech-specific processing by presenting both intelligible and non-intelligible stimuli at three levels of acoustic clarity (unprocessed, 16-channel vocoded, and vocoded with spectral distortion). Auditory attention was sustained using a newly-developed and calibrated repeated-phrase detection task for all listening conditions. Decoding models were trained to reconstruct a set of acoustic features, including the speech envelope, from held-out neural response data, with correlations between reconstructed and true stimulus features forming the dependent variable. Significance was ascertained using random permutation tests (n = 1000).
Results: Whereas speech envelope reconstruction did not vary by spectral resolution, intelligible speech was associated with slightly better decoding accuracy in general. Results were similar across subject-specific and group analyses, with less consistent effects of spectral degradation in group decoding. Permutation tests revealed possible differences in decoder statistical significance by experimental condition. Further analyses suggest that multiple acoustic features from the spectral domain can be significantly decoded from the brain, and that patterns of decoding accuracy across listening conditions are distinct from envelope-based decoding. In particular, decoding of spectral flux and harmonic ratio, as measures of spectral variability and

over time, are sensitive to speech intelligibility and acoustic detail. Other features, such as spectral skewness, are less robustly decoded, despite their use in phonetic research.
Conclusions: Robust neural decoding was observed for the speech envelope at the individual and group level, but large variability within participants would most likely prevent the clinical use of such a measure to differentiate levels of spectral degradation and intelligibility on an individual basis. Spectral feature decoding may offer further neurophysiological insights into auditory speech perception beyond low-level acoustic processing and shows potential as a neural correlate of the auditory processing of comprehended speech.

The Impact of Musical Expertise on Disentangled and Contextual Neural Encoding of Music Revealed by Generative Music Models

Yinghao Li[1], Gavin Mischler*[1], Stephan Bickel[2], Ashesh D. Mehta[3], Nima Mesgarani[1]

[1]*Columbia University,* [2]*The Feinstein Institute for Medical Research,* [3]*Hofstra Northwell School of Medicine*

Background: Music perception requires the auditory cortex to decode individual notes and integrate them into coherent musical narratives over various timescales. Processing contextual cues is crucial for appreciating music, influencing the predictability and surprisal of musical events. While the auditory cortex's selectivity for features like pitch, rhythm, and timbre is known, the precise neural mechanisms underlying context-dependent encoding and the impact of musical expertise remain unclear. Understanding how the brain constructs coherent musical experiences by encoding both disentangled and contextual information is essential for elucidating the neural basis of music perception.
Methods: We investigated the neural basis of music perception and the impact of musical expertise using both noninvasive electroencephalography (EEG) and invasive intracranial EEG (iEEG) recordings. Twenty participants (10 musicians with extensive training and 10 non-musicians) underwent EEG recording while listening to 30 minutes of Bach piano pieces. Six additional non-musician subjects provided iEEG data. We employed a 13-layer transformer model trained for music generation (Musicautobot) to extract embeddings representing musical context. These embeddings were reduced in dimensionality using non-negative matrix factorization and used to predict neural responses via temporal response functions with cross-validation.
Results: The transformer model's layers progressively encoded more disentangled and contextually rich musical features, with deeper layers showing increased separability of musical attributes such as pitch, duration, and piece identity. Neural prediction correlations increased over transformer layers for both musicians and non-musicians, indicating alignment between the brain's encoding of musical features and the model's hierarchical processing. However, musicians exhibited significantly higher prediction correlations than non-musicians, especially in the later layers and with longer context windows, suggesting that musical training enhances neural encoding of complex musical structures. EEG data revealed greater left-hemispheric lateralization in musicians, indicating an augmented role of the left hemisphere in processing disentangled and contextual musical features due to expertise. iEEG recordings showed that electrodes farther from primary auditory cortex, particularly in higher-order temporal and frontal regions, encoded more extended musical context. Electrodes in musicians demonstrated a more

significant increase in prediction correlations with both transformer layers and context length, especially in left temporal and frontal areas. These findings support a hierarchical processing network for music in the brain, modulated by musical expertise and involving increased contextual integration over longer timescales.
Conclusions: Musical training enhances the brain's processing of complex, context-dependent musical structures, leading to more precise neural encoding. The hierarchical nature of music processing is evident, with higher-order regions integrating extended context, contributing to appreciation of predictability and surprisal. Differences between musicians and non-musicians highlight the impact of expertise on auditory system plasticity. Utilizing advanced transformer models offers valuable insights into neural mechanisms of music perception and the influence of experience, deepening our understanding of how musical training shapes auditory processing.

Podium 10: Binaural Hearing and Sound Localization

Moderators: Chris Stecker and Josh McDermott

10:30 a.m. - 12:30 p.m.

Ocean Ballroom 9 - 12

Age-Related Myelin Deficits in the Auditory Brainstem Contribute to Central Hearing Loss in Gerbils

Ben-Zheng Li*[1], Shani Poleg[1], Matthew Ridenour[1], Daniel Tollin[1], Tim Lei[2], Achim Klug[1]

[1]*University of Colorado Anschutz Medical Campus,* [2]*University of Colorado Denver*

Background: Age-related hearing loss impacts a large portion of the elderly population. Unlike peripheral hearing loss, central hearing loss is not well understood and no treatment options are available to date. The condition is thought to result from compromised information processing in the central auditory pathways and results in a listener's inability to follow a conversation of interest in background noise often despite normal hearing thresholds. Several neural substrates have been proposed to contribute to central hearing loss, including synaptopathy at the level of the auditory nerve, impaired temporal coding of sound including binaural cues in the brainstem, and age-related alteration in higher cortical areas. In this project, we focus on age-related changes in sound localization abilities, a process that highly relies on the precise temporal integration of binaural inputs in the auditory brainstem. Specifically, we investigate age related changes in a particularly heavily myelinated pathway from cochlear nucleus globular bushy cells (GBCs) to the medial nucleus of the trapezoid body (MNTB). We hypothesized that age related myelin deficits in this pathway might contribute to central hearing loss in elderly listeners.
Methods: Myelination patterns in young gerbils (68 ± 3 days old; N = 62) and aging gerbils (1034 ± 30 days old; N = 30) were quantified through electron microscopy (EM), coherent anti-stokes Raman spectroscopy (CARS), and immunohistochemistry, with their hearing ability assessed by recording auditory brainstem response (ABR) and binaural interaction components (BIC). A computational model was developed to simulate ABR and BIC waveforms and to link changes in waveform morphology to underlying neuronal alterations and myelin patterns.

Results: Aged gerbils exhibited significant alterations in ABR morphologies, including degraded wave III, wave IV, and BIC, indicating reduced and/or desynchronized MNTB activity. CARS and EM imaging showed reduced myelin thickness and axon diameter in the GBC to MNTB fiber bundles, along with a deficit in oligodendrocyte maturation. The computational model accurately replicated these ABR changes and supported the hypothesis that reduced MNTB activity contributes to central hearing loss in aging.
Conclusions: : This study reveals that age-related myelin deficits in the auditory brainstem impair binaural brainstem mechanisms of sound localization that could be a cause of central hearing loss. These findings offer insights into the underlying neuronal mechanisms and suggest novel therapeutic approaches for improving spatial hearing ability in aging populations with central hearing loss.

Auditory Brainstem Responses in Nine Wild Rodent Species with Different Social Behavior Organizations

Luberson Joseph*[1], Elizabeth McCullagh[1]

[1]*Oklahoma State University*

Background: Hearing is critical for the survival and fitness of all taxa. Previous studies determined that sex, ear shape, body size, habitat type, and environmental factors are important in influencing mammalian hearing. However, few studies have explored whether social grouping strategies influence mammalian hearing as well.
Methods: In this study, we compared the threshold, amplitude, and latency of auditory brainstem responses (ABRs) recorded from 90 wild rodents of 9 species, grouping into three broad social classifications (solitary, monogamous, social).
Results: The ABR thresholds across frequencies of these wild rodent species ranged from 25 to 40 dB SPL with maximal sensitivity around 8kHz. Social species have slightly better low-frequency hearing thresholds than other groups measured. The amplitude of wave I and IV increased with click stimulus intensity and differed considerably among different species at each intensity. Peak latency of wave I and IV decreased with click increase intensity in each species. No significant difference was detected for wave I and IV latency with increased intensity for each species. Significant differences were detected in the latency shift of the DN1 component of the BIC in relation to ITD normalized for ITD at zero across species and social groups while no significant differences were detected in relative DN1 amplitude across ITD normalized to zero ITD.
Conclusions: This data is valuable for understanding the various factors that influence hearing across species.

Auditory Competition or Binaural Decorrelation? A Comparison Between Midbrain Space Maps in the Barn Owl

Roland Ferger*[1], Andrea Bae[1], Jose Luis Pena[1]

[1]*Albert Einstein College of Medicine*

Background: The natural environment challenges the brain to prioritize the processing of salient stimuli. This is especially difficult for concurrent auditory stimuli because sound waves interact in a way that the phase of each contained frequency can be altered prior to arrival at the eardrum. Therefore, phase information is altered whenever concurrent sounds consist of overlapping frequency spectra. This interaction depends on the relative phase of two signals and, thus, is different at each ear for spatially separated sound sources, leading to binaural decorrelation. The barn owl, a sound localization specialist, uses interaural time difference (ITD) as primary cue for localizing the azimuth of a sound source. The detection of ITD relies on the binaural correlation and consequently suffers from binaural decorrelation. However, the owl exhibits a circuit called the midbrain stimulus selection network, dedicated to representing locations of the most salient stimulus in circumstances of concurrent stimuli. Previous competition studies using unimodal (visual) and bimodal (visual and auditory) stimuli have shown that relative strength is encoded in the spike response rates. Open questions remained concerning competition between concurrent auditory signals on coding.
Methods: To this end, we presented diverse auditory competitors (concurrent flat noise and amplitude modulated noise) and recorded neural responses of awake barn owls in subsequent midbrain space maps, the external nucleus of the inferior colliculus (ICx) and optic tectum (OT, homologue to the mammalian superior colliculus). Other work has shown that binaural decorrelation can explain a decrease in spike response rates in ICx. In this study, we expanded the above experiments to use competing stimuli that were spectrally non-overlapping, ruling out binaural decorrelation, but contained enough frequencies across the owls hearing range to be unambiguously localized.
Results: While both ICx and OT exhibit a topographic map of auditory space, OT also integrates visual input and is part of the global-inhibitory midbrain stimulus selection network. Through comparative investigation of these regions, we show that while increasing strength of a competitor sound decreases spike response rates of spatially distant neurons in both regions, relative strength determines spike train synchrony of nearby units only in OT. Furthermore, changes in synchrony by sound competition in OT are correlated to gamma range oscillations of local field potentials (LFPs), associated with input from the midbrain stimulus selection network. Our results suggest that modulations in spiking synchrony between units by gamma oscillations are an emergent coding scheme representing relative strength of concurrent stimuli, which may have relevant implications for downstream forebrain read out.
Conclusions: We compare results in both midbrain maps according to the effect of spectrally overlapping and non-overlapping stimuli on spike rates and features of the LFP. This further elucidates the essential role of the midbrain stimulus selection network for selecting the most salient stimulus.

Myelination Changes During Development Underlying Auditory Dysfunction in the Auditory Brainstem in Fragile X Syndrome.

Amita Chawla*[1]

[1]*Oklahoma State University*

Background: Fragile X Syndrome (FXS) and autism are neurodevelopmental and communication disorders. FXS is a monogenic form of autism. Hypersensitivity to sound and

altered binaural hearing are two common symptoms in these disorders. Binaural hearing and spatial acuity are important for localizing a sound source and separating sounds of interest from noisy backgrounds. Sound information computation starts with the auditory brainstem which compares interaural timing differences (ITD) and interaural level differences (IID) from both ears. Highly myelinated axons in the auditory brainstem encode ITD and IID sound information quickly and precisely. Whether there are changes to myelination that underly auditory sensitivity and dysfunction at the level of the auditory brainstem in FXS is unknown and important for understanding auditory symptomology and treatment.
The study of myelination during critical developmental timepoints will help to establish when during development auditory dysfunction arises using amouse model of FXS, Fmr1 knockout (KO) mice (male and female C57BL/6J wildtype, Fmr1 KO and heterozygote female mice).

Methods: We analyzed anatomical markers of myelin including diameter and thickness of myelination, spacing and size of sodium channels (nodes/paranodes), in Fmr1 KO mice and controls at several critical developmental timepoints, P9, P12-14, and P21-23 and P60-90 using immunohistochemistry and electron microscopy. We are measuring Auditory Brainstem Responses (ABRs) to show brainstem specific auditory function across the same developmental timepoints. The ABR is a non-invasive electrophysiological measure that records a pattern of waveforms that is directly related to auditory brainstem function.
Results: Preliminary data suggests that similar to adult phenotypes, FXS mice have altered auditory brainstem development as measured by ABR that may be related to myelination
Conclusions: These findings are important for understanding mechanisms underlying FXS related to myelination and when during development they arise.

Sound Localization Accuracy During the First Years of Life in Children Born With Unilateral Sensorineural Hearing Loss

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Background: Sound localization accuracy (SLA) is generally impaired in children with unilateral hearing loss (UHL), but it varies widely between individuals. Factors such as the degree and type of hearing loss (congenital or acquired, sensorineural or conductive) remain unclear in their impact on SLA development. SLA has been minimally studied in infants, particularly those with unilateral sensorineural hearing loss (uSNHL). This study aimed to conduct the first longitudinal investigation of SLA in congenital uSNHL during the first years of life, also evaluating the diagnostic value of the SLA test at 1, 2 and 2.5 years of age.
Methods: Horizontal SLA was measured every six months from 0.5 to 2.5 years of age in 19 infants with congenital unilateral sensorineural hearing loss (uSNHL) using eye-tracking. The infants were recruited consecutively from the universal newborn hearing-screening program in Region Stockholm over two years. Auditory brainstem response thresholds (ABRthrs) were measured at 2 months to describe baseline hearing loss (Johansson et al., 2022).
The SLA setup used 12 equidistant loudspeaker/video pairs in the frontal horizontal plane (±55º, positioned at ear level, 1.2 m in front). During the test, sound (63 dB SPL broadband stimulus) shifted between loudspeakers, with a 1.6-second sound-only period before visual stimuli

reappeared. The subject's perceived azimuth was defined as the pupil's position relative to the active loudspeaker. SLA was quantified using an error index (EI), where EI=0 corresponded to a perfect match and EI=1 indicated guessing (CI: 0.72 to 1.28). The cutoff (EI=0.72) was used to calculate the sensitivity and specificity of the SLA test as a predictor of single-sided deafness (SSD) vs non-SSD.

Results: All 19 children had ABRthr ≤20 dB nHL in their normal-hearing ear. Seven had ABRthr GREATER THAN 80 dB nHL in their impaired ear (SSD), and 12 had ABRthr LESS THAN 80 dB nHL (non-SSD). EI decreased with age in non-SSD infants, but not in SSD infants. For non-SSD, linear regression of EI as a function of age revealed a slope significantly different from 0 (EI = 0.70−0.11 × Age, r = −0.36, p LESS THAN 0.05, n = 12), more similar to children with normal hearing than SSD. At the 1-year visit, the SLA test had 86% sensitivity and 67% specificity for diagnosing SSD (n=13). At 2 years, it had 100% sensitivity and 83% specificity (n=11), and at 2.5 years, 83% sensitivity and 100% specificity (n=13).
Conclusions: The study of early SLA development in congenital uSNHL clearly distinguished the performance of infants with SSD from non-SSD. Infants with non-SSD benefit from residual hearing in the impaired ear for SLA development, while infants with SSD did not develop SLA. The SLA test has moderately high to high diagnostic value in distinguishing SSD from non-SSD, especially at 2–2.5 years of age.

Characterizing Spatial Hearing in Unilateral Hearing Loss: Effects on Spatial Cues and Adaptation

Sara Momtaz Bokharaei*[1], Ryan McCreery[1], Elizabeth Heinrichs-Graham[1], Dawna Lewis[1], G. Christopher Stecker[1]

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Background: Unilateral hearing loss (UHL) affects 7.2-7.8% of adults and impairs spatial awareness and communication by reducing access to binaural cues, including interaural time differences (ITD) and interaural level differences (ILD). This study examines how auditory asymmetry and hearing experience shape the use of spatial cues, including both binaural (ITD, ILD) and monaural cues, providing a perception of how listeners with UHL adapt to auditory asymmetry.
Methods: Forty-three adults (aged 18-55) were divided into four groups: 12 individuals with different levels of UHL, 11 individuals with normal limit hearing (WNL), and two simulated UHL groups (RE and LE), each with ten WNL participants wore an earplug to simulate unilateral hearing loss. Participants completed pure-tone audiometry, the Speech, Spatial, and Hearing Qualities Scale (SSQ), and a series of spatial listening tasks. These tasks comprised ITD and ILD threshold tests, minimum audible angle (MAA) assessments, and lateralization and localization tasks to investigate how binaural and monaural cues were used in different groups.
Results: Both the UHL and simulated UHL groups showed significantly decreased binaural sensitivity than WNL group. The WNL group had the lowest ITD thresholds (mean = 49.18 µs, SD = 54.58), whereas UHL participants had higher thresholds (mean = 318.12 µs, SD = 182.68). Simulated UHL participants had significantly higher (elevated) thresholds, especially in the RE group (mean = 411.46 µs). ILD thresholds followed a similar pattern, with the NH group having

the lowest (mean = 1.56, SD = 1.53) and the LE group having the highest (mean = 4.06, SD = 3.09). MAA tests revealed that both the UHL and the simulated UHL groups had lower spatial resolution, with the LE group showing the most substantial spatial deficit. Localization tests also suggested that participants in UHL and simulated UHL groups used spatial cues to complete the tasks, though with less spatial precision. These findings show that, while UHL has an impact on binaural hearing, listeners continue to engage in spatial hearing using other mechanisms.
Conclusions: This study emphasizes the negative effect of hearing asymmetry on spatial hearing and demonstrates that even with impaired/inaudible ITD and ILD sensitivity, individuals with UHL and simulated UHL retain some capacity for spatial hearing. Chronic UHL participants exhibited better spatial localization compared to those with acute, simulated UHL, suggesting that long-term exposure to auditory asymmetry may foster adaptation mechanisms. These findings emphasize the importance of experience-dependent plasticity and offer new perspectives for understanding how listeners adjust to auditory asymmetry. The results provide a fresh viewpoint for further research into the neural and perceptual adaptations that occur with long-term UHL.

Spatial Hearing With Active Hearables: Evaluation of the Transparency Hearing Mode

Seba Ausili*[1], Nathan Erthal[1], Christopher Bennett[1], Hillary Snapp[1]

[1]*University of Miami*

Background: Active hearables, such as wireless earbuds and in-ear devices, are used by millions globally for applications like music streaming and phone calls. These devices now include features like “transparency” mode, allowing ambient sounds to pass through to maintain spatial awareness. Due to this capability and enhanced audio processing, the FDA has approved their use as over-the-counter hearing aids for people with mild-to-moderate hearing loss, opening a new era of accessible hearing healthcare. While transparency mode aims to preserve spatial awareness using built-in microphones and real-time processing, prior research has shown these devices can alter spatial hearing cues. Specifically, disruption of binaural cues and monaural spectral-pinnae cues can degrade the ability to localize sounds in both horizontal and vertical planes. However, the effect of active hearables on real-world sound localization remains under explored. This study evaluates the impact of the transparency hearing mode on spatial hearing by objectively measuring spatial cues and assessing sound localization in quiet and noisy environments.
Methods: We conducted both objective and behavioral evaluations using AirPods Pro (1st gen). Head-related impulse responses (HRIRs) were recorded without background noise and in three signal-to-noise ratios (-5, +5, and +15 dB SNRs) with 55 dBA white noise. ILDs, ITDs, monaural spectral pinna cues, and processing delays were extracted. Sound localization was assessed in ten normal-hearing adults (mean age 25 years; 3 female). Participants had to localize a 150 ms broad-band buzzer in noise (as for HRIR measurements) and a 150 ms filtered white noise burst in silence (BB = 0.2–20 kHz, LP LESS THAN 1.5 kHz, HP GREATER THAN 3 kHz). Head movements were tracked to assess localization accuracy and reaction times. All experiments spanned azimuth angles from -90° to 90° and elevation angles from -30° to 30°.

Results: Objective measurements showed that transparency mode disrupted ILDs and, as expected, monaural spectral-pinnae cues. Moreover, processing delays were asymmetric, averaging up to 91 μs difference. Azimuth sound localization performance in silence was significantly worse with AirPods compared to normal hearing for BB and HP (p LESS THAN 0.05). Furthermore, the use of the AirPods showed a significant performance deterioration for all SNRs (p LESS THAN 0.05). Elevation localization was poor in all conditions and significantly different than normal (p LESS THAN 0.001). Overall, sound localization with background noise increased significantly the response reaction times (p LESS THAN 0.001).
Conclusions: Transparency mode in active hearables disrupts spatial perception, specifically affecting localization cues and user behavior. These limitations have implications for safety and situational awareness, especially for individuals relying heavily on auditory cues. Future research should focus on refining these technologies to enhance spatial accuracy, potentially by better utilizing personalized spatial audio techniques. Improved algorithms and processing for transparency mode could benefit not only general users but also individuals with hearing impairments in complex and challenging acoustic environments.

The Influence of Binaural Cues on Auditory Stream Segregation in Younger and Older Normal- Hearing Listeners

Nathan Higgins*[1], Carrie Secor[1], Erol J. Ozmeral[1]

[1]*University of South Florida*

Background: Spatial information, represented by binaural cues, provides one of the primary features used to separate or integrate streams of acoustic information. Unlike binaural cue discrimination thresholds, much less is known about how these cues influence auditory stream segregation, or how that influence might change in older compared to younger individuals.
Methods: To further explore the effect of binaural cues on auditory stream segregation in younger (N=30) and older (N=30) normal-hearing listeners, the ABA stream segregation paradigm (Bregman, 1990) was adapted and used to measure behavioral perception and EEG (electroencephalography) responses to periodic modulation of binaural cues. Listeners were presented with triplets of low(A)-high(B)-low(A) frequency sounds at a rate of 2 Hz, and instructed to continuously hold down button-1 (integrated) or button-2 (segregated) to indicate their perception. Over the course of minutes, participants' perception spontaneously switched back and forth between these percepts at equal proportions, referred to as bistable perception. In the experimental conditions, the response paradigm was the same, but the A-components of the ABA-triplets were slowly modulated lateral-to-midline and back, as a function of binaural cue while the B-component was maintained at the midline, or diotically. Perceptual and neural responses were recorded as a function of binaural cue, and a segregation boundary metric of was defined and used to delineate changes in percept. For comparison purposes, discrimination thresholds were also collected separately for each participant.
Results: In the control condition of the ABA paradigm, both groups reported comparable bistable percepts, and both groups demonstrated a strong influence of all binaural cues on stream segregation. For ILD (interaural level difference) and ITD (interaural time difference) cues, segregation boundaries were 7.7 dB-ILD and 222 μs-ITD on average with high individual variability, and response functions were characterized by significantly more segregated percepts

reported for lateral- compared to midline-cues. Significant differences in perception were also observed relative to perceived sound movement (toward the midline versus away from midline). EEG results showed significant differences for triplets where a switch in perception was reported, this effect was localized to electrode positions clustered around FCz.
Conclusions: Discrimination thresholds did not show a strong relationship to the segregation boundary, indicating that sensitivity to small differences in a binaural cue does not necessarily translate to use of that cue for stream segregation and highlighting the importance and difficulty of defining ecologically valid functional measures of perception. Results did establish the functional influence of binaural cues to promote and inhibit auditory segregation, and evidence of perceptual maintenance by the central auditory system in the face of changing stimuli. EEG results provide further insight into the neural mechanisms underlying the cognitive processing that enables listeners to integrate or separate auditory objects.

Poster Session III

1:30 p.m. - 3:00 p.m.

Peninsula Ballroom

M1. OPEN BOARD

M2. Computational Model of Human Auditory Evoked Potentials at the Peripheral and Brainstem Levels

Miguel Temboury Gutiérrez*[1], Gerard Encina-Llamas[2], Torsten Dau[1]

[1]*Technical University of Denmark,* [2]*University of Vic - Central University of Catalonia*

Category: Auditory Nerve
Background: Auditory evoked potentials (AEP) are used as objective diagnostic tools in certain clinical populations, such as for threshold estimation in infants. With the discovery of age-related auditory nerve (AN) degeneration in humans, AEPs are becoming increasingly important for assessing cochlear pathology. However, the relationship between the AEP morphology and cochlear status is complex, making interpretation challenging. AEP models based on cochlear function can help explain how interactions within the cochlea produce far-field responses and could enable clinicians to link individual differences in AEP morphology to specific cochlear pathologies.
Methods: An AEP modeling framework was developed using a state-of-the-art computational human AN model as the front-end stage and a convolution with a unitary response (UR) function as the back-end stage. The stimulus-independent UR was fixed for a given electrode configuration: one set for peripheral potentials, measured using a tympanic membrane (TM) electrode referenced to the ipsilateral mastoid, and another for brainstem potentials, measured

from the ipsilateral mastoid to the vertex. Simulated responses were compared with existing datasets of AEPs to transient stimuli (e.g. clicks) and periodic stimuli (e.g. pure tones and amplitude-modulated tones, representing frequency-following responses, FFR, and envelope-following responses, EFR, respectively), across a range of levels. Additionally, we recorded compound action potentials (CAP) and auditory brainstem responses (ABR) simultaneously in 20 young participants with clinically normal thresholds in response to clicks and level-specific chirps, ranging from 10 to 80 dB nHL.
Results: The model successfully replicated full AEP waveforms for transient (CAPs and ABRs) and periodic stimuli (FFRs and EFRs) across various stimuli levels. The framework was used to explore AEP generation mechanisms in the cochlea, with the activation and interaction of distinct AN fibers explaining several phenomena observed in experimental AEPs, such as the 'chirp' benefit and stepwise amplitude growth functions. However, some discrepancies were noted between the simulated and experimental responses. The latency-level functions of simulated CAP N1 and ABR wave V were too shallow, and the amplitude growth functions plateaued at the highest levels. These results suggest that the relative contribution and spread of excitation from different AN fibers at higher levels may not be accurately modeled in the current human AN model.
Conclusions: The computational modeling framework successfully simulated a range of AEPs measured at different electrode locations, capturing many stimulus- and level-dependent differences observed in experimental data, though some discrepancies remained. The human front-end AN model could be refined and validated indirectly using this framework, along with electrocochleographic recordings from TM electrodes. This modeling work has the potential to test hypotheses and interpret experimental findings related to the effects of specific cochlear damage, such as neural versus outer hair cell loss, on AEP patterns.

M3. Effect of Auditory Attention on Otoacoustic Emission Delay

Yuri Dowaki*[1], Sho Otsuka[2], Seiji Nakagawa[2]

[1]*Chiba University,* [2]*Center for Frontier Medical Engineering, Chiba University*

Category: Auditory Nerve
Background: Medial olivocochlear bundle suppresses outer hair cell (OHC) amplification in response to acoustic stimulation. This response is called medial olivocochlear reflex (MOCR). The MOCR-induced gain reduction is manifested as acoustic stimulation induced suppression of otoacoustic emission (OAE). The OHC gain reduction simultaneously widens the frequency tuning of the basilar membrane (BM). This effect is manifested as acoustic stimulation induced reduction in OAE delay, which is proportional to the sharpness of the frequency tuning of the BM.
Centrifugal nerves are connected from cortical areas to MOC neurons. In line with this, acoustic stimulation induced OAE suppression is reported to be modulated by auditory attention. However, it is not clear whether and how auditory attention modulates the OAE delay, i.e., the frequency tuning of the BM. In this study, we examined whether OAE delay is changed by imposing a signal-in-noise detection task and its relation to the task performance.

Methods: OAEs were elicited by clicks presented before and after a noise that elicits MOCR. The OAE waveforms measured during the 250-ms period just before and after the noise presentation were averaged, respectively. Scalograms were calculated for each averaged waveform. OAE delay was defined as the interval between the stimulus onset and the peak of the energy in each frequency band on the scalograms. OAE suppression was defined as the amount of reduction in the sound pressure level of the averaged OAE waveform after the noise presentation. Subjects performed a task to detect a tone burst in the noise presented to the right ear. Noise with and without the target were presented randomly each on 50% of the trials. OAEs were also measured in the non-attentive condition, in which the same stimuli were presented, but participants neglected them.
Results: OAE delay in the 1000-2000 Hz was significantly decreased by the noise presentation ($T = 2.03$-2.37, $p = 0.01$-0.03), and the amounts of reduction at 1500 Hz was used as a representative for later analysis. The reduction in OAE delay was significantly greater during the listening task than in the non-attentive condition ($T = 2.33$, $p=0.01$). The OAE delay reduction and OAE suppression during the listening task were marginally and significantly correlated with the detection threshold, respectively ($r = -0.36$, $p=0.10$; $r = -0.58$,$p=0.07$).
Conclusions: We found (1) MOCR-related frequency tuning widening is increased during a signal-in-noise detection task (2) individuals with the larger frequency tuning widening shows better signal detection performance, although wider frequency tuning is assumed to prevent the detection of a tonal sound in noise. Because the OHC gain reduction manifested as OAE suppression is positively correlated with signal-in-noise detection performance as shown in this study, the frequency tuning widening accompanied with the gain reduction may be spuriously correlated with the detection performance.

M4. Introducing Different Spontaneous-Rate Classes of Auditory Nerve Fibers to the CARFAC v3 Cochlear Model

Dick Lyon[1], Jason Mikiel-Hunter*[2], Rob Schonberger[1], Honglin Yu[1]

[1]*Google Australia,* [2]*Macquarie University*

Category: Auditory Nerve
Background: The CARFAC (Cascade of Asymmetric Resonators with Fast-Acting Compression) cochlear model uses digital filter cascades to model the motion of the human basilar membrane in response to sound. By incorporating modules that represent inner and outer hair cell (IHC/OHC) stages that are themselves connected via automatic-gain-control loop filters (equivalent to efferent feedback in the biological system), the CARFAC can efficiently and accurately reproduce many physiological phenomena observed in the auditory periphery in vivo. However the output of the CARFAC's IHC stage, otherwise known as the Neural Activity Pattern or NAP, has to date been considered an estimate of the average instantaneous auditory nerve (AN) firing rate in each frequency channel and has therefore ignored the contribution of different AN fiber classes.
Methods: To generate diverging NAPs for low-, medium- and high-spontaneous-rate AN fibers, we adapted CARFAC v2's two-capacitor IHC model that distinguishes between the IHC receptor potential (voltage at first capacitor) and neurotransmitter release/recovery at the IHC-AN synapse (where the second-capacitor loops represent the synapse-current gain control). Distinct

(typically 3) sigmoidal transfer functions, differing mainly in their offsets, were implemented between the two capacitors for the different AN fiber classes. This effectively represents the altered relationship between the IHC receptor potential and the neurotransmitter release onto the AN fiber class in question. A scaling factor before the final low-pass smoothing filter scales to appropriate instantaneous firing probabilities per sample time, for each AN class. Care was taken to preserve CARFAC's functioning automatic gain control behavior by weighting the summed population responses across the three AN fiber classes.
Results: By comparing NAP output to in vivo auditory nerve data, we demonstrate the continued "good fit" of the CARFAC v3's NAP output to the physiological data. Furthermore, using a neurometric model of auditory perception that compares neurogram outputs in response to silence and pure-tones, we approximate a "middle-ear filter" that, implemented alongside CARFAC v2 or v3, can reproduce normal-hearing pure-tone audiogram thresholds.
Conclusions: We discuss the potential impact of selective, parametrized synaptopathy (reduction in AN fiber number, especially in the low- and medium-spontaneous rate classes) in the CARFAC v3 as a hearing impairment, either by itself (i.e. "hidden-hearing loss") or in conjunction with outer-hair-cell impairment. This work forms the basis for a machine-learning framework that aims to individualize the CARFAC model with respect to individual hearing losses.

M5. Effects of Cortical Activation on Medial Olivocochlear Reflex

Kandai Uchiyama*[1], Sho Otsuka[2], Seiji Nakagawa[2]

[1]*Graduate school of Chiba University,* [2]*Center for Frontier Medical Engineering, Chiba University*

Category: Auditory Nerve
Background: It has been reported that there are individual differences in the susceptibility to noise-induced hearing loss (NIHL). Because NIHL progresses unnoticeably slowly, assessing the risk beforehand is very useful for raising individual awareness of prevention. One of the promising predictors of the susceptibility to loud sounds is medial olivocochlear reflex (MOCR). MOCR inhibits outer hair cell gain and is thought to protect the cochlear from acoustic overexposure. In general, MOCR has been assumed to be a passive brainstem reflex response, but accumulating evidence suggests that MOCR fluctuates associated with cognitive processing such as attention and arousal. However, the underlying neural mechanisms of the MOCR variations have not yet been elucidated.
To address this question, we modulated cortical activities and observed whether and how MOCR changes associated with the manipulations. Cortical activities were modulated endogenously by imposing mental arithmetic tasks (Experiment 1) and exogenously by applying transcranial direct current stimulation (tDCS) (Experiment 2).

Methods: MOCR strength was evaluated by the amount of suppression of click-evoked otoacoustic emissions induced by contralateral noise presentation. Experiment 1: By repeating the short-duration mental arithmetic task and MOCR measurements, we measured the changes in MOCR over the repetitions of the task. We measured slow vertex response (SVR) evoked by the MOCR eliciting noise simultaneously and compared the fluctuation patterns of MOCR strength

and N1-P2 amplitude of SVR. Experiment 2: MOCR and SVR were measured before and after tDCS (1.25 mA, 10 min). For stimulation of the left auditory cortex, the active and reference electrode were placed over a temporal location (T7) and contralateral supraorbital temporo-parietal area, respectively. The variation patterns of MOCR strength and N1-P2 amplitude across before and 0, 30, 60, 90 min after the stimulation were compared for active and sham stimulation.
Results: Experiment 1: MOCR weakened monotonically along with the execution of the calculation task. N1-P2 amplitude decreased after the first calculation task but later increased across the task repetitions.
Experiment 2: The stimulation to the surrounding area of auditory cortex did not significantly changes MOCR, while cortical EEG increased immediately after the stimulation.

Conclusions: The results suggest (1) the enhancement of cortical activity caused endogenously by calculation tasks, which require large-scale cortical network activity, weaken MOCR, (2) the enhancement of the auditory cortex activities caused exogenously by electrical stimulation did not significantly change MOCR. It can be assumed that the modulation of MOCR requires endogenous and/or large-scale cortical network activity.

M6. The Relationship of the Cortilymph Wave to the Traveling Wave, Auditory-Nerve Responses, and Low-Frequency Downward Glides

John Guinan*[1]

[1]*Mass Eye and Ear, Harvard Medical School*

Category: Auditory Nerve
Background: It was thought that traveling-wave amplification comes from outer-hair-cell (OHC) forces acting on the basilar membrane (BM). However, data from the cochlear base show that OHC motion is at the wrong phase to amplify BM motion by forces acting through the Deiters cells. An alternate hypothesis is that traveling-wave amplification comes from OHCs producing cyclic cortilymph-fluid flow along the organ-of-Corti (OoC) tunnels (the “cortilymph wave”) that changes OoC cross-section area and adds energy to the scala-media-fluid traveling wave. This hypothesis fits the data for the cochlear base, but not the apex.
Methods: One base-to-apex difference comes from the OHC-membrane resistance and capacitance (RC) low-pass filter. This filter sets the phase difference from OHC-stereocilia current to OHC voltage and the resulting OHC lengthwise motion. From live-animal measurements near the 20-kHz region, the OHC-RC corner frequency, Fc, was reported to be ~3 kHz. At tone frequencies GREATER THAN GREATER THAN Fc, the OHC RC filter delays the cortilymph wave from the traveling wave by ¼ cycle and provides the correct timing for OoC-area-change traveling-wave amplification. However, at frequencies LESS THAN LESS THAN Fc, the RC-filter delay is small so that, relative to the traveling wave, the cortilymph wave arrives earlier at low than at high frequencies. Here we consider how the interacting cortilymph wave and traveling wave, with phase relationships that change with tone frequency, affect responses in the low-frequency cochlear apex.
Results: The interaction of the two waves helps in understanding low-frequency auditory-nerve (AN) fiber data, particularly tuning-curve “side lobes.” Side lobes have shorter group delays than the lower-threshold, characteristic-frequency (CF) main lobe. AN-fiber response delays and rate-

saturations are consistent with the hypothesis that AN fibers are excited (a) at low sound levels by a combination of the traveling and cortilymph waves, (b) in high-level tuning-curve side lobes by the cortilymph wave alone, and (c) at high levels near CF, by the passive traveling wave.

In the low-frequency apex, the instantaneous frequencies of AN click responses have been found to start at a frequency higher than CF and "glide" downward to the fiber CF. A hypothesis consistent with these data is that downward glides are produced by a cortilymph wave that is driven by more basal OHCs, with the cortilymph wave arriving at the measurement site before the traveling wave.

Conclusions: AN responses in the cochlear apex are shaped by the interaction of drives from both the classic traveling wave and the cortilymph wave. The relationship of these waves changes with frequency, even within the response area of a single AN fiber. The presence of a downward glide is hypothesized to indicate the presence of a cortilymph wave. [Supported by R01 DC07910 from the NIDCD of NIH (to Sunil Puria).]

M7. Afferent Connection From Cochlea to Flocculus/Parafloccular Complex

Max Gattie*[1], Xiaodong Tan[1], Gabriella Sekerková[1], Marco Martina[1], Claus-Peter Richter[1]

[1]*Northwestern University*

Category: Auditory Nerve

Background: There is growing recognition of the cerebellum's importance in sensory processing. This has included identification of the unipolar brush cell (UBC), an excitatory interneuron enriched in the flocculus/parafloccular complex. This area is already known to receive vestibular innervation. We tested the hypothesis that it is also innervated monosynaptically from the cochlea, with UBCs playing a central role.

Methods: Recordings were made in 41 anaesthetised gerbils. The bulla was opened, and a silver electrode hooked onto the bony rim of the round window to measure compound action potentials (CAP). The flocculus/parafloccular complex was then accessed by exposing the anterior semicircular canal and carefully picking away the bone covering the region. With sharp glass electrodes the parafloccular was penetrated in an anterior trajectory towards the flocculus. While the electrode was advanced, broadband noise bursts were played through an earphone coupled to ear canal tubing. Responsive neurons were identified through the juxtacellular technique, with tone burst stimuli used to characterise best frequency and measure firing properties. After recording some neurons were intopherically filled with biocytin to facilitate anatomical identification.

Additional experiments included tract tracing and measurement of c-Fos expression in the cerebellum following acoustic stimulation. Anterograde tracing was through injection of biotinylated dextran amines to the spiral ganglion or modiolus (care was taken to avoid spillover to the vestibular system) and retrograde tracing was via injection of fluoro-gold to the flocculus/parafloccular complex.

Results: Of 31 neurons having well-characterised responses at their best frequencies, 16 had a mean first spike time within 5 ms of stimulus onset. Five of these neurons responded at or below CAP threshold, and a further 8 responded less than 16 dB above CAP threshold. Neurons were

categorised based on similarities in their spike train data. Example behaviours included phase locking to the stimulus frequency, change in firing pattern above versus below best frequency, and presence of burst activity. Tract tracing showed a connection between the cochlea and flocculus/paraflocculus complex, and c-Fos expression demonstrated activity in the flocculus/paraflocculus complex following auditory stimulation.
Conclusions: These data reveal a hitherto unknown auditory pathway in which the flocculus/paraflocculus complex receives primary auditory input from the cochlea. We propose that UBCs are an integral component of this novel ascending route. Follow-up work will use optical stimulation to further characterise flocculus/paraflocculus neurons through selective stimulation of cochlear and vestibular peripheries. This includes a prospective role for the flocculus/paraflocculus in tinnitus generation, since UBCs are known to fire autonomously and to extend a mesh of newly-formed intrinsic mossy fibres following deafferation. It will also investigate cochlear-vestibular interaction at auditory-vibratory stimulation levels capable of activating the vestibular system, as is the case with an animal's own vocalisations.

M8. Integration of Functional Human Auditory Neural Circuits Based on a Three-Dimensional Carbon Nanotube System

Yiyun Lou[1], Jiaoyao Ma[1], Mingyu Xia[1], Wenyan Li[1], Yiyun Lou*[1]

[1]*Eye and ENT Hospital, Shanghai Medical College, Fudan University*

Category: Auditory Nerve
Background: Sensorineural hearing loss (SNHL) is primarily caused by the loss or dysfunction of auditory hair cells or neurons, affecting the daily lives of hundreds of millions of people worldwide. The physiological interaction between the peripheral and central auditory systems is essential for auditory information transmission and perception. Any malfunction within the auditory circuit can lead to SNHL. While most research has focused on peripheral auditory organs, there remains a shortage of effective research models or therapeutic strategies for disorders affecting the auditory neural pathway. To address this gap, we developed a human auditory neural circuit model featuring functional synaptic connections using super-aligned carbon nanotube sheets (SA-CNTs).
Methods: In this study, SA-CNTs were used as scaffolds to co-culture peripheral and central auditory neurons. Neural progenitor cells derived from the cochlea and auditory cortex of human embryos were utilized to generate region-specific organoids. These organoids were then assembled into a nanofiber-integrated three-dimensional system. We employed optogenetic stimulation, calcium imaging, and whole-cell patch-clamp recordings to investigate the electrophysiological properties of the connections between spiral ganglion neuron (SGN) and auditory cortex neuron (ACN) organoids.
Results: SA-CNTs facilitated the guided axonal growth of primary peripheral and central auditory neurons, derived from both mouse and human sources, in a controlled, directed manner. The electrical conductivity of the SA-CNTs maintained the electrophysiological activity of the primary auditory neurons. Additionally, we established a protocol to generate human auditory region-specific neural organoids from primary peripheral and central neural progenitor cells. When assembled in the 3D SA-CNT system, the differentiated SGNs and ACNs formed both physiological and functional connections. Optogenetic stimulation, calcium imaging, and whole-

cell patch-clamp recordings revealed the electrophysiological characteristics of the connections between SGN and ACN organoids. Functional synaptic connections between peripheral and central neurons were observed, as indicated by calcium spiking and postsynaptic currents.
Conclusions: This study introduced the first auditory neural circuit model that connects the originating neurons (SGNs) with the terminating neurons (ACNs). Human neural spheroids co-cultured in the SA-CNT-based system provided a valuable model for exploring the mechanisms of neural interaction within the auditory pathway. Our research offers insights into the reconstruction of auditory projections and presents an innovative platform for investigating auditory neural pathway dysfunctions in neurodegenerative diseases. Furthermore, this system holds promise as a tool for screening pharmaceutical and genetic strategies aimed at restoring auditory pathways.

M9. Multiple Sources of Cholinergic Input to the Nuclei of the Lateral Lemniscus

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Category: Brainstem: Structure & Function
Background: Acetylcholine (ACh) has substantial effects on neuronal activity in the auditory brainstem and is thought to modulate neuronal processing in the nuclei of the lateral lemniscus (NLL, including ventral, intermediate and dorsal nuclei (VNLL, INLL, DNLL)). We previously identified multiple sources of cholinergic input to the VNLL, but sources of cholinergic input to the remaining NLL are unknown. Here, we expand our study to characterize cholinergic inputs to the NLL overall.
Methods: We placed red RetroBeads into the NLL and subsequently stained the tissue with anti-VAChT to identify cholinergic cells that project to the NLL. VAChT-immunopositive, retrogradely-labeled cells were present bilaterally in the pontomesencephalic tegmentum (PMT, comprising pedunculopontine and laterodorsal tegmental nuclei), the superior olivary complex (SOC) and the lateral paragigantocellular nucleus (LPGi). Of these sources, the LPGi had the fewest labeled cells.
Results: We then injected adeno-associated viral vectors into each cholinergic region in normal-hearing adult male or female ChAT-cre mice. The vectors delivered a gene for cre-dependent expression of red or green fluorescent protein that selectively labeled cholinergic cells and axons. We analyzed projections from each of the extrinsic sources: PMT, SOC and LPGi. Briefly, each cholinergic region provided input to every NLL, but the density of input could vary by side or by target nucleus. Projections from the PMT were roughly equal on ipsilateral and contralateral sides, with similarly dense terminations in each of the NLL. Projections from the LPGi were remarkable for the amount of axonal label; despite relatively few cholinergic cells in the LPGi, the density of cholinergic terminations in the NLL was comparable to that seen after labeling PMT projections. The LPGi projections terminated equally among the NLL, with the contralateral terminations often slightly denser than ipsilateral ones (especially in the VNLL). Finally, cholinergic SOC neurons also project to the NLL. Unlike projections from PMT or

LPGi, terminations from the SOC were heavier on the ipsilateral side and on both sides were densest in the VNLL and sparsest in the DNLL.
Conclusions: Each of the NLL receive cholinergic input from multiple sources: the PMT, LPGi and SOC. The different sources are likely to be active under different conditions. The PMT has been associated with arousal, sleep-wake cycle, reward and cortically-driven plasticity. SOC neurons are likely to be more narrowly tuned for auditory stimuli than PMT neurons, and could serve for auditory feedback and perhaps extraction of signals from noise. Little is known about the LPGi, though extensive auditory connections of the LPGi support a hearing-focused modulation. Future experiments manipulating the different cholinergic inputs independently are needed to better understand the likely varied roles for cholinergic modulation of auditory responses in the NLL.
Supported by NIH R01 DC004391.

M10. Comparative Physiology of Action Potential Generation in Neurons of the Mntb

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Category: Brainstem: Structure & Function
Background: In the auditory system, the input-output structures within the medial nucleus of the trapezoid body (MNTB) are highly conserved. The input to these globular cells is dominated by the calyx of Held synapse that forms a one-to-one connectivity. Due to its large size, the calyx of Held triggers in a one-to-one fashion a postsynaptic action potential. Biophysically MNTB neurons are known for their onset generation of action potentials with short latency and high temporal precision. Despite this conserved structure-function relationship, small but significant differences in synaptic size and action potential threshold were reported. Here we examine whether slight differences in the biophysical properties and action potential generation between different mammals are rather the rule than the exception or can be explained by differences in soma sizes.
Methods: Whole-cell recordings from MNTB neurons in acute brain slices were obtained from Mongolian gerbils, Etruscan shrews, and mouse lemurs. The recordings determined the soma size from charging transients and extracted the biophysical properties of MNTB neurons focusing on action potential generation. Moreover, DTX-k application investigated further similarities in the influence of low voltage-activated potassium channels in voltage signaling between the different species.
Results: Soma sizes of MNTB neurons from gerbils were slightly larger compared to mouse lemurs and about double the size compared to Etruscan shrews. The input resistance of MNTB neurons was about twice as large in Etruscan shrews and mouse lemurs compared to gerbils. The membrane time constant was fastest in gerbils and slowest in mouse lemurs. Action potential current thresholds was lowest while the voltage threshold was highest in Etruscan shrew and similar between gerbils and mouse lemurs. The action potential halftime was shortest in gerbils. The de- and repolarization speed was largest in gerbils and smallest in mouse lemurs. The after hyperpolarization in gerbils showed a single deflection, while in mouse lemurs and Etruscan shrews, a second slow after-hyperpolarization was observed. Assaying the temporal precision at

rheobase showed that gerbil MNTB neurons are most precise. The DTX-sensitive current supports temporal precision of action potential generation in all species, while its role in quenching the number of action potentials is largest in gerbils. Taken together, different cellular adaptations exist and are likely based on different subsets of ion channels. Nevertheless, the overall function of MNTB neurons is conserved while the amount of temporal precision is species-dependent.
Conclusions: Our findings demonstrate that despite the highly conserved function of the MNTB, the biophysical properties of these neurons differ between mammals. The observed differences cannot be attributed to cellular scaling and therefore represent genuine functional adaptations. So far, the relevance of these adaptations remains elusive, yet the transfer of gained cellular insights between species might be taken with caution.

M11. An Investigation on Mitochondrial Protein Makers in Mouse Cochlear Nucleus Changing During Aging

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Category: Brainstem: Structure & Function
Background: Age related hearing loss (ARHL) is one of the most prevalent health conditions that affect the elders. However, there is limited knowledge regarding the aging progress in the cochlear nucleus (CN), which is the first central auditory nucleus. In our previous work, we had used mass spectrometry to broaden our knowledge on the proteomic changes in CN during aging. Interestingly, the significantly changed proteins were enriched in mitochondria related profiles. To further understand the mechanisms beneath this phenomenon, here we performed immunostaining to show their localization related to different CN cells and their altering progress during aging.
Methods: In this study, three age groups of C57BL/6J mice, namely 3, 10, and 16 months of age, were utilized. The CNs of the mice were dissected, then fixed in PBS with 4% paraformaldehyde, cryo-protected in 30% sucrose, embedded in Cryo-Gel, then sectioned with a cryostat slicer at 20 μm thickness. Immunofluorescence staining was performed with the corresponding antibodies for each mitochondrial biomarker we investigated. We used confocal microscope to image the stained makers, then analyzed their expression level and localization.
Results: The biomarkers affected by age were located mostly in bushy cell, a principle neuron type at the area of anteroventral CN. Their expression level was varied in the changing during aging. The increased markers might reflect the degraded mitochondria number was increasing, and the decreased markers might reflect the declined vitality of the mitochondria.
Conclusions: The current study provided a histologic view on dozens of mitochondrion related biomarkers changing in CN aging. And the results showed the bushy cells might be more affected by the mitochondrial malfunctions comparing to other cell types. This would provide informative references to understand the components of CN mitochondria in ARHL mechanisms. in CN.

M12. Noise-Induced Hearing Loss Enhances Ca2+-Dependent Spontaneous Bursting Activity in Lateral Cochlear Efferents

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Category: Brainstem: Structure & Function

Background: Lateral olivocochlear (LOC) efferent neurons may protect hearing sensitivity. Previously we showed that LOC neurons from juvenile mice exhibit an infra-slow (~0.1 Hz) burst firing pattern, whose genesis is dependent on L-type Ca2+ channels. However, it remains unclear what sets burst duration, or how burst firing and the ion channels that control bursts change with noise-induced hearing loss.

Methods: The auditory brainstem response (ABR) of transgenic mice (ChAT-Cre × tdTomato) was tested before and ~6 days after a broad-spectrum noise exposure at 110 dB SPL for 2 hours. Brain slices from deaf mice were harvested for electrophysiological recordings: cell-attached and whole-cell patch-clamp to examine burst firing pattern and Ca2+ current, respectively. A model LOC neuron was constructed to assess the roles of different ionic currents in regulating the firing pattern.

Results: We first examined the role of K+ channels in terminating the spike burst. Spontaneous burst firing in LOC neurons persisted despite the application of Ca2+-activated or voltage-gated K+ channel antagonists, indicating these channels are not involved in burst pattern. Instead, Ba2+-sensitive K+ currents, unaffected by these antagonists, had profound effects on firing activity, pointing to two-pore domain K+ (K2P) channels as likely drivers. This inference is supported by two pieces of evidence: first, immunostaining confirmed the expression of TREK-1 channels in the LOC; and second, reducing K2P conductance in the model neuron prolonged burst duration until the neuron exhibited tonic firing when K2P conductance was reduced to zero. Given that K2P currents are voltage insensitive, the rhythm of neuronal activity likely depends on alternating cycles of Ca2+ channel activation and inactivation. Ca2+ inactivation kinetics were slowed when intracellular Ca2+ chelation power was enhanced or when extracellular Ca2+ was replaced with Ba2+, indicating that Ca2+-dependent Ca2+ channel inactivation (CDI) may contribute to burst termination. Indeed, in the model, slowing Ca2+ inactivation to simulate CDI attenuation resulted in prolonged burst duration. Noise-induced hearing loss significantly increased burst duration and reduced burst frequency. It differentially affected Ca2+ channel subtypes, increasing high-voltage-activated Ca2+ currents without affecting low-voltage-activated ones. In the model, increasing Ca2+ conductance led to longer bursts, supporting a causal relationship. Additionally, noise exposure compromised CDI, as neither enhanced chelation nor Ba2+ could prolong Ca2+ channel inactivation as in control neurons.

Conclusions: K2P and CDI work synergistically to terminate bursts in LOC neurons. Noise-induced hearing loss caused increased Ca2+ channel conductances with altered inactivation properties. Such changes might be a result of upregulated Ca2+ channel expression and altered Ca2+ signaling molecules like calmodulin. The changes in firing pattern of LOC neurons might play an important role in amplifying neuropeptide release in the cochlea following noise exposure.

M13. Mitochondrial Morphology Differences in the Auditory Brainstem of Fragile X Syndrome Mice Using Electron Microscopy

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Category: Brainstem: Structure & Function
Background: Our previous work has shown sex-specific mitochondrial morphology and integrity alterations in the auditory brainstem of Fragile X syndrome (FXS) mice. Mitochondria are crucial in neuronal survival and energy generation in the form of ATP. Neurons in the auditory brainstem, especially in the medial nucleus of the trapezoid body (MNTB), need a lot of energy for sound processing. FXS is a genetic form of autism, and in other brain areas, has been linked to mitochondrial dysfunction. However, there are no studies that have quantified mitochondria in the auditory brainstem of FXS mice using transmission electron microscopy (TEM). We hypothesize that based on previous work, there will be sex-specific differences in integrity of mitochondria in mice with FXS.
Methods: Sixteen total FXS mice were used for experiments (four of each genotype and sex). Mice under 100 days old were used. Using the Fiji ImageJ (NIH) software, length and width of the outer mitochondrial membrane (OMM) were measured as well as length and width of each mitochondrion.
Results: Results are still in progress. Using TEM to quantify the images of mitochondria in FXS, we expect to see morphological and integrity changes in the MNTB like our work using confocal. Previous work has also shown altered morphologies and function in mitochondria in FXS mice compared to control non-auditory areas.
Conclusions: Understanding morphological differences in mitochondria of the auditory brainstem of FXS mice is important to understanding how morphology and integrity affect sound processing information. Auditory difficulties are one of the most predominant symptoms in FXS patients, making this study relevant to common symptomology in patients. Future research on how mitochondrial function is altered in FXS will help us better understand how mitochondria work in processing sound information and are related to auditory symptoms in patients.

M14. Synaptic Variation of Auditory Nerve Inputs Decreases Temporal Precision but Improves Stimulus Reproduction in Globular Bushy Cells

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Category: Brainstem: Structure & Function
Background: Globular bushy cells (GBCs) in the cochlear nucleus encode temporal information of sounds with high fidelity essential for binaural processing. At low-frequency sounds, their spiking pattern depends on sound frequency resulting in temporally precise phase-locking, while at high-frequency sounds primary-like responses with less regular spiking are elicited. The GBCs receive converging auditory nerve inputs which form axosomatic synaptic terminals that vary in size and strength. Such variability of sub- and suprathreshold inputs is expected to enable GBC to operate as coincidence detectors or mixed integrators. However, how input variation

influences sound encoding over ecologically relevant frequencies remains elusive. We address this question by performing conductance clamp recordings from GBC of Mongolian gerbils. The experimental results were complemented by simulations of GBC processing using an adaptive threshold-crossing detection model.
Methods: Conductance clamp recordings from GBC were obtained from acute brain slices of P21-25 gerbils. The stimulus was generated by convolving experimentally measured EPSCs with auditory nerve (AN) trains obtained from a mammalian auditory periphery model. The simulated conductance was implemented as the linear sum of 10 ANF inputs with varying synaptic strength profiles: no variation, medium variation, and high variation.
A single-compartment model was designed based on a previously published coincidence counting model of cat GBCs. Model parameters, including the duration of synaptic inputs and the strength and time scale of threshold adaptation, were adjusted to match the experimental data of gerbil GBCs. The simulated inputs to the model, as well as the output measures, were identical to the experimental ones allowing for a direct comparison. The variability of synaptic strength was changed systematically to cover the entire range tested in the experiments.

Results: The impact of input variability on GBC spiking was tested for pure tones at 350 Hz, 1 kHz, and 3.5 kHz. Experimental and modeling data consistently showed that an increase in input variability elevates firing rates during 1 kHz and 3.5 kHz stimulation, but reduced temporal precision, quantified as vector strength, for 350 Hz and 1 kHz stimulation. Increased input variability led to reduced onset firing rates with longer first-spike latency and larger temporal jitter, while increasing sustained firing rates during sound stimulation. For amplitude-modulated sounds, a common feature of environmental sounds, input variability increased firing rates and resulted in a better reproduction of the stimulus waveform but at the loss of temporal precision.
Conclusions: Our experimental and modeling results demonstrate that increased input variability elevates the spiking rate and improves the GBCs' reproduction of the stimulus at the expense of phase-locking accuracy. These findings suggest that changes in input variability can shift the emphasis from temporal coding to sound level encoding and thus may play a role in enhancing GBCs' ability to encode multiple features of complex acoustic signals.

M15. Characterizing Novel Candidate Molecular Markers of Inferior Colliculus Neuron Types

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Category: Midbrain: Structure & Function
Background: The ability to isolate and selectively manipulate neuronal subpopulations is critical for understanding how those subpopulations contribute to circuit-based functions. Neurons in the inferior colliculus (IC), the midbrain hub of the central auditory pathway, can be divided into glutamatergic (excitatory) and GABAergic (inhibitory) neurons. Excitatory and inhibitory IC neurons can be further subdivided into neuronal types based on molecular markers. Current molecular markers for IC neurons include vasoactive intestinal peptide (VIP), cholecystokinin (CCK), and somatostatin (SST) for excitatory neurons and neuropeptide Y (NPY) for inhibitory neurons. Using the Allen Brain Cell Atlas, a single cell RNA-sequencing

(scRNA-seq) atlas of the mouse brain, we have identified two novel candidate molecular markers for IC neuron types, secreted phosphoprotein (SPP1) and prodynorphin (PDYN).
Methods: To determine whether SPP1 and PDYN are useful molecular markers for IC neuron populations we conducted fluorescent in situ hybridization on IC sections from C57BL/6J and Cdh23Ahl+ mice and analyzed the distributions of Spp1 and Pdyn mRNA across subdivisions of the IC. Next, we used Pdyn-IRES-Cre x Ai14 and Spp1-IRES-tdTomato mice to perform whole-cell patch clamp recordings in brain slices targeted to each neuron type and reconstructed neuronal morphology based on biocytin cell fills.
Results: Our findings demonstrate that Pdyn mRNA expression labels exclusively a subset of excitatory neurons that are roughly ~80% distinct from previously identified excitatory neuron types in the IC. Furthermore, our findings suggest PDYN neurons have consistent intrinsic physiological properties, including moderate membrane resistance and sustained firing pattern in response to sustained current steps. Our in situ hybridization results indicate that PDYN cells are found throughout all major IC subdivisions. In addition, we demonstrate that roughly one-third of Spp1 mRNA expression labels a subpopulation of excitatory neurons that are approximately ~95% distinct from previously identified molecular markers for IC excitatory neurons. The distribution of Spp1 positive cells is also much more biased to the central nucleus of the IC (ICc) than cells labeled with other IC excitatory neuron markers.
Conclusions: We find that PDYN and SPP1 are promising candidates for molecular markers for IC neuron types. Our results suggest that PDYN and SPP1 label subpopulations of excitatory IC neurons largely distinct from each other and from previously identified groups of IC neurons. We also find that Spp1 positive cells are localized in the ICc. To determine the functional connectivity of PDYN and SPP1 neurons to IC circuits, we will next use channelrhodopsin-assisted circuit mapping (CRACM) to assess the inputs and outputs of PDYN and SPP1 neurons.

M16. Serotonergic Modulation of Inhibitory and Excitatory Neurons in the Inferior Colliculus

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Category: Midbrain: Structure & Function
Background: Neuromodulatory inputs, such as serotonergic signaling, influence the activity of neurons in the central auditory pathway, affecting our ability to perceive sound. The inferior colliculus (IC), which is considered the midbrain hub of the auditory system, receives a dense serotonergic projection from the dorsal raphe nuclei. Serotonin has been proposed as an important regulator of enhanced central gain, a change in inhibitory and excitatory circuits that favors excitability after hearing loss. However, because of the diversity of neuron types and complex circuitry in the IC, the mechanisms by which serotonin leads to changes in auditory responses remain poorly understood. We previously identified that neuropeptide Y (NPY) expression is

a marker for the first class of IC GABAergic neurons. On the other hand, neurons that express the receptor for NPY (NPY Y1 receptor, Y1R) are glutamatergic neurons. Here we hypothesize that serotoninergic signaling alters the inhibitory and excitatory balance of circuits in the IC by enhancing activity in NPY neurons and diminishing activity in Y1R neurons.

Methods: To test this hypothesis, we performed whole-cell patch clamp recordings targeted to either NPY or Y1R neurons in IC coronal brain slices. NPY neurons were identified by the hrGFP fluorescence in the NPY-hrGFP mouse line and Y1R neurons were identified by the tdTomato fluorescence in the Y1R-Cre x Ai14 mouse line. We used a puff pipette to deliver serotonin and selective antagonists to identify the receptors mediating the serotonergic effect. Next, we performed in situ hybridization to detect mRNA transcripts for the main types of serotonergic receptors previously described in the IC: 5-HT1A/B 5-HT2A/C and 5-HT3A/B in Npy and Npy1r expressing neurons.

Results: Application of serotonin in brain slices resulted in a depolarization of NPY neurons and hyperpolarization of Y1R neurons. The depolarizing responses were mediated by activation of 5-HT2A and/or 5-HT2C serotonergic receptors. In agreement with these results, the in situ hybridization showed that 40% of Npy neurons expressed 5-HT2A and 45% expressed 5-HT2C. Around 11% of Npy neurons expressed 5-HT1A and only 4% expressed 5-HT1B. Interesting, no expression of 5-HT3A/B was observed in the IC. Additionally, our data also showed that serotonin enhances inhibitory inputs to Y1R neurons and this effect is mediated by 5-HT2A receptors. Future experiments will be performed to determine the subtypes of serotonergic receptors expressed by Y1R neurons.

Conclusions: Our data shows that serotonin differentially modulate the activity of NPY and Y1R neurons in the IC. Since age-related alters the balance of excitation and inhibition in the IC, future experiments will determine whether serotonergic signaling to NPY and Y1R neurons are changed in a mouse model of age-related hearing loss.

M17. Social Experience Dependent Plasticity in Micro-Organization and Population Coding of Sequences of Mouse Vocalizations in the Mouse Auditory Cortex

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Category: Auditory Cortex and Thalamus: Structure & Function

Background: Mouse ultrasonic vocalizations (USVs) are produced in a context specific manner with communicative significance. Our earlier work shows social experience related increased single neuron selectivity of predictive natural USVs produced by male mice during courtship compared to randomly ordered syllable sequences. Here we investigate the effects of social experience on micro-organization of neurons with different sequence selectivity properties and how plasticity alters representation of entire sequences in micro-networks of neurons in the mouse auditory cortex (ACX). Based on the above we also investigate the effect of the plasticity in coding and discrimination of sequences by different populations of neurons.

Methods: Single unit extracellular recordings and 2-photon Ca2+ imaging was performed in the ACX awake and (as well as) anesthetized female (and male) mice. Responses to previously derived random (SR) and predictive USV sequences (natural, SN) produced by male mice in different courtship situations were obtained. Responses were collected from excitatory (EX) and classes of inhibitory (IN) neurons with 2-photon Ca2+ imaging. The USV sequences are created with 5 specific syllable types: noisy (N), single pitch contour (S), pitch jump type (J), with harmonic content (H) and multiple pitch jumps and other complex calls (O) as in our previous work.

Results: We show that in the local circuitry (in ~100 um), SN selective neurons had high signal correlations with other such neurons. The same was observed for SR selective neurons. However, the signal correlations between neurons selective to SR with those selective to SN, were near 0. These indicate that selectivity to natural sequences or lack thereof occurred in local clusters of neurons indicating homogeneity in an otherwise functionally heterogeneous ACX. Further, pairs of neurons selective to SR had comparatively higher noise correlations than other types of pairs of neurons. With social experience the increased selectivity to SN over SR was accompanied with lowering of noise correlations in pairs of SR selective neurons while in other types of pairs they remained similar as a function of distance between pairs. An overall lowering of signal correlations along with the above show that in the population, coding of SR became worse with experience, leading to a relative improvement in coding of SN. We quantify the coding performance by different types of populations of neurons of different sizes to understand the alterations in local correlations of neurons due to plasticity with social experience.

Conclusions: Our results further support the idea of mouse vocalization sequences being encoded as a whole and their overall representation alterations due to plasticity associated with social behaviour provide a way to understand mechanisms in alterations in plasticity required in developing social communications present. Such alterations and lack of learning and social communications are present in autism spectrum and other neurodevelopmental disorders.

M18. Stimulus-Specific Suppression Distinguishes Layer 5 From Layer 6b Extratelencephalic Neurons

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Category: Auditory Cortex and Thalamus: Structure & Function
Background: Extratelencephalic (ET) neurons of the auditory cortex (ACtx) receive both ascending and intra/inter-cortical inputs, forming an intricate feedback circuit to higher-order subcortical targets. Located in both layer (L) 5, and L6b of ACtx, they play important roles in learning-induced plasticity. L5 and L6b ET neurons are morphologically and physiologically distinct. Unlike L5 ET neurons that have large pyramidal-shaped cell bodies with a single prominent apical dendrite extending towards L2/3 and L1, L6b ET neurons have radially oriented soma with profusely branched dendrites extending over a millimeter, allowing them to gather information across longer distances. In vitro studies have found that a significant fraction of L5 ET neurons are burst spiking while L6b ET neurons are largely regular spiking. The in vivo ramifications and functional significance of such ET diversity remains poorly understood. To address this, our current study focused on characterizing single-cell and population-level sound processing by both L5 and L6b ET neurons.
Methods: Using an intersectional viral strategy, we expressed GCaMP8s in both L5 and L6b ET neurons and used two-photon microscopy to record calcium activity in response to pure tones (PT), sinusoidally amplitude modulated (sAM) noise, and dynamic ripples.
Results: Using hierarchical clustering, distinct neuronal response motifs were characterized in an unsupervised manner. PT evoked a response in over 33% of the L5 ET neurons (24% enhanced, 9% suppressed), while only 23% of L6b ET neurons were responsive (10% enhanced, 13% suppressed). In contrast, sAM responses demonstrated greater diversity, with clusters representing distinct patterns of both enhanced and suppressed firing. Most L5 ET neurons exhibited excitatory responses (45% excited, 26% suppressed), while the dominant response motif in L6b ET neurons was suppression (26% enhanced, 46% suppressed). Like sAM noise, dynamic ripples evoked excitatory responses in most responsive L5 ET neurons (34% excited, 14% suppressed), while most L6b ET neurons were suppressed (25% excited, 39% suppressed). We then used multinomial logistic regression to analyze the ability of L5 and L6b neurons to decode stimulus identity. L5 ET neurons could decode all 3 stimuli (PT, sAM noise, and dynamic ripples) with higher accuracy than L6b ET neurons.
Conclusions: These results demonstrate a dichotomy in stimulus decoding and processing between L5 and L6b ET neurons, potentially leading to differential impacts on downstream targets that modulate auditory-guided behavior.

M19. Functional in Vivo Characterization of Layer 6 Corticothalamic Neurons in Primary Auditory Cortex

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Category: Auditory Cortex and Thalamus: Structure & Function
Background: Corticothalamic (CT) neurons in layer 6 (L6) are glutamatergic pyramidal neurons that provide modulatory input to upper cortical layers through disynaptic inhibition onto local interneurons or direct modulatory glutamatergic synapses. They also send long-range reciprocal projections to all thalamic nuclei, exerting a role in modulating thalamic responses. In the auditory cortex, these features could be significant in controlling the gain of upper cortical layers and modulating neuronal responses toward enhanced sound detection against a noisy

background. However, the functional properties of L6 CT neurons and their distinct subpopulations have yet to be described, and it is still unknown how they coordinate to modulate both thalamic and intracortical activity to account for the response gain of upper layer neurons during listening in noisy backgrounds.
Methods: We used in vivo two-photon microscopy to image the primary auditory cortex of mice using the Ntsr1-Cre (B6.FVB(Cg)-Tg(Ntsr1-cre)GN220Gsat/Mmucd) mouse line, which selectively labels L6 CT neurons, crossed with a reporter line expressing GCaMP6s (B6.Cg-Igs7tm162.1(tetO-GCaMP6s,CAG-tTA2)Hze/J) on a fixed hearing background (B6.CAST-Cdh23Ahl+/Kjn). We first imaged adult mice (6 – 20 weeks of age) while presenting pure tones (PTs) (4 – 64 k Hz) at different sound levels (40 to 70 dB SPL), followed by a combination of two PTs at 63 dB SPL to obtain the tonotopic maps of L6 CT neurons, frequency response areas (FRAs) and inhibitory sidebands. To study the possible contribution of L6 CT neurons in gain control, we also imaged while presenting PTs followed by PTs within a fixed white noise (WN) background (50 dB SPL) to assess changes in FRAs at different signal-to-noise ratios (SNRs).
Results: We observed that the L6 CT neurons exhibit a heterogeneous tonotopic organization with diverse FRAs. FRAs could be classified into six groups, ranging from narrowly to more broadly tuned responses. Analysis of two-tone interactions revealed broad inhibitory sidebands. Consistent with this finding, we find that the tuning curves of L6 CT neurons narrow when PTs are presented against a WN background, regardless of SNR. We also found that the tonotopic organization of L6 CT neurons becomes more homogeneous in WN, with adjacent neurons showing similar frequency properties.
Conclusions: Our results show that L6 CT neurons have a heterogeneous tonotopic organization, even though they receive significant inputs from first-order thalamic nuclei. That might indicate that intracortical inputs play a role in defining the L6 CT neuron's responses. Our two-tone experiment showed that inhibition shapes neuronal responses, and when presented with WN, tuning curves are narrowed, which enhances frequency selectivity. Moreover, the changes in the tonotopic map when PTs are presented against a WN background suggest that nearby neurons have similar functional properties and thus inputs, which might enhance their ability to synergistically modulate the activity upper cortical layers.

M20. Neural Mechanisms of Rhythm Perception and Encoding in the Marmoset Auditory Cortex

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Category: Primary Auditory Cortex
Background: This study aims to investigate the neural mechanisms underlying rhythm perception and encoding in the marmoset brain, focusing on how neuronal populations in the auditory cortex respond to deviations in rhythmic sequences. Using the oddball paradigm, the research explores how the marmoset auditory system perceives and encodes changes in periodic sounds, particularly when an expected rhythm is disrupted.
Methods: In the experiment, marmosets were exposed to regular rhythmic sound sequences with consistent intervals, occasionally interrupted by a deviant tone. Two-photon imaging was used to count and monitor large populations of neurons in the auditory cortex, providing a precise

measure of their responses to both standard and deviant stimuli. This method allowed for detailed tracking of neuronal activity across the auditory cortex during rhythm perception and detection of deviations.
Results: The findings revealed that different neuronal populations exhibited distinct responses to standard and deviant stimuli. Certain neurons showed enhanced activity when detecting rhythm deviations, suggesting their critical role in identifying changes in rhythmic patterns.
Conclusions: The activity patterns of these neurons provide insight into how the brain predicts and encodes rhythms. The study further demonstrates that rhythm perception and processing are not driven by individual neurons but rely on the collective action of neuronal ensembles.

M21. Immediate Upregulation of Nr4a1 Mrna in Response to Blast-Induced Cochlear Injury in Mice

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Category: Inner Ear: Anatomy & Physiology
Background: Blast-induced cochlear degeneration is primarily driven by mechanical and metabolic damage, including the generation of reactive oxygen species, the release of inflammatory cytokines, and the activation of apoptosis. However, the immediate acute-phase responses that trigger metabolic cochlear degeneration remain poorly understood. A comprehensive understanding of the molecular and structural changes occurring in the immediate acute phase following blast shockwave injury is crucial for the development of effective therapies to mitigate cochlear degeneration. The present study aims to elucidate the acute molecular events and structural alterations in the cochlea associated with auditory dysfunction following blast exposure.
Methods: Male CBA/J mice at 3 months of age were exposed to blast overpressure using a blast-tube. The peak pressure of blast shock wave was set to 25 kPa and irradiated to the mouse from diagonally upward. We have employed RNA-sequencing (RNA-seq), quantitative real-time polymerase chain reaction (qRT-PCR) to provide the comprehensive molecular profile immediately in response to blast exposure. The auditory brainstem response (ABR) were measured to confirm cochlear function before and after blast exposure. Cochleae were examined to measure the survival of hair cells (HCs), spiral ganglion neurons (SGNs), and synaptic properties of auditory neurons following blast exposure.
Results: In blast exposed mice, a significant reduction in ABR wave I amplitudes was observed, accompanied by a slight elevation in ABR thresholds one month post-exposure. Consistent with these findings, a marked reduction in cochlear synapse numbers was detected, despite the absence of substantial loss of inner and outer hair cells. RNA-seq analysis was performed on cochlear samples collected within one minute post-blast exposure, as well as from non-exposed controls. Comparative analysis of mRNA expression identified eight differentially expressed genes, with two (25%; Nr4a1, Ddit4) upregulated and six (75%; Gstm7, Myo7a, Fam83b, Zfp867, Tnfrs19, Ttr) downregulated in the blast-exposed cochleae. Among these, Nr4a1 is recognized as a key regulator of cochlear inflammation, apoptosis, and cell survival and death. qRT-PCR results demonstrated that Nr4a1 mRNA expression increased immediately following blast exposure and subsequently declined over time. Immunohistochemistry revealed prominent

Nr4a1 expression in specific regions of the cochlea, including the organ of Corti, spiral ganglion neurons (SGNs), and the cochlear lateral wall, particularly in the stria vascularis and spiral ligament.
Conclusions: Eight differentially expressed genes were identified immediately following blast exposure, which may be associated with blast-induced cochlear synaptopathy. Notably, Nr4a1 was widely expressed throughout the inner ear, strongly suggesting its involvement in the pathogenesis of cochlear degeneration. Further investigation into the altered gene expression in the cochleae of blast-injured mice may offer valuable insights into potential therapeutic targets and strategies.

M22. Developmental Expression of the Ha Tagged α9 Nachr Subunit in the Mouse Cochlea

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Category: Inner Ear: Anatomy & Physiology
Background: In the developing inner ear the efferents play a critical role in the maturation of cochlear function. The release of acetylcholine from efferent endings activates α9α10 nAChRs early in postnatal development, making a transition from inner to outer hair cells prior to the onset of hearing. In the present work we describe the expression of HA-tagged α9 nAChRs in inner and outer hair cells during this critical period of cochlear maturation.
Methods: Heterozygote A9HA neonatal and 3-week old mouse inner ears were fixed with 4% paraformaldehyde. Whole-mount cochlear sensory epithelia were dissected out and incubated with a mixture of rabbit anti-HA and goat anti-ChAT antibodies. Fluorophore conjugated secondary antibodies were used to visualize α9HA immunopuncta and the efferent synapses. Wild-type cochlear sensory epithelia were used as control. Confocal microscope images were collected for visualization and subsequent quantitative analysis.
Results: The mice were divided into 6 post-natal groups: P5, P7-8, P10, P12, P14 and P18. Small α9HA immunopuncta were visible as early as P5 on IHCs throughout the cochlea, but were absent from all OHCs. By P7-8 the number of immunopuncta per IHC increased with the most per IHC in the apex. By P10 and P12 α9HA immuno-puncta are present throughout the cochlea on all 3 rows of OHCs with only few remaining on apical IHCs. Thus, nAChR expression revealed by HA labeling corresponds with previous descriptions of the developmental transition of efferents from inner to outer hair cells at the onset of hearing (Simmons et al. 1996 J. comp. Neurol. 370:5561). At P14 α9HA immunolabel on OHCs was less intense and spread within the subnuclear, post-synaptic area. By P17 and P18 HA immunolabel was restricted to bright, circumscribed puncta aligned with efferent endings. By P18 α9 HA immunopuncta have disappeared from IHCs and were present only on OHCs, echoing the adult pattern of efferent innervation.
Conclusions: Our observations show that as the cochlea matures, α9HA nAChR subunit expression shifts from IHCs to OHCs at the onset of hearing. We also observed that the postsynaptic distribution of α9HA protein becomes increasingly localized opposite the efferent contacts. It will be of interest to determine if this transition is related to the expression of SK channels that accompanies synaptic function (Roux et al., 2011, J. Neurosci. 31:15092).

M23. Transcriptomic Heterogeneity in Young C57BL/6N Mice Due to the Presence of the Cdh23ahl Allele

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Category: Inner Ear: Anatomy & Physiology

Background: The mouse continues to be an essential model organism for our understanding of the development, function, and maintenance of the mammalian auditory system. However, issues relating to reproducibility of studies across different laboratories has been a cause of concern, and this is likely to continue as we move into a new era of therapeutic interventional studies. Cadherin23 (Cdh23) is an integral component of tip links, which are essential for mechanical gating of the transducer channels in response to sound-induced deflection of the stereocilia bundle. In mouse, the hypomorphic Cdh23ahl (Cdh23c.753A) allele is present in GREATER THAN 20 different inbred strains. This includes the commonly utilised C57BL/6N and C57BL/6J strains, predisposing these mice to progressive hearing loss starting at around 3-months of age with high-frequency hearing lost first. Although the association between Cadherin23 and tip-links is well established, mice homozygous for the Cdh23ahl allele display a wide range of cochlear pathologies, some of which occur before hair-cells begin to degenerate. Moreover, we have recently demonstrated that genetic interaction between Cdh23ahl and an Embigin-null allele not only causes early-onset, progressive hearing loss but also causes developmental defects in the brain and heart. Thus, we hypothesised that subclinical molecular changes occur within the auditory system of mice homozygous for the Cdh23ahl allele prior to the onset of hearing loss in these mice.

Methods: To investigate this, we have have utlised bulk RNA-sequecencing to assess the cochlear transcriptomes of C57BL/6N (C57BL/6N-Cdh23ahl) mice compared with a highly-genetically controlled, co-isogenic C57BL/6N-Cdh23753A GREATER THAN G strain in which Cdh23 has been “corrected” using CRISPR/Cas9-mediated homology-directed repair. For each strain, cochlear transcriptomes were generated utilising male (n=4) and female (n=4) mice, at both P16 and at P30.

Results: At P16, we identify 187 differentially expressed genes (DEGs) (151 upregulated, 36 downregulated in C57BL/6N-Cdh23ahl) with evidence of an upregulated immune response. At P30, the number of DEGs is increased to 657 (620 upregulated, 37 downregulated in C57BL/6N-Cdh23ahl) with changes to genes associated with Ca2+ homeostasis. Motif enrichement analysis reveals that more than a third of the upregulated genes at P30 contain binding motifs associated with a single transcription factor family. Moreover, we also find that C57BL/6N cochlear transcriptomes are more heterogeneous at both timepoints compared with the homogeneous C57BL/6N-Cdh23753A GREATER THAN G cochlear transcriptomes.

Conclusions: Our findings demonstrate that the Cdh23ahl allele causes a number of transcriptomic changes in the cochleae of young C57BL/6N mice. The transcriptomic heterogenity present in young C57BL/6N mice may represent their individual trajectory along a common pathway towards hearing loss. Importantly, these changes are occuring long before

overt increases in hearing thresholds become evident. These findings have important ramificaitions for studies involving mice that carry the ahl allele, and could help explain some of the phenotype variation observed across studies.

M24. Functional Role of FLRT3 in Mammalian Auditory Hair Cells

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Category: Inner Ear: Anatomy & Physiology

Background: Fibronectin leucine-rich transmembrane proteins (FLRTs) in mammals comprise a family of three proteins—FLRT1, FLRT2, and FLRT3—identified through genomic screening. These proteins are implicated in cell adhesion and receptor signaling. Although mutations in human FLRT3 have been linked to hearing loss via an unknown mechanism, the functions of FLRT3 in auditory hair cells remain largely unexplored. In this study, we propose that FLRT3 may play a crucial role as a component of hair cell bundles.
Methods: Here, immunostaining of hair cells and auditory brainstem response tests were performed.
Results: Immunostaining for FLRT3 revealed its expression in the peripheral subpopulation of microvilli at embryonic day 16, with enrichment observed at the base of stereocilia in hair cells from postnatal days 0 to 2. By postnatal days 7 and 13, FLRT3 was no longer detectable on the hair bundles of outer and inner hair cells, respectively. Notably, two-thirds of the FLRT3 conditional knockout mice exhibited significant high-frequency hearing deficits by two months of age, as indicated by auditory brainstem response tests, with these deficits becoming more pronounced by six months.In addition, significant outer hair cell loss was observed in the basal turn of the cochlear hair cells in the affected mice.
Conclusions: These findings suggest that FLRT3 may be involved as a constituent of the ankle region complex and plays a vital role in the normal development of mouse hair bundles. This work was supported by Hong Kong RGC GRF16102417, GRF16100218, GRF16102720, GRF16101321, and GRF16104223, NSFC-RGC Joint Research Scheme N_HKUST614/18, SMSEGL20SC01-K (all to P.H.), and in part by the Innovation and Technology Commission (ITCPD/17-9) and HKUST (Z1056).

M25. Single-Nucleus RNA-Sequencing Profiling of Mouse Cochlea in Response to Cisplatin

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Category: Inner Ear: Anatomy & Physiology

Background: Cisplatin is a widely used anti-cancer drug with significant ototoxic effects that leads to the death of mechanosensory hair cells resulting in permanent hearing loss. The major route of cisplatin entry into the cochlea is thought to be through the capillaries in the stria vascularis (SV). Our previous findings indicate highest cisplatin accumulation and retention in the stria vascularis, with significant increase also observed in hair cells of the organ of Corti following cisplatin treatment. Therefore, using our previously developed clinically relevant mouse model of cisplatin-induced ototoxicity, we performed single-nucleus RNA-Sequencing (snRNA-Seq) to examine transcriptional changes specifically in cells within the stria vascularis (marginal, intermediate, basal cells) and hair cells in the mouse cochlea following cisplatin exposure to understand transcriptional changes associated with cisplatin treatment and accumulation.
Methods: Our mouse model involves three cycles of cisplatin treatment, each cycle consisting of once-daily cisplatin injection for 4 days followed by a 10-day recovery period. We conducted snRNA-Seq using the 10X Genomics platform on cochleae obtained during the first cycle of cisplatin treatment from control mice, mice treated with cisplatin for four days (cisplatin group), and mice treated with cisplatin for four days followed by a 10-day recovery period (recovery group). The snRNA-Seq datasets were analyzed using the Seurat R package to cluster cells according to their expression profiles. Expression profiles were further analyzed using differential gene analysis and gene ontology to understand transcriptional changes in response to cisplatin treatment and recovery.
Results: Within the stria vascularis, the marginal and intermediate cells displayed transcriptomic changes in response to cisplatin treatment while the basal cells exhibited only minimal changes. Both cell types displayed upregulation of genes associated with apoptosis and leukocyte migration in response to cisplatin treatment. During the recovery period, these cell types upregulated genes associated with regulation of DNA repair and recombination. The cell types in the organ of Corti also displayed cell-type specific responses to cisplatin. Hair cells upregulated gene related to metabolism of reactive oxygen species and homeostasis while spiral ganglion neurons (SGN) displayed upregulation of genes related to regulation of membrane potential, neurotransmitter secretion, and synapse assembly. The recovery time point displayed upregulation of cell polarity establishment and DNA repair in hair cells and SGN respectively.
Conclusions: Our results demonstrate dynamic transcriptomic changes occurring in the stria vascularis and organ of Corti. Further analyses will help identify transcriptional changes in response to cisplatin across other cochlear cell types and facilitate further investigation of the roles of identified signaling molecules in the cochlear response to cisplatin in animal models.

M26. Adriamycin Nephropathy Causes Sensorineural Hearing Loss via Blood-Labyrinth Barrier Disruption and Hyperpermeability in Balb/c Mice

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Category: Inner Ear: Anatomy & Physiology
Background: Sensorineural hearing loss (SNHL) is considerably more prevalent in patients with chronic kidney disease (CKD) than in the general population. The blood-labyrinth barrier (BLB), the barrier between the vasculature and fluids of the inner ear, controls the exchange between the

blood and interstitial space in the cochlea. Vascular hyperpermeability is a feature of various pathological conditions, including chronic inflammatory diseases, neurological disorders, and cisplatin-induced hearing loss.
Methods: To investigate the roles of cochlear BLB in CKD, we used adriamycin (10 mg/kg and 12 mg/kg) to induce an animal model of nephropathy in male BALB/c mice. Mice treated with adriamycin showed significant kidney injury (nephrotoxicity) as well as hearing loss (ototoxicity). Adriamycin-treated animals showed a marked increase in glomerular injury, podocyte damage, and renal neutrophil gelatinase-associated lipocalin (NGAL) as well as elevated levels of serum creatinine and blood urea nitrogen (BUN).
Results: Adriamycin resulted in significant hearing impairment measured by auditory brainstem responses (ABR) and distortion-product otoacoustic emissions (DPOAE) accompanied by loss of cochlear hair cells and ribbon synapses (synaptopathy). Adriamycin nephropathy impacted ion channel expression on the stria vascularis and induced BLB hyperpermeability along with changes in endothelial cells, pericytes, and perivascular-resident macrophage-like melanocytes. Importantly, adriamycin nephropathy significantly increased cochlear NGAL and NLRP3 levels, but not IL-1β and TNF-α at 4- or 8 weeks post adriamycin administration. The expression of NGAL was highly expressed in the tectorial membrane, cochlear neurons, and Organ of Corti, and its receptor, 24p3r, was co-localized with NGAL.
Conclusions: Taken together, these data indicate that sensorineural hearing loss induced by adriamycin nephropathy is modulated by periphery-derived NGAL and disruption of the blood-labyrinth barrier.

M27. Gonad-Derived Hormones Mediate Sex Differences in the Maturation of Peripheral Auditory Sensitivity in C57BL/6J Mice From Adolescence to Adulthood

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Category: Inner Ear: Anatomy & Physiology
Background: Sex differences in peripheral and central auditory processing have been reported in numerous studies, often measured with auditory brainstem responses (ABR), where females have lower thresholds and higher wave I-IV amplitudes than males. Studies in multiple vertebrate species suggest that at least thresholds and wave I amplitude are driven by gonad-derived hormones. Less is known, however, about the timing of the onset and duration of these sex differences and whether sex chromosome-linked genes also contribute. Resolving these issues is important because it may explain why recent studies using hormone replacement in both rodent models and human patients have yielded conflicting results as to the benefits of hormone replacement therapy (HRT) for auditory sensitivity.
Methods: To characterize the sex differences in the maturation of auditory sensitivity, we performed longitudinal ABR recordings from pre-pubescent adolescence (postnatal day 25 (P25)), through late adolescence after the onset of puberty (P38), and into early adulthood (P65 and P95) in the presence and absence of gonad-derived sex hormones in wildtype (WT) C57BL/6J mice. We analyzed ABR thresholds and wave I-II latencies and amplitudes. To test potential contributions of sex chromosome-linked genes on peripheral auditory processing, at

P65 we recorded ABRs in gonadectomized and intact four core genotypes (FCG) C57BL/6J mice.
Results: In sham and gonadectomized female and male WT mice, we did not find differences in hearing thresholds at any of the ages tested. We found that from P25 to P95, wave I amplitude decreased by 50%. However, wave I amplitude in sham females was 22% and 11% higher than sham males at P38 and P65, respectively. In gonadectomized mice, there was no sex difference in wave I amplitude at any of the ages tested, due to a decrease in gonadectomized females. Results from P65 FCG mice corroborated those from WT mice since mice with ovaries had higher wave I amplitudes than mice with testes, independent of genetic sex, and the sex difference was lost in gonadectomized mice. Unlike wave I, wave II amplitude slightly decreased from P25 to P95 and was higher in both WT sham and gonadectomized females compared to males.
Conclusions: Together, the data indicate a maturation-dependent decrease from late adolescence to early adulthood in wave I amplitude in both females and males, though it is delayed in females. Our results also suggest that ovary-derived estradiol drives the maturation delay. On the other hand, gonad-derived hormones may not affect the maturation of amplitude of wave II (cochlear nucleus). We conclude that gonad-derived hormones may affect differently the maturation of neural encoding in the cochlea during adolescence in females vs. males. This may explain why HRT may not be a useful l strategy for increasing auditory sensitivity, especially in older age.

M28. The Diversity of Murine Type II Spiral Ganglion Neurons Biophysical Properties

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Category: Inner Ear: Anatomy & Physiology
Background: Auditory information is transmitted from the cochlea to the central nervous system through spiral ganglion neurons (SGNs). The SGN population has two major divisions based on their synaptic inputs. 95% of SGNs (type I SGNs) contact a single inner hair cell with an unbranched dendrite whereas the remaining SGNs (type II SGNs) branch widely and contact tens of outer hair cells. Heterogeneity within type I SGNs is hypothesized to contribute to sound detection with sub-populations identified based on their firing patterns, synaptic positions, genetic profiles, and ion channel properties. Given their relative sparsity, it is unknown whether similar subdivisions exist within the type II SGN population and whether they have distinct biophysical properties to support their function.
Methods: SGNs from SERTCre;Ai14 and Tac1Cre;Ai14 mice were dissected, enzymatically treated, and triturated to isolate their somata. The expression of a genetically encoded fluorescent reporter, tdTomato, allowed for the identification of type II from type I SGNs despite the absence of their synaptic connections. For both populations, SGNs without tdTomato fluorescence should be type I SGNs, and SGNs with tdTomato fluorescence should be biased toward the type II identity, with SERTCre;Ai14 more exclusive to type II SGNs than Tac1Cre;Ai14. Ionic currents and firing properties were recorded through perforated patch-clamp methods from these isolated somata. SGNs were grouped by a clustering algorithm based

on biophysical properties such as whole-cell conductance and then validated by their tdTomato fluorescence.
Results: We recorded 14 SERT+ (putative type II SGNs), 9 Tac1+ (mixed SGNs), and 28 tdTomato- SGNs (putative type I SGNS). Overall, nearly every SERT+ and half of the Tac1+ SGNs had smaller, rapidly inactivating whole-cell currents, more depolarized resting membrane potentials, and were less capable of maintaining spiking to high stimulus pulse rates as compared to tdTomato- putative type I SGNs. Additionally, we identified three groups of putative type II SGNs based on a multivariate analysis of cell size, whole-cell currents, and spiking properties: 1) 3/18 tdTomato+ cells had extra-large somata (191.9 ± 33.8 μm2) and fired anode break spikes and spikes at current onset, 2) 7/18 tdT+ intermediate-sized SGNs (148.3 ± 26.0 μm2) did not spike from rest but were capable of spiking if first hyperpolarized to relieve sodium channel inactivation, and 3) 8/18 TdT+ small SGNs (114.6 ± 13.6 μm2) fired anode-break spikes and depolarized slowly during current injection.
Conclusions: Previous attempts to record from type II SGNs targeted neurons by chance or by targeting their thin dendrites. Fluorescent reporter lines offer a tractable method to identify and target type II SGNs. We found clear differences in intrinsic excitability between type I and II SGNs. Furthermore, three different biophysical profiles emerged within the type II SGN population, suggesting a previously unappreciated diversity within the type II SGNs.

M29. Pathology of Fresh Human Cochleae Imaged With OCT and Validated With Histological Assessment

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Category: Inner Ear: Anatomy & Physiology
Background: The human cochlea, a small fluid-filled organ, is encapsulated by the hardest bone in our body and is further surrounded by the temporal bone. As a result, currently available clinical assessments of cochlear health are mostly limited to standard audiometric measurements, including otoacoustic emissions. While these methods can diagnose hearing loss and the dysfunction of outer hair cells (which help amplify small sounds), the etiology is generally unknown, and the anatomical state of the cochlea is never directly assessed in clinic. However, recent advances in imaging technologies such as optical coherence tomography (OCT) enable visualization of cochlear structures in situ without invading the cochlea. Here, we carried out a coordinated study of human cochlear anatomy based on OCT imaging of fresh (4-24 hours postmortem), unfixed human cochleae combined with light microscopy imaging of histology prepared from the same donor to better interpret OCT images and assess OCT's ability to detect cochlear pathologies.
Methods: Fresh human temporal bones (4-24 hours postmortem) were obtained from donors with permission at Massachusetts General Hospital. From each case, one ear was prepared for OCT imaging whereas the contralateral ear was immediately fixed in formalin and prepared for histology. Imaging was performed in intact, unfixed cochleae through the round window membrane using a 900-nm center wavelength OCT system (Gan6201C1, Thorlabs, Germany) with an axial resolution of 2.23 μm (in water) and lateral resolution of ~8 μm as previously

described (Cho et al., 2022 JARO 23(2):195-211). Commercially available software (ThorImageOCT 5.4.8) was used to collect cross-sectional images and 3D volumetric images of the cochlear partition. Following imaging, cochleae were fixed and histologically processed along with the contralateral ears using a novel and rapid embedding technique based on a plastic (methyl methacrylate) resin.
Results: OCT was able to image cochlear structures after surgically accessing the middle ear cavity via a mastoidectomy with an expanded facial recess approach. Direct comparisons of OCT images and histology from the same donor and cochlear region facilitated the interpretation of OCT images and identified structural pathologies such sensory hair cell loss. We also investigated the effects of histological processing artifacts due to chemical fixation and dehydration.
Conclusions: The ability to image and identify cochlear structures with OCT may enable us to visualize and diagnose pathologies in patients. Intracochlear imaging would be invaluable for the testing of new pharmaceutical therapies, as the success of these drugs may require that baseline conditions of cochlear health be met, such as the survival of supporting cells or a near-normal appearance of the organ of Corti. Our results aid the accurate interpretation of OCT images and aim to facilitate the use of OCT as a future non-invasive real-time method to diagnose cochlear pathology in the clinic.

M30. Reliability of Potential Biomarkers in the Neurodiagnostic Auditory Brainstem Response

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Category: Inner Ear: Anatomy & Physiology
Background: The physiology of the auditory nerve and brainstem can be assessed with the suprathreshold, or neurodiagnostic, auditory brainstem response (ABR). The neurodiagnostic ABR is typically analyzed by visual inspection of waveform features, which requires time, training, and introduces human error into an objective measure of auditory function. Furthermore, visual analysis limits data to that which can easily be seen by the eye (i.e., peaks and troughs). We developed a model-based feature-extraction technique to automate analysis of the neurodiagnostic ABR and extract more features that could be biomarkers for pathophysiology. Extracted features include wave width, wave area, and wave onset latency, which may provide information about neural synchrony and the health of different spontaneous rate populations of the auditory nerve, in addition to peak latency and amplitude. Before these additional features can be studied as potential biomarkers for neural pathology, we must know if they are reliable.
Methods: We recorded ABRs from 50 adults with healthy hearing, as measured via audiometry, tympanometry, and self-report. All participants returned for a second ABR recording at least one week after the first recording, and 20 participants returned over one month later for a third and fourth recording at least one week apart. We elicited ABRs with 100 dB pe SPL clicks, chirps, and 500 and 4000 Hz tone-bursts, for a total of 560 ABR waveforms. The model-based feature extraction technique measured 5 features (peak latency, peak amplitude, wave width, wave area, and wave onset latency) for waves I, III, and V within each waveform.

Results: Two-way mixed effects models of intraclass correlation will determine the absolute agreement of each extracted feature from each wave and from each stimulus, across repeated ABR measures (2-4 visits). This will determine the test-retest reliability of each extracted feature and for each stimulus.
Conclusions: The test-retest reliability of each extracted feature for each stimulus will be vital to determine appropriate sample sizes and create experimental protocols for future studies intending to use the automated feature extraction technique or any waveform feature in their human studies. Without knowledge of test-retest reliability, we cannot draw accurate conclusions regarding group or treatment effects measured by the ABR.

M31. Identification of Soluble Factors Affecting Blood-Labyrinth Barrier Permeability During Cisplatin Treatment via Single Nucleus RNA-Sequencing

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Category: Inner Ear: Anatomy & Physiology
Background: Cisplatin is a widely used anti-cancer drug that can cause permanent hearing loss. Previously we demonstrated that macrophage ablation was correlated with reduced cisplatin levels in the cochlea, suggesting that macrophages contributed to cisplatin entry into the cochlea. A major route of cisplatin entry into the cochlea is through the blood-labyrinth barrier (BLB) into the stria vascularis (SV). Perivascular macrophages are closely associated with capillaries and can regulate BLB permeability either directly or indirectly by altering the expression of soluble factors. Therefore, cisplatin may increase BLB permeability and, consequently, cisplatin entry into the cochlea. TGF-β and VEGF-A are soluble factors that regulate the breakdown of the blood brain barrier, which is comparable the BLB. To identify the specific cell types expressing TGF-β, VEGF-A, and their respective receptors in the cochlea, we performed single nucleus RNA-sequencing (snRNA-seq) on cochleas harvested from control and cisplatin-treated mice.
Methods: Treatment groups included: (1) mice treated with cisplatin for four days and (2) mice treated with cisplatin for four days followed by ten days of recovery. Saline-treated mice served as controls. Whole cochleas were lysed, and single nuclei suspensions were prepared for 10X Genomics Chromium, followed by sequencing. The resulting data were processed and analyzed using Seurat. Differential gene expression analyses were performed for cells within the SV (marginal, intermediate, basal cells), macrophages, and endothelial cells.
Results: TGF-β (encoded by the Tgfb1 gene) can regulate vascular permeability through its receptor, TGFBR1 (encoded by the Tgfbr1 gene). Our snRNA-seq data show a significant increase in Tgfbr1 expression in endothelial cells following cisplatin treatment. Tgfb1 is expressed in cochlear macrophages in both control and cisplatin-treated mice.
Additionally, VEGF-A (encoded by the Vegfa gene) regulates vascular permeability by binding to two receptors, FLT1 and KDR (encoded by the Flt1 and Kdr genes). Our snRNA-seq data indicate that cells within the SV express Vegfa, while endothelial cells express Flt1 and Kdr. Our data suggest that Vegfa expression is significantly increased in cisplatin-treated mice compared

to controls. Although Vegfa expression was not observed in macrophages, macrophages may non-autonomously regulate Vegfa expression in cells within the SV.

Conclusions: Our findings indicate that Vegfa is expressed in the SV, and Tgfb1 is expressed in macrophages. The close proximity of these cells to endothelial cells suggests that the soluble factors they produce may bind directly to their respective receptors expressed on endothelial cells, potentially regulating the BLB permeability. Next steps will include characterizing these soluble factors and receptors at the protein level in cochlear tissues and investigating their roles using transgenic animal models. These data will shed light on the mechanisms that regulate the BLB permeability, and thus controlling drug entry into the cochlea, and they suggest therapeutic approaches to the prevention of cisplatin-induced hearing loss.

M32. Gating of Hair Cell Ca2+ Channels Governs the Activity of Cochlear Neurons

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Category: Inner Ear: Anatomy & Physiology

Background: In the mammalian cochlea, the primary auditory afferent spiral ganglion neurons (SGNs) sample the sound information from the sensory receptor inner hair cells (IHCs). SGNs tuned to a specific frequency and thus potentially receiving input from the same IHC, display a variety spontaneous rates, thresholds and dynamic ranges. This physiological diversity of SGNs is thought to underlie the coding of the wide range of audible sound intensities: SGNs with high spontaneous rates and low thresholds already fire at soft sounds, while SGNs with low spontaneous rates and high thresholds are recruited as the sound levels increase, thereby covering the whole dynamic range of sound and enabling population coding of sound intensities. In this study we tested the hypothesis that heterogeneity of voltage dependent activation of presynaptic CaV1.3 channels among the active zones (AZs) of IHCs contributes to the diversity of spontaneous and evoked SGN firing. To do so, we employed mice expressing the human A749G point mutation in CaV1.3 channels (CaV1.3AG), which, when expressed in heterologous system, was shown to result in channel activation at lower (hyperpolarized) voltages. We hypothesized, that the hyperpolarized activation of the CaV1.3 channels would result in increased spontaneous rates and low thresholds in SGNs.

Methods: IHC physiology and exocytosis was assessed with patch-clamp recordings. The number of the Ca2+ channels in IHCs was estimated electrophysiologically, by performing non-stationary mean-variance analysis, and histologically, by performing confocal and STED superresolution imaging. Ca2+ influx and glutamate release at individual AZs of IHCs were measured by combining patch-clamp from IHCs with Ca2+ (Fluo4-FF or Rhod-FF dyes loaded into the IHCs via patch-pipette) and glutamate imaging (iGluSnFR targeted to the SGNs via viral injection). Auditory function was assessed in-vivo by recording auditory brainstem responses

(ABRs) and single SGN firing. Mitochondrial status of SGNs was obtained from serial block-face electron microscopy reconstruction of cochlear segments.
Results: IHC AZs of CaV1.3AG/AG mice displayed an approximately 15 mV hyperpolarized shift in voltage dependence of CaV1.3 activation and glutamate release. The AZ complement of Ca2+ channels was reduced in CaV1.3AG/AG IHCs, potentially indicating homeostatic compensation of the increased open channel probability of the Ca2+ channels in mutant IHCs. SGNs revealed increased spontaneous rates and the auditory thresholds assessed from the ABRs were reduced by approximately 10 dB in CaV1.3AG/AG mice. Finally, we observed increased mitochondrial content in CaV1.3AG/AG SGN terminals and fibers, potentially reflecting the increased presynaptic activity due to the hyperpolarized activation of CaV1.3 channels.
Conclusions: These results directly demonstrate the regulation of SGN spontaneous and evoked firing by presynaptic CaV1.3 gating and indicate that the heterogeneous voltage dependence of Ca2+ channel activation at IHC AZs contributes to the firing diversity of SGNs.

M33. Investigating Norrin Expression Patterns Within the Cochlea

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Category: Inner Ear: Anatomy & Physiology
Background: Norrin, also known as Norrie Disease Protein (NDP), encoded by the NDP gene, plays a critical role in cochlear development by regulating hair cell maturation and cochlear vasculature formation through the Wnt/β-catenin pathway. NDP knockout (KO) mice exhibit progressive cochlear dysfunction, marked by hair cell loss and severe hearing impairment by 2 months of age. Previous studies have shown that NDP overexpression or upregulation of Wnt signaling can rescue cochlear function.
However, a detailed understanding of Norrin's cellular expression in the cochlea and its effects on cochlear structures remains incomplete. To address these gaps, we focus on characterizing Norrin's distribution in supporting cells and endothelial cells across different genetic models in mice.

Methods: To investigate Norrin expression in the cochlea, we conducted immunostaining on whole-mount preparations of the organ of Corti and cochlear sections, including the stria vascularis. We used specific antibodies to visualize Norrin, supporting cells, and endothelial cells, employing high-resolution confocal microscopy for detailed imaging.
We examined three mouse models: wild-type (WT), NDP KO mice, and Sox2Cre-NDP KO mice with NDP overexpression (OE), where NDP expression is driven by Sox2, meaning the Sox2+ supporting cells specifically express NDP. For the comparison of capillary structures between WT and NDP KO mice, we performed specific immunostaining for endothelial markers.

Our ongoing work also includes using RNAscope to investigate Norrin secretion in the cochlea, aiming to provide further insights into its precise cellular distribution and function.

Results: Our recent findings confirm and expand upon previous observations by Yushi Hayashi (2021), with new insights into vascular changes in NDP KO mice. Using specific markers for supporting cells and endothelial cells, we have mapped Norrin's distribution in key cochlear

structures, including the organ of Corti and the stria vascularis. This analysis reveals differential Norrin distribution in supporting and endothelial cells, underscoring its critical role in cochlear maintenance.
Our ongoing RNAScope efforts will further map Norrin secretion, providing greater insight into its cellular functions within the cochlea.

Conclusions: Our comprehensive analysis of Norrin expression across different genetic models has provided valuable insights into its role in cochlear development and function. The differences in Norrin distribution and capillary structures between wild-type, NDP knockout, and NDP overexpression models, combined with ongoing RNAscope investigations into Norrin secretion, contribute to a deeper understanding of Norrin's precise functions. These findings may ultimately inform future therapeutic strategies for treating Norrie disease.

M34. Investigating DNA Damage Response and Apoptosis in Cisplatin-Induced Ototoxicity

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Category: Inner Ear: Anatomy & Physiology
Background: Cisplatin is a chemotherapeutic drug that is widely used to treat various cancers. However, it is ototoxic and leads to permanent hearing loss in treated cancer patients. Our laboratory has previously developed a clinically relevant mouse model of cisplatin-induced hearing loss, through which we have demonstrated that cisplatin leads to loss of cochlear cells as well as dysfunction of the stria vascularis. Cisplatin binds DNA and form lesions such as intrastrand and interstrand cisplatin-DNA adducts. These DNA lesions block replication and transcription and may also lead to DNA double-strand breaks. An impaired DNA damage repair pathway may ultimately result in apoptosis; however the interactions of signaling proteins involved in these pathways are unclear in the context of cisplatin-induced ototoxicity. After the third cycle of cisplatin administration in our model, there is robust protein expression of the phosphorylated form of the DNA damage marker γH2AX, the . Here, we use the cisplatin mouse model to investigate the localization of these and other markers of apoptotic and DNA damage responses after cisplatin administration.
Methods: Mice were subjected to three cycles of cisplatin treatment, each cycle consisting of once-daily cisplatin injection for 4 days followed by a 10-day recovery period. Cochleae were harvested and cochlear sections were prepared from five different timepoints: cycle 1 cisplatin, cycle 1 recovery, cycle 2 recovery, and cycle 3 recovery periods. Saline-treated mice served as controls. RNAscope, a commercially available in-situ hybridization technique was performed on the cochlear sections to detect the expression of apoptotic and DNA damage response transcripts in all five groups.
Results: In the control tissue sections, Bcl2l1 and H2afx mRNA expression was robust, with both transcripts exhibiting enriched expression in the stria vascularis and spiral ganglion neurons. After one cycle of cisplatin administration, we observed a significant reduction in expression of both markers, followed by upregulation in the expression of both markers at the later timepoints.

Conclusions: Bcl2l1 and H2afx are examples of markers involved in the apoptotic and DNA damage repair pathways, respectively. We observed differential expression of both transcripts showing the same trend of expression throughout the timepoints. Future work includes examination of additional apoptotic and DNA damage response markers with the goal of elucidating the roles of these pathways as mediators of cisplatin-induced hearing loss.

M35. 3D Reconstruction of the Inner Ear Membranous Labyrinth Using 7 Tesla Magnetic Resonance Imaging and Advanced Post-Processing Techniques

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Category: Inner Ear: Anatomy & Physiology
Background: Recent advances in Magnetic resonance imaging (MRI) at 7 Tesla (T) have allowed for greater characterization and resolution of the inner ear's membranous labyrinth. Since inner ear disorders like Meniere's Disease may be associated with swelling of the membranous labyrinth called endolymphatic hydrops, visualizing this space in greater detail may help understand its etiology. We aimed to develop a protocol for 3D segmentation of the inner ear to conduct volumetric analysis and characterize healthy vs. pathologic ears.
Methods: Adult participants without inner ear or neurological disorders were recruited. Axial T2-weighted and 3D-Fluid Attenuated Inversion Recovery (FLAIR) sequences were obtained at 7T MRI before and four hours after intravenous gadolinium-based contrast agent administration. Following image co-registration and subtraction with Statistical Parametric Mapping (SPM12) and MATLAB, images were uploaded into 3D Slicer. Voxels measuring 0.3 x 0.3 x 0.5 mm corresponding to areas of endolymph were manually identified and highlighted to create 3D reconstruction, delineation, and volume quantification of the a) utricle and semicircular canals (SCC), b) saccule, and c) the cochlea.
Results: 3D segmentation was completed in five participants, yielding a volumetric analysis of 10 ears. The mean age of participants was 25.8 years (SD 1.64 years), and the cohort included three (60%) females. Across the ten ears, the mean utricle and SCC volume was 60.73 mm3 (SD 10.78 mm3), the mean saccule volume was 3.62 mm3 (SD 1.45 mm3), and the mean cochlea volume was 31.93 mm3 (SD 9.90 mm3). Of the five participants, the mean total endolymph volume (all three compartments bilaterally) was 192.62 mm3 (SD 36.83 mm3).
Conclusions: Herein, we describe one of the first protocols for 3D reconstruction and characterization of 10 non-pathologic ears of living human subjects. Volumetric analysis of this space can serve as a proxy for endolymphatic hydrops—and, therefore, as a bridge to better understanding the etiology, diagnosis, and treatment of several inner ear disorders. Future directions include comparing the inner ear's membranous labyrinth among patients with Meniere's Disease, Vestibular Schwannoma, and healthy controls to characterize differences in pathologic vs. non-pathologic ears better.

M36. Characterizing a Multi-Dose Kanamycin Ototoxicity Mouse Model

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Category: Inner Ear: Damage and Protection of Hair Cells

Background: Aminoglycosides (AGs) are one of the most widely used antibiotics against infections caused by gram-negative bacteria, such as sinus and pulmonary infections in cystic fibrosis patients and complicated urinary tract infections. Because of their low costs and high efficacy, AGs are designated the first-line agents to treat neonatal and peripartum sepsis in the US, this is despite AGs being known to be ototoxic and cause irreversible hearing loss. At present there is no FDA-approved treatment to prevent aminoglycoside-induced hearing loss. To test whether blocking the AG transporter protects against ototoxicity in a clinically relevant model, we have firstly established a multidose kanamycin mouse model to AG transportation into the cochlea to test candidate drugs that protect against AG-induced hearing loss.

Methods: Kanamycin was administrated to 4-week-old WT female mice twice per day lasting for 15 days. The same amount of saline was administrated on mice as control. All mice received hearing functional tests (ABR and DPOAE) before injection (day 0), on day 23 and 30. Tissues were harvested on day 23 and 30, fixed, prepared as whole mounts and processed for immunostaining for kanamycin, and the hair cell marker Myosin 7a. Hair cell number and fluorescence of kanamycin were quantified to measure kanamycin accumulation in hair cells and hair cell survival. Synapses of inner hair cells were labeled for Ctbp2 and Glur2, number of paired synapses were quantified.

Results: After 15 days of injection and 1-2 weeks of recovery, kanamycin-treated mice had higher ABR thresholds across frequencies on day 23 and 30 than untreated controls. DPOAE threshold also increased to 80 - 85 dB across frequencies on day 23 and 30. Histologic examination of the cochlea showed outer hair cell loss progressing in an apical-to-basal gradient, with more hair cell loss on day 30 than 23, indicating progressive hair cell loss after kanamycin injection. There was no significant inner hair cell loss except in the basal turn on day 30. Inner hair cell synapse loss in three turns was noted on day 23 and 30.

Conclusions: We have established a multidose kanamycin mouse model with progressive hearing dysfunction, outer hair cell loss, inner hair cell synapse loss. Ongoing work will evaluate kanamycin uptake and approaches to reduce AG transport into the cochlea.

M37. The Role of Oncomodulin in HPβCD-Induced Hearing Loss in Mice

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Category: Inner Ear: Damage and Protection of Hair Cells

Background: 2-Hydroxypropyl-β-cyclodextrin (HPβCD) is a cholesterol chelator used as a therapeutic compound to treat Niemann-Pick disease type C (NPC), a lysosomal storage disease due to abnormal intracellular cholesterol transport. Unfortunately, HPβCD treatment is linked to hearing loss associated with rapid outer hair cell (OHC) loss. Intracellular Ca2+ homeostasis is suggested to be a contributory factor making OHCs most vulnerable to environmental insults. The dominant Ca2+-binding protein in OHCs is oncomodulin (OCM). In the cochlea, OCM is

exclusively found in OHCs. Deleting Ocm gene in mice causes progressive hearing loss and OHC degeneration, suggesting that OCM is critical for maintaining cochlear function during aging. OCM also regulates spontaneous Ca2+ signaling and maturation of afferent innervation in developing OHCs. However, it is unknown whether OCM plays a role in protecting OHCs against ototoxic insults. In the current study, we aim to determine OCM's role in HPβCD ototoxicity using a new Ocm-knockout (KO) mouse model (Ocmtm1a/tm1a), which eliminates OCM protein synthesis without deleting the Ocm gene.
Methods: To validate whether the Ocmtm1a/tm1a mice do not express OCM protein in cochleae, we performed immunofluorescence in the cochleae from wild-type (WT, Ocm+/+) and Ocmtm1a/tm1a mice at 2 months of age. Cochlear whole mount tissues were stained for OCM and hair cell marker myosin VIIA or OHC marker prestin. To investigate whether the absence of OCM affects hearing under HPβCD treatment in mice, we measured distortion product otoacoustic emission (DPOAE) thresholds at 8, 16, 24, and 32 kHz in WT and Ocmtm1a/tm1a mice 7 days before and 4 hours after either saline or 8000 mg/kg HPβCD injection at 2 months of age.
Results: OCM protein was not detected in the OHCs of Ocmtm1a/tm1a mice but strongly expressed in the OHCs of WT mice. WT mice did not display changes in DPOAE thresholds after either saline or HPβCD injection. However, Ocmtm1a/tm1a mice displayed significantly elevated DPOAE thresholds 4 hours after HPβCD injection while saline injection did not change DPOAE thresholds in Ocmtm1a/tm1a mice.
Conclusions: These results show that mice without OCM are more susceptible to HPβCD-induced hearing loss, indicating that Ca2+ overload in OHCs could contribute to HPβCD ototoxicity. As OCM affects the auditory system in both adult and developing mice, we will investigate the role of OCM in maintaining OHC's intracellular Ca2+ homeostasis and auditory neural network using conditional KO mice, which allow us to eliminate OCM in hair cells at various stages of life.

M38. Hair Cell Survival Following Selective Denervation of the Spiral Ganglion Neurons in Neonatal Mice

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Category: Inner Ear: Damage and Protection of Hair Cells
Background: In most cases, damage to the spiral ganglion neurons (SGN) occurs secondarily following the loss of trophic support from the hair cells (HC) and supporting cells of the organ of Corti. Auditory neuropathy spectrum disorder (ANSD) describes a type of SNHL characterized by abnormal ABRs with intact cochlear microphonics and otoacoustic emissions. In ANSD, HCs are thought to survive despite significant damage to the SGN. The remaining HCs may be a viable target for regenerated SGN to reconnect with the cochlear nucleus and restore natural hearing in ANSD phenotype. However, the long-term impact of SGN loss during development on the HCs is unclear. Previously, our lab created an AN model combining AAV Cre recombinase-dependent DTA ablation with transgenic mice with selective Cre expression in the SGN. However, this approach remains time and resource intensive. We now overcome this

obstacle by selectively ablating SGN in wildtype (WT) CBA/CaJ or C57BL/6 mice via delivery of a dual AAV-injection utilizing neuron-specific AAV capsids expressing Cre recombinase and Cre recombinase-dependent DTA. This creates a WT murine AN model that allows a longitudinal study of long-term HC survival, and elucidates a therapeutic window for SGN regeneration to restore hearing.
Methods: A dual injection suspension composed of a 1:1 ratio of AAV2/Retro-hSYN-Cre-eGFP and AAV2/Retro-eF1a-flex-DTA-mCherry was delivered to the inner ear of neonatal (P1-2) wild-type C57 and CBA mice strains via the posterior semicircular canal (PSCC) approach. Controls were injected with AAV-eF1a-DTA only. Auditory brainstem responses (ABRs) were measured at 1 month and distortion product otoacoustic emissions (DPOAEs) at 1, 2, 3, and 6 months post-injection. HC and SGN survival was quantified with cochlear cryosections along with cochlear whole-mounts. Immunohistochemistry was performed with anti-beta-tubulinIII (TUJ1) and anti-myosin 7A (myo7a).
Results: WT C57/CBA mice dual-injected with AAV-hSYN-Cre/AAV-eF1a-DTA demonstrated a dramatic decrease of SGN in mid-modiolar cryosection as early as POD-7. We observed 60% SGN loss by POD-7 and 80-85% SGN loss in all cochlear turns in cryosection tissue without hair cell loss at POD-30. Mice injected with our control injection (AAV-eF1a-DTA) demonstrated intact neurites and SGN density. ABR thresholds were significantly elevated at 8, 16, 24, and 32 kHz in the one-month post-dual injections, with comparable DPOAE across dual and control-injected mice. We will continue performing a longitudinal study assessing DPOAE and HC survival at 2, 3, and 6 months.
Conclusions: Our preliminary results demonstrate feasibility of selective SGN ablation without damaging HCs in an AAV dual-injection-mediated WT neonatal mouse model. At one month, the mice are profoundly deaf with elevated ABR thresholds, intact DPOAE recordings, and morphologically intact HC consistent with AN. We will further assess the longevity of HC function and survival in WT CBA and presbycusis model in C57 mice (ahl gene).

M39. Hyperosmotic Sisomicin Infusion: A Mouse Model for Hearing Loss

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Category: Inner Ear: Damage and Protection of Hair Cells
Background: Losing either type of cochlear sensory hair cells leads to hearing impairment. Inner hair cells act as primary mechanoelectrical transducers, while outer hair cells enhance sound-induced vibrations within the organ of Corti. Established inner ear damage models, such as systemic administration of ototoxic aminoglycosides, yield inconsistent and variable hair cell death in mice.
Methods: To overcome this limitation, we developed a method involving surgical delivery of a hyperosmotic sisomicin solution into the posterior semicircular canal of adult mice.
Results: This procedure induced rapid and synchronous apoptotic demise of outer hair cells within 14 h, leading to irreversible hearing loss. The combination of sisomicin and hyperosmotic stress caused consistent and synergistic ototoxic damage. Inner hair cells remained until three days post-treatment, after which deterioration in structure and number was observed, culminating in a complete hair cell loss by day seven.

Conclusions: This robust animal model provides a valuable tool for otoregenerative research, facilitating single-cell and omics-based studies toward exploring preclinical therapeutic strategies.

M40. Evaluation of Hearing Loss Induced by Blast Exposure of Varying Intensity and Frequency in CBA/J Mice

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Category: Inner Ear: Damage and Protection of Hair Cells
Background: Exposure to blast injury from improvised explosive devices has become increasingly common in military and civilian populations since 2001, as the number of terrorist attacks worldwide has dramatically increased. In the Madrid train bombings, half of the injured persons suffered tympanic membrane perforation (Turegano, 2008), and auditory trauma has been cited as the most common injury from civilian blast exposure (Remenschneider, 2014). Presently there are no approved treatments for blast induced hearing loss and the development of countermeasures for hearing damage caused by blast is urgently needed. Models of blast induced hearing loss will aid in the evaluation of potential restorative therapies. In this study, mice were exposed to varied levels and number of blasts to investigate auditory effects.
Methods: Adult male mice were assigned to one of 14 blast exposure groups. Peak blast levels were varied from 165-175 dB and the number of exposures varied from 1-10. We use an blast setup described in Hickman, 2018. Multiple exposures were performed at 1-minute intervals. Cochlear function was assessed by ABR and DPOAE before and after blast 24 hours and 1 week. Cochleae were harvested for histological analysis.
Results: Spectrum analysis of the blast waveform showed major acoustic energy spans across 0.5 to 1 kHz. No tympanic membrane perforation occurred in any groups; animals exhibiting permanent threshold shifts (PTS) were found in groups with high blast intensities above 173 dB or with a high number (4-10 times) of exposures, even at 168 dB. Animals exhibiting temporary threshold shifts (TTS) were found in groups with a small number (LESS THAN 2) exposure, even at 174 dB, or with a moderate number (2-6 times) exposures at 165-168 dB. ABR/DPOAE in animals with PTS (n=29) showed an increase in threshold at all frequencies from 5.66-32 kHz. Box-and-whisker plots indicated many PTS animals suffered from severe hearing loss, with slightly greater threshold variability at higher frequencies (GREATER THAN 11.32 kHz). Histological analysis of one PTS group (168 dB, 10 blast, n=4) showed a significant loss in outer hair cells (OHCs) across all frequencies compared to TTS animals. On the other hand, inner hair cells were preserved.
Conclusions: We described a method for assessing blast induced hearing loss in CBA/J mice. This method could produce PTS and TTS animals by varying the number and intensity of blast exposures. Histological examination of PTS animals suggested that loss of OHCs was primarily responsible for blast-induced SNHL. Our approach provides a useful platform for studying the mechanisms of hearing loss caused by blast exposure and for developing SNHL treatments. We

also find high variability in hearing thresholds between animals subject to a single blast. Further research is needed to understand what caused such differences in hearing despite similar exposure.

M41. FKBP5 Regulates Map Kinase in the Organ of Corti After Noise-Induced Hearing Loss: RNA-Seq Analysis in Mice

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Category: Inner Ear: Damage and Protection of Hair Cells

Background: Sudden sensorineural hearing loss (SSNHL) is a common clinical entity with limited treatment options, with idiopathic SSNHL affecting 60.9 people per 100,000. Molecularly, acute inflammation and immune responses within the inner ear are implicated in SSNHL, with mechanisms elucidated in acoustic trauma, drug-induced, and mitochondrial dysfunction models. Clinicians often administer glucocorticoids intravenously or intratympanically for SSNHL, aiming to suppress inflammation and immune responses. We previously demonstrated that dexamethasone significantly increases 51kDa FK506-binding protein (FKBP5) expression in cultured and in vivo cochlear tissues (Maeda et al., Acta Otolaryngol, 2010, 2012). This study aimed to elucidate the role of FK506-binding protein 5 (FKBP5), a glucocorticoid regulatory factor, in the organ of Corti of cochleae of mice with noise-induced hearing loss (NIHL) using RNA-seq, providing insights into the molecular mechanisms of steroid therapy.

Methods: Homozygous mutant FKBP5 knockout (KO) female mice aged 5-7 weeks and wild-type (WT) C57BL/6J mice (recommended controls by Jackson Laboratory) were exposed to 114 dB SPL octave-band noise for 2 hours. Auditory brainstem responses (ABR) were measured before and after noise exposure. The tissues of sensory epithelia of cochleae were collected for RNA-seq analysis. Differential expression analysis, biological pathway analysis, and protein-protein interaction database analysis were performed for the samples with RNA integrity number (RIN) values ≥ 7.0. The expression of FKBP5 in hair cells was examined using immunostaining and the SHIELD database.

Results: Pre-exposure, FKBP5 homozygous mutant mice exhibited significantly lower hearing thresholds for clicks and 8kHz compared to WT mice. Both genotypes showed significant hearing loss after noise exposure. However, no significant differences were observed between the FKBP5 homozygous KO mice and WT mice at 24 hours and 14 days after noise exposure. Immunostaining and SHIELD analysis revealed Fkbp5 expression in both inner and outer hair cells. Differential expression analysis showed 1426 genes significantly altered in WT mice 12 hours after noise exposure compared to before noise exposure (828 upregulated, 598 downregulated). The top 3 upregulated pathways included MAP kinase. FKBP5 homozygous KO mice showed 3839 genes significantly altered compared to before noise exposure WT mice (2293 upregulated, 1546 downregulated), with MAP kinase pathways also significantly altered. Comparing FKBP5 homozygous mutants and WT 12 hours after noise exposure revealed 2651 genes significantly altered (1343 upregulated, 1308 downregulated). MAP kinase was significantly altered in FKBP5 homozygous KO mice in this comparison as well.

Conclusions: We discovered that the glucocorticoid regulatory factor FKBP5 regulates MAP kinase in the organ of Corti of mice with acoustic trauma-induced hearing loss.
MAPK signaling controls cellular responses to diverse types of extracellular stimuli such as proinflammatory cytokines, heat shock, and acoustic trauma. These findings may play a crucial role in developing tailored therapies and gene therapies for SSNHL.

M42. Identifying Protector Chemicals of Mechanosensory Hair Cell via Measuring Zebrafish Acoustic Startle Response of Lateral Line

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Category: Inner Ear: Damage and Protection of Hair Cells
Background: Larva zebrafish lateral line (LL, 5-7 dpf) has been widely used to study various biology events related to mechanosensory hair cell (HC), which is structurally and functionally analogous to that of mammals. Due to the importance of HCs for auditory/vestibular functions and their fragility/sensitivity, numerous protective chemicals against HC damage have been identified but remain to be approved by FDA.In most screening efforts, the main approach of evaluating HC death/number in zebrafish larvae may not fully capture the intact auditory loop, so screening strategies based on HC function may have greater potential to discover more protectant candidates.
Methods: We developed a zebrafish larval acoustic startle response (ASR) screen system. Twenty fish was placed in a dish and stimulated with the low frequency vibration. The total distance and velocity after stimulus were calculated from the recorded video for evaluating the ASR.
Results: Based on self-built low-frequency evoked acoustic startle response (ASR) detecting system, the known ototoxins, otoprotectors and neural transmission modifiers such as MET, ROS and neural transmission blockers/enhancers are reliably identified, in comparison with LL HC number counting strategy. The low-frequency evoked ASR detector is LL-specific because the testing animals have normal otic functions. The detecting system is automatic, equipped with wireless and deep learning technology to quickly and precisely acquire and process huge image information, and easily scaled up for large sample size like chemical screens. In a pilot screen of a library with 124 CDK modifiers, five hit chemicals were identified to restore HC function upon cisplatin insult in merely 10 hours. Among them, AS2863619 and ribociclib were later confirmed their protector features in mice.
Conclusions: Overall, this ASR-based drug screening system advanced the traditional hair cell counting method by efficiently identifying new compounds that modify LL HC functions, in addition to hair cell death.

M43. The Potential of Natural Therapies to Treat Aminoglycoside-Induced Hearing Loss

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Category: Inner Ear: Damage and Protection of Hair Cells
Background: The use of aminoglycoside antibiotics to treat severe infections has led to high rates of permanent sensorineural hearing loss among patients. The incidence of high frequency hearing loss due to aminoglycoside therapy can vary between 10-50% depending on the aminoglycoside and the protocol of administration. Given this, the identification and inclusion of alternative therapies against aminoglycoside-induced hearing loss (AGIHL) as preventive care will potentially enable patients to complete their treatment without the detrimental side-effect of hearing loss. An otoprotective and well-tolerated drug that can be orally administered and not interfere with the aminoglycoside treatment would significantly improve the quality of life and reduce the cost burden of the patients in the United States and worldwide. No such current technology exists. This is important since most of the compounds that are currently under study are either mechanotransduction channel blockers (i.e., ORC-13661, resulting in transient hearing loss) or antioxidants (i.e., ebselen, D-methionine) that act at later stages when irreversible oxidative damage to the auditory hair cells may have already occurred.
Methods: We obtained and tested extracts from six different plants known to have medicinal properties: Celastrus paniculatus, Hydrocotyl umbellate, Cassia fistula, Curcuma zanthorhiza, Ocimum tenuifolium and Mangifera indica. Various concentrations of the extracts were tested in a zebrafish model for kanamycin ototoxicity. Animals were fixed, immunostained for otoferlin, and neuromast hair cells were counted under a fluorescent microscope. FM1-43 uptake was used to test mechanotransduction (MET) channel activity. The redox state of the hair cells in the presence of kanamycin alone or together with the natural extracts was tested by the use of vital dyes.
Results: Our initial screening showed that Curcuma zanthorhiza, Ocimum tenuifolium and Mangifera indica have the potential to protect against AGIHL without blocking MET channel activity. The redox state of the hair cells is currently being evaluated under different extract conditions.
Conclusions: Although we still need to characterize the active compound(s) within these extracts, we are demonstrating the potential of natural therapy for the treatment of hearing loss.

M44. Investigating the Combined Detrimental Effects of Noise Exposure and Electrode Insertion Trauma for Hearing Preservation Outcomes

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Category: Inner Ear: Damage and Protection of Hair Cells
Background: In our increasingly noisy world, the general population experiences damaging environmental noise exposures on a daily basis that contribute to noise-induced hearing loss (NIHL). It is known that acoustic trauma leads to auditory dysfunction known as NIHL and

eventually manifests into permanent sensorineural hearing loss (SNHL). While those with mild to moderate SNHL may be treated with hearing aids, those with more severe hearing loss profiles may only qualify for cochlear implants (CIs). CIs are one of the most successful neuroprotheses to date and have benefitted a vast number of patients, including those with normal low frequency hearing function (dual stimulation CI qualification). However, CI outcomes are complicated and confounded by numerous factors, often resulting in post-implantation loss of low frequency residual hearing. Therefore, this study aims to ascertain how combined noise and electrode insertion trauma (EIT) contributes to poor residual hearing outcomes in a controlled preclinical environment.
Methods: Awake Male Brown Norway rats (n=16) were exposed to broadband noise (4-16 kHz) for 1 hour at 110 dB SPL to induce SNHL. After 3-months post-noise exposure, unilateral electrode insertion trauma (EIT) was performed. Auditory brainstem response (ABR) was performed at multiple frequencies (2, 4, 8, 16, 24, and 32 kHz) after noise exposure and after EIT to be compared to an EIT-only (no-noise) control group (n=16) for up to 3-months following EIT (total of 6-months). Additionally, timepoint-based histology studies were conducted at 3-months post-noise (prior to EIT), 1-month post-EIT, and 3-months post-EIT to elucidate morphological changes that may be responsible for differential residual hearing profiles. Histology results will be compared between double-insult (Noise + EIT) groups and EIT-only groups. The contralateral ears were used as internal controls for all data analysis.
Results: Following 3-months noise exposure, significant elevation in ABR thresholds were detected at all frequencies tested except low frequency 2 kHz, as compared to nonexposed control thresholds. This loss was permanent, with increased damage towards higher frequencies mimicking human CI candidacy. ABR thresholds were collected following EIT, showing that the double-insult group had significantly elevated thresholds at 2, 4, and 8 kHz at 1-month post-EIT compared to (no noise) EIT-only implanted ears. No statistical significance was found at this timepoint in higher frequency regions – 16, 24, and 32 kHz. This trend persisted only at 2 kHz frequency by 3-months post-EIT, suggesting that prior noise exposure does have an effect on post-operative lower frequency hearing. We anticipate that our histology studies will show similar trends in damage between groups over time.
Conclusions: Our results demonstrate that underlying electrophysiological mechanism are at play following noise-induced SNHL with CI-driven post-operative residual hearing loss.

M45. Exploring New Frontiers in Otoprotection: Evaluating the Efficacy of Novel Compounds in an Ex Vivo Cochlear Implant Trauma Model

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Category: Inner Ear: Damage and Protection of Hair Cells
Background: The process of inserting an electrode array during cochlear implantation can cause trauma to the inner ear, leading to oxidative stress, inflammatory processes, and the activation of intrinsic and extrinsic pathways of programmed cell death in sensory hair cells, ultimately compromising residual hearing. This study was initiated to evaluate the protective effects of two

novel compounds, referred to as E1 and E2, against electrode insertion trauma (EIT) in an ex vivo model of cochlear implantation. Additionally, the research aimed to determine the effective dosages of these compounds for optimal otoprotection.
Methods: The organ of Corti (OC) was extracted from rats on postnatal day 3, and divided into four groups: (1) control, (2) EIT only, (3) EIT treated with various concentrations of E1, and (4) EIT treated with various concentrations of E2. The EIT was induced by inserting a custom-designed electrode through a small cochleostomy near the round window, angled between 110° and 150°. We assessed hair cell survival using FITC phalloidin staining and measured inflammatory and apoptotic responses by detecting levels of proinflammatory cytokines and cleaved caspase-3, respectively.
Results: Significant loss of hair cells was observed in the OCs subjected to EIT. E1 proved more effective than E2 in preserving hair cells in this model. Samples treated with E1 displayed a higher count of surviving hair cells compared to those treated with E2. Further, OCs exposed to EIT showed increased production of proinflammatory cytokines and apoptosis, with E1 significantly reducing these effects more effectively than E2.
Conclusions: The findings suggest that Compound E1 may be a promising therapeutic agent for preserving hearing in implanted individuals. It significantly protects against sensory cell damage induced by EIT and could potentially enhance clinical outcomes after cochlear implantation. The ex vivo model used in this study serves as a useful tool for testing new otoprotective agents and sets the stage for further investigations to assess the efficacy of compound E1 in an in vivo animal model cochlear implantation.

M46. Identification of Protective Molecules against Cisplatin Induced Ototoxicity

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Category: Inner Ear: Damage and Protection of Hair Cells
Background: Since its FDA approval in 1978, cisplatin has become a cornerstone of cancer chemotherapy, either used alone or in combination with other agents. Its antineoplastic efficacy has been well established over time. However, as cancer treatments improve and survival rates rise (Miller KD et al., 2019), addressing long-term side effects becomes increasingly important. One significant issue is cisplatin-induced sensorineural hearing loss, affecting 40-80% of adults and at least 50% of children treated with the drug (Laverdière C et al., 2005; Patatt FSA et al., 2022). This hearing loss can severely impact quality of life and hinder the psychosocial development of pediatric patients (Gurney JG et al., 2007; Gurney JG et al., 2009). The loss is primarily due to the destruction of cochlear hair cells, which are responsible for converting mechanical sound into electrical signals in the inner ear. Unfortunately, the only FDA-approved treatment, sodium thiosulfate, is limited to pediatric patients with localized non-metastatic solid tumors, leaving many patients without effective protection (A. Saillant and L. Campebel, 2024).
Methods: Our project seeks to identify molecules that can protect against cisplatin-induced hearing loss. In collaboration with Perha Pharmaceuticals, we are testing libraries (MRT13/14)

of distant roscovitine analogues, which target a broad range of kinases and G-protein coupled receptors. Initial in vitro studies on the HEI-OC1 (House Ear Institute-Organ of Corti 1) cell line have identified candidate molecules that reduce cochlear cell death caused by cisplatin. Encouraged by these results, we are moving to more clinically relevant models, including an ex vivo murine Organ of Corti culture model and an in vivo mouse model treated with cisplatin. We also plan to investigate the role of the target receptor in hearing development and maintenance using a CRISPR/CAS9 knockout model.
Results: In vitro, our molecule significantly increased HEI-OC1 cell survival by nearly 20% when cells were treated with 50 μM cisplatin and 10 μM of the molecule. This otoprotective effect was also observed ex vivo in postnatal mouse Organ of Corti cultures (P0-P3), where 20 μM of cisplatin typically caused about 50% hair cell loss. The addition of 0.01 μM of the molecule reduced cisplatin-induced hair cell death by nearly 25%. We also observed an increase in receptor expression following cisplatin treatment. Preliminary results from knockout models show no significant hearing impairment in four-week-old mice, suggesting the receptor's non-critical role in basic hearing function.
Conclusions: Our molecule demonstrates protective effects both in vitro and ex vivo and increases receptor expression in response to cisplatin. We plan to further evaluate its potential in vivo, testing its ability to protect hearing after cisplatin treatment. Knockout mice, which do not exhibit major hearing deficits, will be used to assess their sensitivity to cisplatin and the protective effects of our molecule.

M47. Expression of Aryl Hydrocarbon Receptor in Supporting Cells and Glia: Protective Role in Cochlear Hair Cells

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Category: Inner Ear: Damage and Protection of Hair Cells
Background: Hair cell damage in the cochlea, caused by factors such as ototoxins and acoustic trauma, remains a significant challenge in auditory field, with limited therapeutic options. Investigation of potential genes and signaling pathways that could mediate protective cellular responses to oxidative stress and injury is crucial. The Aryl Hydrocarbon Receptor (AhR) pathway is well-documented for its protective role in diverse systems such as the skin and brain, where it modulates responses to xenobiotics. However, its expression and role in cochlear pathology has not been explored. Using a cochlear explant model, we previously found that treatment with an endogenous AhR ligand, 24 hours after neomycin exposure resulted in a significant increase in outer hair cell numbers in the middle and basal turns compared to controls treated with neomycin only. Fate-mapping studies did not show any evidence of traced hair cells, which suggests that AhR activation can protect hair cells. Here, we investigated the expression pattern of AhR in the cochlea, both under physiological and damaged conditions
Methods: We utilized the knockin AhRtdTomato mouse model to investigate the expression patterns of AhR in the cochlea by performing cochlear whole-mount microdissections and mid-modiolar sections at key postnatal stages. In this mouse line, expression of the tdTomato reporter is controlled by endogenous AhR regulatory elements. We also examined AhR expression in the context of hair cell damage using cochlear explant cultures that were exposed to 600 μM

neomycin for 24 hours. Changes in AhR expression were then assessed by measuring the fluorescence intensity of tdTomato using IMARIS software, comparing neomycin-treated samples to media-only controls.
Results: We found that AhR is strongly expressed in neonatal cochlear tissues at postnatal day (P)0, particularly in supporting cells that surround hair cells, Schwann cells, and other glial cells located between the organ of Corti and the spiral ganglion neurons. This expression pattern continues at P14, P21 and 8 weeks of age, although the expression level is reduced in the supporting cells surrounding hair cells. Our previous results using an AhR ligand suggests that AhR has a protective role against neomycin-induced toxicity to hair cells, and preliminary results using neomycin-treated cochlear explants shows increased fluorescence intensity of the tdTomato reporter in the supporting cells 1 hour after neomycin treatment compared to media only controls.
Conclusions: Our work is the first to define AhR expression in the cochlea and changes that occur after neomycin-induced damage. Our results suggest that activation of AhR in supporting cells can protect cochlear hair cells from neomycin-induced damage, which is a novel pathway that has not been explored and could be crucial in developing therapeutic strategies to prevent hair cell damage.

M48. Characterizing Antimicrobial and Ototoxic Properties of Novel Gentamicin Derivative

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Category: Inner Ear: Damage and Protection of Hair Cells
Background: Although aminoglycosides are effective, inexpensive, and commonly prescribed antibiotics, they can cause permanent hearing loss in patients. Mechanoelectrical transduction (MET) channels have been shown to mediate aminoglycoside uptake from the endolymph into hair cells, leading to drug accumulation and hair cell death. While researchers have identified aminoglycoside co-treatments that offer otoprotection, there is a lack of new aminoglycoside derivatives that exhibit both strong antimicrobial activity and minimal ototoxic properties. This study characterizes a novel gentamicin derivative, c1a-HABA, with size and charge properties less favorable for MET channel-mediated entry. This novel compound is a derivative of c1a, a gentamicin C-subtype and component of hospital gentamicin. We hypothesize that the novel aminoglycoside would retain antimicrobial potency while exhibiting less ototoxicity than its parent compound.
Methods: In this study, organotypic cochlear explants from postnatal-day-4 (P4) C56BL/6 mice of both sexes were harvested, dissected and cultured in supplemented Dulbecco's Modified Eagle Medium. Aminoglycosides were added to the culture media in technical duplicates at 10μM, 300μM, 500μM, 750μM, 1 mM, 3 mM, and 6 mM for 1 hour, after which explants were cultured in aminoglycoside-free media for an additional 48 hours. Cultures were fixed and stained with Myosin7a, Sox2, and DAPI and confocal microscopy was used to image the three turns (apex, middle, and base) of each culture. A dose-response curve was established to calculate the IC50 for each compound. In vitro minimum inhibitory assays were performed against Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Staphylococcus aureus. c1a and c1a-

HABA were tested on reference strains and at least five clinical isolates of each bacteria species. A minimum inhibitory concentration (MIC) LESS THAN 4 μg/ml was used as the criteria to determine antibiotic efficacy, as defined for gentamicin by the Clinical and Laboratory Standards Institute.
Results: At each concentration administered, c1a-HABA induced less OHC loss compared to c1a. In vitro IC50 of c1a-HABA was higher than that of the parent compound c1a, suggesting lower ototoxicity in c1a-HABA. Consistent with previous findings showing that hair cells at the cochlear base more readily uptake aminoglycosides, there was a basal-apical gradient of hair cell loss in both c1a and c1a-HABA.
All E. coli isolates (8/8) tested were susceptible to c1a-HABA compared to half (4/8) to c1a. Similarly, more K. pneumoniae (4/5 vs 1/5) and S. aureus (10/10 vs 6/10) isolates were susceptible to c1a-HABA than c1a. P. aeruginosa isolates were similarly resistant to c1a-HABA (5/7) and c1a (5/7). Therefore, compared to c1a, c1a-HABA demonstrates either increased or comparable antimicrobial potency against bacteria species of interest.

Conclusions: Chemical modification of gentamicin C-subtypes may reduce its ototoxic side-effects while maintaining its antimicrobial activities. Additional in vivo testing can further elucidate their potential as novel aminoglycoside alternatives that preserves hearing.

M49. Ototoxic Effects of Tobramycin and Lipopolysaccharide: Auditory Impact in Cdh23 Mice

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Category: Inner Ear: Damage and Protection of Hair Cells
Background: Tobramycin is an essential drug used to treat bacterial infections but is associated with serious adverse effects. Tobramycin (TM) is an aminoglycoside which is a class of bactericidal antibiotics with broad-spectrum activity against Gram-negative and some Gram-positive bacteria. Widely utilized for addressing serious and life-threatening infections in both adults and neonates, aminoglycosides, including TM, have maintained their efficacy amidst the emergence of multidrug-resistant bacterial strains, rendering them invaluable in the realm of antibiotics. Treatment with aminoglycosides, however, carries significant health risks including reversible nephrotoxicity and irreversible cochlear and vestibular toxicities.
Methods: We first performed baseline auditory tests before the start of tobramycin/LPS protocol on Cdh23 strain mice. Testing included auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE) testing. Frequencies including 4, 5.6, 8, 11.3, 16, 22.6, and 32, 45.0 and 64 kHz were tested. We followed a two-week long, twice-a-day TM treatment protocol beginning when the mice were 10-12 weeks old. In addition, animals were exposed to lipopolysaccharide (LPS) derived from Pseudomonas aeruginosa at low and moderate (mod.) amounts via intraperitoneal injection three times over the two weeks. LPS administration began one day before the start of TM administration to stimulate infection and subsequent treatment of infection. We tested 5 treatment groups including TM + LPS (low), TM + LPS (mod.), LPS (low), LPS (mod.), and TM only as well as an age-matched control group. After treatment with

LPS and Tobramycin, to monitor the development of ototoxicity, we performed follow-up auditory function tests 3 weeks after the induction of ototoxicity.
Results: We found that animals that received LPS (mod.) and TM show significant threshold shifts on ABR compared to the age matched controls at 16, 22.6, 32, 45.0, and 64 Hz. Animals that received LPS (low), LPS and TM only did not show any threshold shift on ABR. DPOAE threshold shifts were also significantly different in the moderate dose LPS +TM group. All other groups showed no significant difference in DPOAE testing.
Conclusions: The study demonstrated that the combined treatment of TM and LPS, particularly at a moderate dose, resulted in significant threshold shifts in auditory function as measured by ABR and DPOAE at specific frequencies. This finding underscores the potential ototoxic effects of TM and highlights the importance of further investigating the impact of its use on hearing health.

M50. Effects of Cholesterol Modulation on Cisplatin-Induced Hearing Loss

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Category: Inner Ear: Damage and Protection of Hair Cells
Background: Cisplatin is a widely used and effective anticancer drug. However, it can induce permanent, bilateral, sensorineural hearing loss in over 50% of patients, and there are currently no FDA-approved therapies for the treatment or prevention of cisplatin-induced hearing loss (CIHL) in adults. Previous studies suggest statins, FDA-approved drugs used to treat hypercholesterolemia, may reduce the incidence and severity of CIHL. However, the mechanism(s) by which statins confer protection against CIHL are unknown. Proprotein convertase subtilisin/kexin type 9 (PCSK9) is an enzyme that degrades low-density lipoprotein cholesterol receptors, and PCSK9 knockout mice exhibit reduced plasma cholesterol levels. The purpose of this study was to measure CIHL in PCSK9 knockout mice utilizing a clinically relevant model of cisplatin administration in mice to determine if reduced plasma cholesterol is protective against CIHL.
Methods: Adult PCSK9 wildtype and knockout mice were assigned to saline-treated and cisplatin-treated groups. Cisplatin-treated mice received 3 consecutive cycles of once-daily IP injections of 3-3.5 mg/kg cisplatin for 4 days followed by a 10-day recovery period. Auditory brainstem responses (ABRs) were measured pre-, immediately post-, and 2 months post-cisplatin protocol. Blood was collected immediately post- and 2 months post-cisplatin protocol. Cochleae were collected following auditory testing and blood collection. Fluorometric total cholesterol assays were performed on blood plasma and cochlear lysate samples.
Results: PCSK9 knockout mice exhibited significantly reduced plasma cholesterol levels compared to PCSK9 wildtype mice. No significant genotypic differences in cochlear lysate cholesterol levels were observed. While there were sex differences in the extent of CIHL observed in both genotypes, PCSK9 knockout mice exhibited reduced ABR threshold shifts compared to PCSK9 wildtype mice. ABR threshold shifts were moderately and positively correlated with plasma cholesterol levels in male and female cisplatin-treated mice.

Conclusions: Our results indicate that PCSK9 knockout mice have reduced serum cholesterol compared to WT
controls. PCSK9 KO mice also have reduced susceptibility to CIHL, suggesting that reduced serum

cholesterol results in protection against CIHL. Our data are consistent with a model in which the protective effect of statins against CIHL is mediated by reduced serum cholesterol.

M51. Anti-FAM19A5 Antibody Enhances Auditory Function in a Noise-Induced Hearing Loss Mouse Model by Restoring Ribbon Synapses

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Category: Inner Ear: Damage and Protection of Neurons & Synapses
Background: FAM19A5, a secretory protein expressed in the brain, binds to the post-synaptic adhesion protein LRRC4B and plays a crucial role in regulating the balance between synapse formation and elimination. Although synapse loss is commonly associated with noise-induced hearing loss, the specific role of FAM19A5 in this process remains unclear. In this study, we explored the effects of FAM19A5 inhibition on noise-induced hearing loss.
Methods: In situ hybridization was performed to assess the mRNA expression of FAM19A5 and LRRC4B, and ELISA was used to quantify FAM19A5 protein levels in the cochlear perilymph. An ex vivo study was performed in which cochlear cultures were treated with FAM19A5 and/or the anti-FAM19A5 antibody, NS101. In a mouse model of noise-induced temporary threshold shift (TTS), NS101 was administered intravenously (IV) either before or after noise exposure, followed by weekly doses for 8 weeks. Auditory function was assessed through auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE) tests. The animals were then euthanized and ribbon synapses were analyzed using immunohistochemistry.
Results: FAM19A5 and LRRC4B mRNA expression was observed in spiral ganglion neurons, and FAM19A5 protein was detected in the cochlear perilymph. In cochlear cultures, treatment with FAM19A5 protein reduced ribbon synapses, which was reversed by co-treatment with the anti-FAM19A5 antibody NS101. Intravenous administration of NS101 to mice confirmed its presence in the cochlear perilymph, demonstrating its ability to reach the target area. In the TTS mouse model, ABR thresholds were significantly elevated one day after noise exposure. However, pre-treatment with NS101 significantly attenuated these threshold elevations compared to hIgG-injected control mice. While the threshold elevations subsided naturally 2-4 weeks after noise exposure in both control and NS101-treated groups, control mice continued to exhibit reduced ABR wave I amplitudes, indicative of synaptic damage leading to hidden hearing loss. However, NS101 treatment restored the ABR threshold to pre-noise exposure levels and reinstated the number of ribbon synapses in both inner and outer hair cells. In addition, NS101 restored DPOAE amplitudes to pre-noise levels, suggesting improved outer hair cell function. Notably, even when NS101 was administered 2 hours after noise exposure, ABR wave I

amplitudes and ribbon synapse numbers still recovered, although to a lesser extent than pre-exposure treatment.
Conclusions: These results suggest that NS101 effectively restores synaptic structure and improves auditory function after noise-induced hearing loss, positioning it as a promising therapeutic candidate for preventing synapse loss and promoting hearing recovery.

M52. A Mouse Model of Unilateral Stereotactic Radiosurgery-Induced Hearing Loss

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Category: Inner Ear: Damage and Protection of Neurons & Synapses
Background: Stereotactic radiosurgery (SRS) is a precise, single-session irradiation technique commonly used to treat vestibular schwannomas. However, SRS can lead to irreversible hearing loss, mainly due to irradiation-induced damage to the nearby inner ear. Currently, no preventive or therapeutic options exist, highlighting the need for the development and experimental testing of novel treatments. To enable this research, we developed a protocol for inducing unilateral hearing loss in mice through targeted unilateral cochlear irradiation.
Methods: We used 6-week-old C57BL/6J mice to administer precise unilateral irradiation in the vicinity of the cochlea using a Leksell Gamma Knife Icon device. The precision and reproducibility of the targeted area were ensured through radiological imaging for each mouse using the integrated cone beam CT scan and co-registering these images with MRI and CT mouse atlas images. To ensure meaningful translational data, we placed a single isocenter lateral to the cochlea with the 80% isodose line passing through the medial edge of the cochlea to deliver 8 (n=3), 16 (n=5), 24 (n=8), and 32 (n=8) Gray (Gy). Auditory brainstem responses were measured one day prior to irradiation (baseline) and on days 7 and 28 post-irradiation. Statistical analysis was performed using two-way repeated measures ANOVA with Bonferroni correction.
Results: In all experimental groups, the irradiation dose received by the non-irradiated cochlea was less than 15% of that received by the irradiated cochlea. In the 32 Gy group, irradiation of the cochlea yielded significant threshold shifts, compared to the non-irradiated cochlea, at 22.6 and 32 kHz on day 7 and to a greater degree on day 28. Similar but less pronounced effects were observed in the 24 Gy group. Furthermore, we observed a unilateral decrease in wave I amplitudes following 72-78 dB SPL click stimulation in both groups. No hearing loss was detected in the 8 and 16 Gy groups. The histological analysis corroborated these functional changes. In the 32 Gy group, quantification analysis of the outer hair cells (OHC) found a loss, only in the irradiated cochlea, of an average of 44% and 14% at the cochlea frequency regions of 45 and 32 kHz, respectively. In the same experimental group, counts of CtBP2-positive puncta per inner hair cell (IHC) yielded an average reduction of 35% at the irradiated cochlea when compared to the non-irradiation cochlea at both the basal and apical turns. Comparable yet less

marked histological findings were noted in the 24 Gy group. There was no IHC loss across all groups.
Conclusions: Targeted near-cochlear irradiation in mice induces unilateral dose-dependent high-frequency hearing loss associated with OHC loss and a reduction of CtBP2-positive puncta per IHC. This model provides a valuable tool for exploring the radiobiological mechanisms underlying SRS-induced hearing loss and for testing potential radioprotective agents.

M53. Efficacy of a Lipid Regulator Fenofibrate Against Agihl in Mice

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Category: Inner Ear: Damage and Protection of Neurons & Synapses
Background: Aminoglycosides (AGs) are frequently used to treat a variety of life-threatening infections caused due to Gram negative bacteria. Unfortunately, they can cause permanent sensorineural hearing loss. Systemic administration of AG can cause ototoxicity, neurotoxicity, and nephrotoxicity. The incidence of AG-induced hearing loss (AGIHL) accounts for 10-50% incidence among patients with severe sepsis. One of the reasons of the severe ototoxicity is the accumulation and retention of these ototoxin in the inner ear compartment. Despite the long-term ototoxic effect of AGs, there is no FDA approved drug to treat it. Our project aims to screen and characterize FDA-approved drugs (approved to treat metabolic diseases and that are in long term and wide use) using zebrafish and mice as the animal models for AG ototoxicity. The primary goal is to fast track the selected candidate drug(s) for its use as a cotreatment to prevent acquired hearing loss.
Methods: FDA approved drugs were shortlisted based on 1) their broad human use for long term conditions, 2) permeability, 3) target disease, and 4) level of toxicity. Initial screening was done in zebrafish. Fenofibrate, a lipid regulator, performed best in terms of dosage, mechanotransduction channel blocking ability, swimming behavior and lack of interaction with kanamycin. For those reasons, fenofibrate was advanced in the pipeline and used in pharmacokinetics and efficacy studies in mice. Six-to-seven-week old CBA/CaJ were co-treated with 600mg/kg of kanamycin twice a day and fenofibrate 20mg/kg/day for 14 consecutive days. ABRs and DPOAEs were recorded before and at 30 days post kanamycin and fenofibrate co-treatment. Mice were then sacrificed, and cochleae isolated for the analyses of IHC, OHC, and synapses. Mid-modiolar sections were also prepared, and filipin staining was performed to assess the lipid distribution. The presence and distribution of low-density lipid receptors were assessed by immunohistochemistry. To elucidate the mechanism of action, mice were similarly co-treated for 10 consecutive days, and cochlear lysates prepared to estimate the levels of fenofibrate, kanamycin, total cholesterol and triglycerides.
Results: Fifty FDA-approved drugs currently use for hypertension and/or lipidemia were screened. Among them, telmisartan, nicardipine and fenofibrate showed the maximum hair cell protection against kanamycin ototoxicity. Fenofibrate did not interact with kanamycin nor blocked the mechanotransduction channels, shown by NMR and FM1-43 uptake studies, respectively. Fenofibrate treatment prevented zebrafish abnormal swimming behavior due to kanamycin exposure. Moreover, fenofibrate was able to reach the cochlea 1-hour post-administration and was still detectable 6 hours post-systemic administration. At 30 days post

recovery, fenofibrate prevented kanamycin ototoxicity, assessed by ABR and DPOAE recordings. There was no significant shift in DPOAE gram and number of outer hair cells.
Conclusions: Fenofibrate could be fast tracked to treat AGIHL. However, its clear mechanism of action, efficacy in other models need to be studied.

M54. Noise-Induced Cochlear Synaptopathy in C57BL/6N Mice as a Function of Trauma Strength: Ribbons Are More Vulnerable than Postsynapses

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Category: Inner Ear: Damage and Protection of Neurons & Synapses
Background: Noise-induced cochlear synaptopathy is characterized by irreversible loss of synapses between inner hair cells (IHC) and spiral ganglion neurons despite normal hearing thresholds. We analyzed hearing performance and cochlear structure in C57BL/6N mice that had been exposed to a noise trauma ranging from 100 dB SPL to 112 dB SPL for 2 h.
Methods: Eight-week-old mice of both sexes were exposed to 100, 106, or 112 dB SPL broadband noise (8-16 kHz) for 2 h. Auditory brainstem responses (ABR) were assessed before, directly after and up to 28 days post trauma. Finally, the number, size, and pairing of IHC presynaptic (CtBP2-positive) ribbons and postsynaptic AMPA receptor scaffold (Homer1-positive) clusters were analyzed along the cochlea. Further, the number of OHCs was determined.
Results: Four weeks after the 100 dB SPL trauma, a permanent threshold shift (PTS) was observed at 45 kHz, which after the higher traumata extended towards middle to low frequencies. Frequency-specific loss of ABR wave I amplitudes scaled with trauma strength indicating loss of functional IHC synaptic connections. No trauma-related OHC loss was found. Frequency-specific latencies of wave I mostly increased with trauma strength in the mid- to high-frequency region. Synaptic pairs were reduced in the midbasal and basal cochlear region in all trauma conditions with ribbon loss amounting up to 46 % of the control group. The loss of ribbons observed four weeks after the 100 dB SPL trauma did not further increase with trauma strength. Ribbons surviving the trauma were enlarged and paired with a postsynapse. In contrast, 4 to 6 unpaired postsynapses/IHC were found in the medial, midbasal and basal region irrespective of trauma strength, which contrasts findings in CBA/CaJ mice.
Conclusions: These data confirm the susceptibility of ribbon synapses and ABR wave I amplitudes to a noise trauma of 100 dB SPL and above. In C57BL/6N mice, IHC postsynapses were more resistant than ribbons to the noise traumata applied. Our findings identify the ribbon as a novel target structure for protection measures and novel therapeutic strategies in noise-induced hearing loss.

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M55. Diagnostic Methods for Potential Cochlear Synaptopathy in Humans

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Category: Inner Ear: Damage and Protection of Neurons & Synapses

Background: At the early stages of age-related hearing loss (ARHL), patients often report difficulty with speech recognition in background noise, despite normal pure-tone audiograms. This may be due to cochlear synaptopathy (CS), which can affect stapedius reflex thresholds and amplitude, as shown in animal models. Studies suggest that declines in extended high-frequency thresholds (EHT) (10–16 kHz) may indicate CS, particularly in the apical and middle cochlear turns, as outer hair cells (OHCs) in the basal turn are more resistant to damage compared to synapses in the apical and middle turns. Therefore, when extended high-frequency hearing thresholds deteriorate due to OHC damage, CS in the apical and middle regions is likely present. This study aims to investigate whether a reduction in speech discrimination in background noise during the early stages of age-related hearing loss is associated with changes in stapedius reflexes and decreased EHT.

Methods: We recruited 38 participants with an average age of 26.2 ± 3.3 years. All participants underwent a routine physical examination by an ENT consultant and a pure-tone audiogram covering frequencies from 0.125 to 16 kHz, as well as stapedius reflex measurements at 0.5, 1.0, 2.0, and 4.0 kHz. Participants were also tested using speech recognition tasks in background noise at varying signal-to-noise ratios (SNRs). None of the participants reported prior ear complaints, and their hearing thresholds from 0.125 to 8 kHz were no greater than 20 dB, with no individual frequency exceeding 30 dB [HL]. Speech discrimination in background noise at different SNRs was analyzed using a sigmoidal fit, characterized by the slope of the curve and the speech recognition threshold at 50% discrimination (SRT50). These parameters were then correlated with stapedius reflex thresholds and extended high-frequency hearing thresholds (EHT).

Results: The average SRT50 was -5.8 ± 1.1 dB SNR with a slope of 0.74 ± 0.149%/dB. The average EHT was 10.45 ± 15.6 dB HL, with 14 ears showing values worse than 20 dB [HL]. Stapedius reflex thresholds averaged between 75.4 and 80.6 dB [HL]. No significant correlations were found between age, EHT, and speech recognition. However, stapedius reflex thresholds at 0.5 kHz correlated with both the SRT50 ($R = 0.27$, $p = 0.02$) and the slope of the speech discrimination function ($R = -0.37$, $p = 0.001$), as well as with the EHT ($R = -0.29$, $p = 0.01$). Stapedius reflex thresholds at 4 kHz did not correlate with any parameter.

Conclusions: These results underline the hypothesis that reduced speech recognition linked to cochlear synaptopathy (CS) could be accompanied by changes in stapedius reflexes. Additionally, it demonstrates that extended high-frequency thresholds (EHT) correlate with these processes, which supports the idea that EHT can serve as an early indicator of CS.

M56. Assessment of Liraglutide's Therapeutic Effect on Hearing Function of Chinchillas Exposed to Recurring High-Intensity Blasts

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Category: Inner Ear: Damage and Protection of Neurons & Synapses
Background: Repeated exposure to blasts, which leads to the prevailing hearing loss and complaints, are inevitable situations for Service members. The therapeutic effect of liraglutide, a glucagon-like peptide-1 receptor agonist, has been reported to mitigate the acute hearing damage in earplug-protected chinchillas and facilitate the post-injury hearing restoration in open-ear chinchillas after one-time blasts in recent studies. However, the effect of liraglutide on auditory injuries accumulated over recurring blast exposures with ears open without protection across multiple times has not been investigated. This study aims to monitor the hearing function changes after recuring blast incidents and assess the effect of liraglutide treatment under this condition of more severe and persistent auditory injuries.
Methods: Chinchillas were divided into two groups: blast control without treatment (N=5) and post-blast treatment with liraglutide (N=8). All animals were exposed to 3 blasts at a level of 15-25 psi (103-172 kPa) on Day 1 with ears open without protection, and another 3 blasts of the same level on Day 4 as recurred blast incidents. The 7-day-long subcutaneous liraglutide injection (0.25 mg/kg/day) started 2 hours after blasts on Day 1 in the post-blast liraglutide treatment group. The blast control chinchillas were injected with an equivalent amount of saline. The hearing function change was monitored by measuring the auditory brainstem response (ABR) and middle latency responses (MLRs) pre- and post-blast on Day 1, post-blast on Day 4, and on Days 7 and 14, respectively.
Results: Significant and permanent hearing damage was observed after blasts in all chinchillas. The additional blasts on Day 4 induced less ABR threshold shift than that of blasts on Day 1. The severity of hearing damage decreased over recovery time, but significant ABR threshold shifts were observed at the end of the study. Liraglutide-treated chinchillas showed lower ABR threshold shifts than the blast controls at certain frequencies. The blast-induced changes and the therapeutic function of liraglutide in the central auditory system were reflected by the MLR results.
Conclusions: Liraglutide treatment facilitated the restoration of hearing after exposures to recurring high-intensity blasts in chinchillas, which was consistent with the results observed in the previous one-time blasts. Compared to the previous results obtained from earplug-protected chinchillas, the preventive function of liraglutide was not observed on Day 4, which was possibly shadowed by the severe damage in ears without protection. The post-injury changes and liraglutide's effect on the central auditory system require further investigation.

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M57. Psychedelic Drugs Induce the Formation of New Synapses in the Cochlea

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Category: Inner Ear: Damage and Protection of Neurons & Synapses

Background: Age-related hearing loss (ARHL), a prevalent condition affecting millions worldwide, is primarily caused by the loss of synapses between sensory hair cells and spiral ganglion neurons (SGNs) in the cochlea. This synaptic loss, termed synaptopathy, leads to impaired auditory signal transmission to the brain. Currently, there are no FDA-approved drugs for synaptopathy, highlighting a significant unmet medical need. Recent research has revealed the potential of psychedelic drugs in promoting synaptogenesis (the formation of new synapses) in the brain. This led us to explore their potential impact on cochlear synapses, given the critical role of cochlear synaptopathy in its pathology, and the exciting potential for synaptogenic compounds in reversing synaptopathy and associated hearing loss.

Methods: We analyzed 5-HT2 receptor expression in the mouse cochlea using publicly available RNA-seq datasets in gEAR. To assess the synaptogenic potential of psychedelics in the cochlea, we administered classical psychedelic compounds and non-hallucinogenic analogs to healthy adult mice and SAMP8 mice with accelerated hearing loss and synaptopathy. We quantified synapse numbers in treated vs. vehicle controls using Airyscan confocal immunofluorescence imaging. Additionally, we measured ABR Wave I amplitudes before and after treatment to assess changes in synaptic transmission.

Results: Analysis of RNA-seq datasets revealed robust expression of 5-HT2A/B/C receptors in SGNs, all of which are known to be agonized by psychedelic compounds. Systemic IP administration of psychedelics and non-hallucinogenic analogs in healthy mice resulted in an increase in both cochlear and cortical synapses compared to vehicle controls. We also observed a significant increase in ABR Wave I amplitudes in some animals, consistent with enhanced synaptic transmission, and an increase in synaptic ribbons. We also observed increased neuritogenesis in treated SGN cultures, indicating a direct effect of these compounds on SGNs.

Conclusions: These findings demonstrate the ability of psychedelics to induce synaptogenesis in the cochlea, suggesting their potential for treating synaptopathy-associated hearing loss. The robust expression of 5-HT2 receptors in SGNs, increased neuritogenesis in treated SGN cultures, and the observed increase in synapses and synaptic transmission in vivo following psychedelic treatment support this hypothesis. Ongoing studies in mouse models of ARHL and other forms of synaptopathy (e.g. noise-induced, ototoxic drugs) will further elucidate the therapeutic potential of psychedelics for hearing restoration, offering hope for millions suffering from this debilitating condition. Just as importantly, these results demonstrate for the first time (to our knowledge) that these compounds can directly act on peripheral neurons, greatly expanding their therapeutic potential beyond psychiatric disorders.

M58. Associations Between Physiological Indicators of Cochlear Deafferentation and Listening Effort in Military Veterans With Normal Audiograms

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Category: Inner Ear: Damage and Protection of Neurons & Synapses
Background: Human physiology and temporal bone studies suggest that older age and military or occupational noise exposure are risk factors for cochlear synaptopathy, consistent with animal data. One predicted perceptual impact of synaptopathy is difficulty understanding speech in background noise. Because synaptopathy can only be confirmed through post-mortem temporal bone analysis, it has been difficult to confirm the impacts of this form of cochlear deafferentation on speech-in-noise perception. Several physiological indicators of deafferentation have been identified in animal models including the amplitude of wave I of the auditory brainstem response (ABR), the magnitude of the envelope following response (EFR), and the magnitude of the wideband middle ear muscle reflex (MEMR). However, previous human studies investigating relationships between these physiological measures and speech perception have resulted in mixed findings. One possible explanation is that an individual's performance on a speech perception task may underestimate underlying auditory deficits. If increased cognitive effort can be used to compensate for the degraded speech signal, task performance may be relatively unaffected. However, even if performance on the task is maintained, the increased effort may come at cost to the listener. For example, they may experience greater listening fatigue, impaired ability to recall auditory information, and/or difficulty multitasking while listening. Assessment of the cognitive effort expended to complete a speech perception in noise task (i.e., listening effort) may provide a better indicator of the impact of deafferentation on speech perception than task performance. Listening effort can be estimated by measuring pupil dilation during a speech perception task because pupil dilation increases as cognitive effort increases.
Methods: In a sample of military Veterans with clinically normal hearing, aged 20-50 years, who report hearing complaints (tinnitus and/or difficulty with speech perception in noise), we measured ABR wave I amplitude, EFR magnitude, wideband MEMR magnitude, and pupil dilation during a speech perception in noise task. Distortion product otoacoustic emissions (DPOAEs) were also measured to assess outer hair cell function.
Results: Pupil dilation increased as signal-to-noise ratio decreased (i.e., as the task became more difficult). Poorer DPOAEs were associated with greater pupil dilation. Lower EFR magnitude was associated with greater pupil dilation, even after statistical adjustment for average DPOAE level. There was no clear relationship between ABR wave I amplitude or MEMR magnitude and pupil dilation.
Conclusions: The EFR results suggest that cochlear deafferentation may be associated with increased listening effort during speech-in-noise perception. The lack of a clear association between ABR or MEMR magnitude and pupil dilation may indicate shortcomings of these measures as deafferentation biomarkers. Another possible explanation of the results is that the observed association between EFR magnitude and pupil dilation reflects changes to central auditory processing that impact speech perception, but are not direct consequences of deafferentation.

M59. Combining Multiple ABR and EFR Stimuli to Predict Cochlear Deafferentation in Individual Humans

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Category: Inner Ear: Damage and Protection of Neurons & Synapses

Background: Cochlear deafferentation may underlie common auditory complaints including tinnitus, hyperacusis, and difficulty understanding speech in noise. Evidence from animal models suggests that the magnitude of the auditory brainstem response (ABR) and envelope following response (EFR) are correlated with cochlear synapse loss, a type of cochlear deafferentation. Previously, we used Bayesian regression to combine a computational model of the auditory periphery (CMAP) with prior knowledge of cochlear synapse counts from human temporal bones to predict the number of functional synapses remaining in individual human participants given their auditory brainstem responses (ABRs). This approach allowed us to correct for the impact of cochlear gain loss on the ABR in individual participants by individualizing their CMAP using measured DPOAE amplitudes. Predicted synapse counts were correlated with predicted risk factors and perceptual consequences of deafferentation.
Collecting stimuli at multiple frequencies and levels may make a diagnostic assay more robust; however, clinicians have limited time to assess each patient. Here, we test whether collecting additional ABR and EFR data improves our ability to predict the degree of deafferentation in individual human subjects. Since we have no direct means of validating our predictions, we use age as a proxy since post-mortem histology from temporal bone data in humans shows that cochlear synapses are lost at a rate of approximately 1 synapse per decade of age.

Methods: In each human participant, ABR data were collected at multiple frequencies and levels and EFR data were collected at multiple carrier frequencies. Degree of deafferentation was predicted using various combinations of ABR and EFR stimuli ranging from a single stimulus to all collected ABR and EFR stimuli. For each set of stimuli, cross validation was used to test how well the predicted degree of deafferentation could predict the participant's age.
Results: We have expanded our deafferentation prediction algorithm to generate predictions from the EFR, enabling us to combine data collected using multiple ABR and EFR stimuli to predict deafferentation in individual human participants. When generating predictions from a single ABR or EFR stimulus, predicted deafferentation is generally uniform across cochlear frequency. As additional stimuli are incorporated into the model, predicted deafferentation becomes more focal with greater deafferentation at some cochlear frequencies than others. When using a single stimulus to predict deafferentation, the ability to predict a participant's age varies depending on the stimulus, suggesting that certain stimuli may be better candidates for a deafferentation diagnostic assay than others.
Conclusions: In the absence of a direct means for validating deafferentation predictions in humans, age offers a proxy for exploring putative diagnostic assays of deafferentation. Identifying the optimal set of ABR and/or EFR data for predicting deafferentation will facilitate the design of future cochlear deafferentation studies and the eventual development of a clinical diagnostic assay.

M60. Ebselen-Eluting Silicone Strips Reduce Low Frequency Hearing Loss in a Guinea Pig Model of Cochlear Implantation

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Category: Inner Ear: Drug Delivery

Background: Cochlear implants (CIs) are the primary treatment for restoring auditory function in deaf or profoundly hearing-impaired adults and children. However, insertion of silicone-coated electrodes may cause loss of residual low-frequency hearing, due to the post-insertion inflammation, fibrosis, and cell death that can occur over the following 1 to 12-months. Despite efforts to mitigate this side effect, no FDA-approved solution exists. This study aims to determine if ebselen-eluting silicone strips (CIEBSEL) can reduce residual low-frequency hearing loss after intra-cochlear insertion in adult guinea pigs.

Methods: Silicone strips (0.75mm length, 1mm width, and 0.1mm thickness) containing 0% or 30% ebselen that sustainably eluted ebselen over 90-days in PBS in vitro were utilized. Guinea pigs (N=60) two months of age were surgically implanted with CIEBSEL into the scala tympani through the round window unilaterally. In the first cohort, control 0% (N=12) and ebselen 30% (N=12) groups had follow-up ABRs (4, 8, 16, 24, 32 kHz) at 1-week, 1, 2, 3, and 6-months post-implantation. Threshold shifts were calculated by subtracting the shift in the implanted ear from the shift in the un-implanted ear from baseline. In the second cohort, guinea pigs (N=36) were all implanted with CIEBSEL (30% ebselen), unilaterally, and then treated with ebselen subcutaneously (sc) between 3 to 6-months post implantation. Control (DMSO) (n=12), ebselen 10 mg/kg (N=12), or ebselen 20 mg/kg (N=12) groups received daily sc injections for 3-months. Threshold shifts were compared between the groups. Cochlear histopathology was quantified for hair cell loss at 6-month post-implantation.

Results: In the first cohort, threshold shifts were highest at 1-week (20-35 dB) and decreased steadily at 1, 2, and 3-months (5-10 dB) post-implantation. At 6-months, threshold shifts increased in both control and CIEBSEL-implanted guinea pigs (25-30 dB), and in the un-implanted ears (5-10 dB). CIEBSEL-implanted GPs showed improvements at 4kHz (15-20 dB) at 2 and 6-months when compared to 0% controls (p-values LESS THAN 0.05). Hair cell loss was limited to the site of implantation (2mm) and no significant difference between 30% ebselen and 0% controls was observed. In the second cohort, systemic ebselen treatment (10 or 20 mg/kg/d, sc) significantly reduced the threshold shifts at 6-months post-CIEBSEL-implantation at 4 kHz (10 mg/kg, -22.50 dB, p-value LESS THAN 0.05) and at 32 kHz (10 mg/kg, -14.58 dB, p-value LESS THAN 0.05) when compared to DMSO controls.

Conclusions: CIEBSEL, a novel silicone formulation of ebselen, improved low frequency hearing when compared to controls following intra-cochlear insertion in adult guinea pigs. Furthermore, subsequent treatment with systemic ebselen improved residual hearing at low and high frequencies. These data support the co-administration of ebselen in adult patients about to receive a cochlear implant.

M61. Enhancing Round Window Membrane Permeability: Efficacy and Safety of Adjuvants for Intratympanic Dexamethasone Delivery

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Category: Inner Ear: Drug Delivery

Background: Dexamethasone (DEX) is a commonly used medication for treating acute inner ear conditions. Intratympanic DEX (IT-DEX) injections provide the benefit of high inner ear penetration with minimal systemic side effects. However, a major drawback of IT-DEX is its limited ability to adequately penetrate the round window membrane (RWM), resulting in drug loss. This study aimed to evaluate the safety and effectiveness of adjuvant agents that enhance RWM permeability.
Methods: Three injectable agents (Histamine 0.01g/ml, 3% hypertonic saline, sodium caprate 1.94mg/ml) previously reported to increase RWM permeability were selected. These agents were administered intratympanically in C57BL/6J (B6) mice, followed by DEX injection 15 minutes later. After an additional 15 minutes, perilymph was collected by puncturing the posterior semicircular canal for uHPLC analysis and organ of Corti was harvested for IF analysis. RWM samples were also collected on the day of IT injection and one week later for transmission electron microscopy (TEM) examination. In addition, light microscopic study of organ of Corti was conducted to evaluate the inner ear toxicity of enhancer agents.
Results: The highest concentration of DEX in the perilymph was observed when it was administered after 3% hypertonic saline. This group also showed the greatest increase in DEX receptor expression. Structural changes in the RWM were noted in the following order: histamine, hypertonic saline, and sodium caprate, with significant recovery seen after one week. No morphological changes were detected in the organ of Corti in any of the groups.
Conclusions: This study demonstrated that 3% hypertonic saline was the most effective adjuvant for enhancing round window membrane (RWM) permeability, leading to the highest concentration of dexamethasone (DEX) in the perilymph and the greatest increase in DEX receptor expression. Although structural changes in the RWM were observed across all adjuvant groups, significant recovery occurred within one week, indicating that these changes were temporary. None of the adjuvant agents caused any morphological damage to the organ of Corti, suggesting that they are safe for inner ear structures. These findings suggest that 3% hypertonic saline is a promising adjuvant for improving the efficacy of intratympanic DEX therapy in treating inner ear conditions.

M62. Evaluation of the Efficacy and Safety of the High Molecular Weight Hyaluronic Acid Vehicle for the Prevention of Ototoxic Hearing Loss

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Category: Inner Ear: Drug Delivery
Background: Ototoxic hearing loss is a side effect of medications that are inevitably used for treatments, such as chemotherapy and drugs for treating multidrug-resistant tuberculosis. Reducing the concentration of cisplatin decreases its anticancer effectiveness, highlighting the need for solutions to overcome this issue. Sodium thiosulfate (STS) has been approved by the FDA as a therapeutic agent to reduce the risk of hearing loss caused by cisplatin. However, despite its market potential, research on drug delivery therapy for hearing loss using STS and its carrier, High Molecular Hyaluronic Acid (HHA), has not been actively conducted. Therefore,

this study aims to verify the biocompatibility and therapeutic effects of a drug delivery system for ototoxic hearing loss.
Methods: Six-week-old SD rats with normal hearing were used for the experiment. The rats were divided into four groups: three treated with Saline + 0.5M STS, 2.0% HHA + 0.1M STS, and 2.0% HHA + 0.5M STS, and a control group without intratympanic drug administration. Ototoxic hearing loss was induced by administering a combination of gentamicin, furosemide, and cisplatin via the tail vein for two consecutive days, starting three days after the intratympanic injection of each drug/carrier. Endoscopy, MicroCT and Auditory Brainstem Response (ABR) tests were conducted at Click, 8, 16, and 32 kHz on the 1st, 3rd, 7th, 14th, and 21st days after inducing ototoxic hearing loss. After the experiment, histological verification of the middle ear and tympanic membrane was conducted.
Results: In the HHA + STS group, it was observed that the drug/carrier remained effective for up to three weeks, compared to the Saline + STS group, where it lasted for a maximum of two days. TM perforations in all groups healed without side effects. At the Click stimulus, the hearing thresholds were 40.0 ± 14.1 dB SPL in the HHA + 0.5M STS group, compared to 27.5 ± 5.3 dB SPL in the control group. At 8 kHz, the values were 37.5 ± 10.6 dB SPL and 28.3 ± 6.1 dB SPL, respectively, indicating better hearing recovery in the HHA + 0.5M STS group.
Conclusions: HHA has been confirmed as an excellent carrier for intratympanic drug delivery in the treatment of ototoxic hearing loss. It prolongs the duration of drug retention in the middle ear without causing any inflammatory reactions or side effects. The results of this experiment demonstrate that HHA + STS has potential as a drug/carrier system to prevent ototoxic hearing loss.

M63. Comparison of Long-Term in Vivo Pharmacokinetics Between Fully Loaded and Strip-Coated Electrode Carrier Dummies With Dexamethasone

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Category: Inner Ear: Drug Delivery
Background: Cochlear implant placement often results in electrode insertion trauma (EIT), causing physical and cellular damage to the cochlea. A common consequence of EIT is the formation of a fibrotic capsule around the implant, which increases electrical impedance and reduces the efficiency of the device. Glucocorticoid receptor agonists, such as dexamethasone, are effective in mitigating this fibrotic response by suppressing the synthesis of pro-fibrotic mediators. Local delivery of dexamethasone, achieved by incorporating the drug into the silicone of the electrode array, offers superior therapeutic benefits by maintaining functional concentrations within the cochlea while minimizing systemic side effects associated with chronic systemic administration.
Methods: This study evaluated the pharmacokinetics of dexamethasone released from silicone electrode-carrier dummies in a guinea pig model, focusing on two different loading techniques: fully loaded electrodes with dexamethasone (0.55 µg, 5.5 µg, and 55 µg) and strip-coated electrodes (1.313 µg, 2.625 µg, and 5.25 µg). Dexamethasone release into the perilymph fluid of

the cochlea was monitored to compare how each loading type influenced drug concentration levels over time. The goal was to identify which loading method could maintain therapeutic concentrations most effectively.
Results: Both the fully loaded and strip-coated electrode carriers achieved therapeutic steady-state dexamethasone concentrations of approximately 10-50 ng/ml for extended periods. The fully loaded electrodes exhibited a burst release phase lasting between 3 and 7 days, with dexamethasone concentrations ranging between 150 and 450 ng/ml. In contrast, the strip-coated electrodes, despite containing lower total amounts of dexamethasone, showed a more pronounced and prolonged burst release lasting up to 30 days, with concentrations similarly ranging between 100 and 450 ng/ml.
Conclusions: The study suggests that strip-coated electrode carriers may offer greater clinical advantages due to their robust initial burst phase and sustained therapeutic concentrations over an extended period. This controlled release profile could provide effective long-term anti-inflammatory protection following cochlear implant surgery.

M64. Development and Verifying the Therapeutic Effect of Intra-Tympanic Controlled Nanoparticle Drug Delivery Carrier for Inner Ear Disorders

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Category: Inner Ear: Drug Delivery
Background: Intra-tympanic (IT) drug delivery offers a powerful approach to the research and treatment of sensorineural hearing loss. The development of nanoparticle drug delivery carriers has the potential to significantly improve the quality of treatment by enabling the delayed release of drugs. This study evaluated the potential of nanoparticle drug carriers as intratympanic (IT) drug delivery vehicles for nanoparticle-dexamethasone sodium phosphate (N-Ds) and hyaluronic acid-dexamethasone palmitate (HA-Dp) in treating acute hearing loss. We compared the efficacy, safety, and residence time of these nanoparticle drug carriers to the standard IT drug delivery method.
Methods: A total of 26 male Sprague Dawley rats (51 ears) weighing 130-160g with normal hearing and intact tympanic membranes (TM) were used in this study. We compared the effects of Dex-sal (14 ears), N-Cs (10 ears), HA-Dp (14 ears), and HL-control (13 ears) groups. Auditory brainstem responses (ABR) were measured using a Smart EP system immediately after injection and at 0, 4, 7, and 14 days following the induction of ototoxic hearing loss. Additionally, we performed animal computed tomography (CT) and observed the tympanic membrane (TM) using otoscopy at the baseline and 0, 4, 7, and 14 days following IT injection. Histological analyses included hair cell counts.
Results: The HA-Dp showed significantly better ABR results compared to baseline at 14 days. However, no improvements were observed in the Dex-saline, N-Cs, and control groups after the treatment. Using an otoscope to observe the TM, it was noted that inflammation and otitis externa gradually worsened on days 7 and 14 post-IT injection in 3 subjects from the N-Cs group, while no inflammatory response was observed in any other group. Perforations created during injection healed within 21 days, with perforation closure occurring at 11.0 ± 3.6 days

post-IT injection in the Dex-saline group, 8.7 ± 3.9 days in the N-Cs group, and 9.5 ± 5.2 days in the HA-Dp group. Soft tissue density, indicative of vehicle/drug presence, was identified in the bulla, and the persistence of this signal was monitored. In the N-Cs group, the vehicle/drug remained in the bulla for 7.17 ± 5.7 days, while in the HA-Dp group, it persisted for 7.1 ± 4.9 days. The observed duration in the bulla was similar in both the N-Cs and HA-Dp groups. Using confocal microscopy, the number of lost hair cells was quantified. In the all cochlea turn (apical, middle, basal), no significant differences were found between groups.
Conclusions: In the in vivo animal study, a beneficial effect on hearing recovery was observed with the drug delivery carrier injection in the HA-Dp group, while no treatment effect was found in the Dex-saline, N-Ds, and HL-control groups. The HA-Dp group may be more effective than the Dex-saline, N-Ds, and HL-control groups in controlling hearing loss.

M65. Hearing Impairment in the Fmr1 Knockout Mouse Model for Fragile X Syndrome

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Category: Development: Cellular/Systems
Background: *These authors contributed equally.
Hearing is essential for communication and social development and hearing deficits could be an important target in treating neurodevelopmental disorders. The Fmr1 knockout (KO) mouse model for fragile X syndrome (FXS) exhibits hearing impairment and recapitulates social and communication deficits found in FXS. We sought to investigate the pathophysiological changes in the Fmr1 KO cochlea.

Methods: We performed auditory brainstem response (ABR) analysis to determine thresholds for click, 8, 16, 32 and 45 kHz in groups of Fmr1 KO mice and wildtype control mice including males and females, as well as Fmr1 heterozygous female mice. To assess structural correlates of hearing function we performed multiplex immunofluorescence analysis of Myo7a+ hair cells, stria vascularis thickness and vessel density, Ctbp2+ ribbon synapses and Mbp+ myelinated peripheral auditory nerve fibers in the cochlea.
Results: Our main finding is that ABR thresholds are significantly greater at 45 kHz in Fmr1 KO mice than those in wildtype controls. Fmr1 heterozygous females show intermediate threshold levels. Moreover, we found that Ctbp2+ synaptic ribbons of high-frequency inner hair cells are smaller in volume in the Fmr1 KO than those in wildtype controls and exhibit a disordered distribution. By contrast, hair cells are present in normal numbers and patterning in the Fmr1 KO cochlea.
Conclusions: We are currently investigating additional synapse markers as well as the implications of hearing impairment for social behavior, given that 45 kHz is within the range of ultrasonic vocalizations that mice emit during social interactions. Fmr1 KO mice exhibit a unique pattern of synaptopathy with implications for understanding the basis for synaptic patterning in the cochlea.

M66. Auditory System Development in Genetically Distinct Rat Models of Autism

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Category: Development: Cellular/Systems
Background: Auditory hypersensitivity is a pervasive, quality-of-life-altering symptom that contributes to the social and communicative deficits experienced by individuals with autism spectrum disorders (ASD). Many studies have identified the auditory cortex as a crucial site for atypical sound processing in ASD. However, the challenge remains to determine how functionally distinct genetic mutations linked to ASD may converge on common mechanisms that can account for these auditory processing differences. One potential point of convergence is disruption to early life critical periods—a phase in early post-natal development when cortical circuits are highly plastic and are significantly shaped by sensory experience. To test this hypothesis, we characterized the development of critical period markers in rat models of the two most common genetically defined causes of ASD, Fragile X syndrome (FXS) and Tuberous Sclerosis Complex (TSC), which have previously been shown to exhibit opposite cellular phenotypes but also present with similar auditory processing deficits
Methods: Specifically, we used neuroanatomical analysis to examine parvalbumin positive (PV+) interneuron and perineuronal nets (PNN) expression in the auditory cortex of a Fmr1KO rat model of FXS and a Tsc2+/- Eker rat model of TSC across several developmental time-points (P14, P21, P28 and adult). We chose to focus on these structures because the maturation of PV+ inhibitory interneurons and their envelopment by PNNs are thought to be major drivers of critical period closure.
Results: Preliminary results suggest that there are indeed differences in PV+ expression, PNN expression, and PV+/PNN co-localization in the auditory cortex of both Tsc2+/- and Fmr1KO rat models, highlighting a possible shared pathway underlying auditory challenges in ASD.
Conclusions: Our findings are clinically relevant as they could help identify optimal treatment windows, emphasizing the importance of targeting specific developmental stages for therapeutic intervention.

M67. Developing a 3D Motor Neuron Organoid and Schwann Cell Co-Culture in a Microfluidic Device for Facial Nerve Injury Model

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Category: Development: Cellular/Systems
Background: Preclinical models for peripheral nerve regeneration often fail to predict the therapeutic outcomes in human clinical trials due to biological differences and ethical concerns associated with animal models. To address these challenges, we developed a 3D motor neuron (MN) organoid system using human induced pluripotent stem cell (hiPSC)-derived motor

neurons and primary human Schwann cells (SCs) within a microfluidic device. This model aims to simulate facial nerve injury and enhance the understanding of nerve regeneration processes.
Methods: Human hiPSCs were differentiated into motor neurons using a small molecule protocol, incorporating inducing factors (SB431542, LDN-193189, RA, SAG) and acceleration factors (SU5402, DAPT), with CHIR99021 to promote neural induction. These motor neuron organoids were co-cultured with primary human Schwann cells within a microfluidic device to simulate myelination. The differentiation efficiency and axonal growth were evaluated through immunofluorescence staining, RNA-seq, and microelectrode array (MEA) analysis. Additionally, the effects of glial-derived neurotrophic factor (GDNF) on axon growth and neuronal function were assessed.
Results: CHIR99021 significantly enhanced neural induction, with a marked increase in neuroprogenitor cell markers (SOX1, Nestin). By day 12, there was an increase in the motor neuron marker HB9, indicating the progression toward motor neuron differentiation. After co-culturing with Schwann cells, enhanced neural outgrowth was observed by day 28, evidenced by increased expression of TuJ1. 3D spheroid cultures demonstrated superior motor neuron differentiation and maturation compared to 2D cultures, as indicated by higher expression of key motor neuron markers (ISL2, SHH, CHAT, and SLC18A3) and enriched synaptic pathways. MEA analysis revealed increased action potential frequency and synchronized neuronal activity in the 3D cultures. The addition of GDNF further enhanced axon formation and promoted faster neuronal synchronization.
Conclusions: This study establishes a robust 3D motor neuron organoid co-culture system with Schwann cells in a microfluidic device, providing an advanced model for facial nerve injury research. The addition of CHIR99021 accelerates neural induction, and GDNF significantly improves axon functionality and maturation, making this model a valuable tool for exploring nerve regeneration and potential therapeutic strategies.

M68. Raman Spectroscopic Label-Free Microscopy to Detect Biochemical Property Changes in Pluripotent Stem Cells Induced Toward Human Early Otic Lineage

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Category: Development: Cellular/Systems
Background: Background: Recent advances in generating otic organoids from pluripotent stem cells has provided a valuable cell model of human early otic lineage. However, differences exist in the endogenous expression of key signalling molecules, such as bone morphogenetic protein 4 (BMP4), between different pluripotent stem cell lines. It is therefore necessary to optimize the concentration of BMP4 to be applied to each cell line during otic induction of stem cells to generate correct cell fates. Neural fate is induced when BMP4 concentration is too low, while differentiation is driven towards surface epidermis if BMP4 concentration is too high. Current methods for optimizing BMP4 concentration for otic induction are time-consuming and provide little information on the mechanisms by which otic fate is induced. Raman spectroscopy is a

label-free method of imaging based solely on the biochemical properties of cells. We therefore investigated whether Raman imaging could be used to distinguish between different cell fates following application of different BMP4 concentrations to pluripotent stem cells, and which biochemical signatures could potentially be used to characterize cells undergoing otic induction as opposed to off-target populations.
Methods: We combined Raman spectroscopy with 2D-culture system of otic induction from human induced pluripotent stem cells (hiPSCs) in the presence of various concentrations of BMP4 over the time course of 8 days in vitro. Differentiation towards neuronal, surface epidermis, and otic fates was validated using qPCR and immunocytochemistry.
Results: Differentiation of 3 hiPSC lines was performed in the presence of 0 to 5 ng/ml BMP4. Loss of pluripotency was confirmed using qPCR, while immunostaining for non-neural ectoderm and otic markers revealed an optimal concentration of BMP4 for otic induction of each hiPSC line. PCA of Raman spectra revealed differences between cells based on the concentration of BMP4 applied, suggesting differences in the biochemical properties of the differentiated cells. Indeed, Raman signatures indicated several of the peaks for collagen (i.e. at 856, 920, 937, and 1035 raman shift (cm-1)) were lowest for cells undergoing otic differentiation than for cells being driven towards off-target fates, suggesting that collagen may be used as a biomarker for human otic induction.
Conclusions: Raman spectroscopy is a powerful imaging label-free technique that can distinguish different cell fates including human early otic lineage and provide additional information on the biochemical properties of cells undergoing differentiation. Moreover, it offers the potential to be used for screening live cells for therapeutic purposes, such as regenerative medicine that would benefit from this non-invasive bioanalytical tool.

M69. Ribbon Synapse Assembly and Refinement During Hair Cell Maturation in Human Inner Ear Organoids

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Category: Development: Cellular/Systems
Background: Ribbon synapses in hair cells of the inner ear play an essential role in precise and persistent neurotransmission in the auditory system. The micro “hair cell-sensory neuron” circuit undergoes dynamic morphological reshaping during prenatal-postnatal development in the mouse inner ear. This reshaping involves ribbon density resizing, an increase in the number of ribbon-tethered synaptic vesicle pools, an increase in ribbon volume, and spatial confinement of constituent proteins near the Active Zone. Although the progression of ribbon synapse formation and maturation has been well characterized using the mouse model, little is known about how these ribbon synapses are assembled and refined during human inner ear development. In the present study, we tested if ribbon synapses in human inner ear organoids recapitulate temporal progression of ribbon synapse development in the mouse inner ear.
Methods: POU4F3-2a-ntdTomato human embryonic stem cells were differentiated into inner ear organoids, and at differentiation day 80, 120, 160 or 200, samples were fixed and subjected to wholemount immunofluorescence followed by tissue clearing based on the AbScale protocol. Three-dimensional reconstructions of confocal serial optical images were performed and the

number and volume of puncta was collected and the pre-synaptic ribbon counts were normalized to the hair cell nuclei counts. We also quantified the sphericity, circularity and area of the CtBP2+ puncta. To obtain high-resolution images of pre-synaptic ribbons, we used expansion microscopy. Imaging and quantification were carried out using the same method used for tissue cleared samples.
Results: The number, size and shape of CTBP2+ puncta, representing pre-synaptic ribbons, underwent temporal changes during hair cell maturation in human inner ear organoids. Quantitative immunofluorescence analyses of Z-stack images of cleared samples revealed a peak of the number of CTBP2+ puncta per hair cell at d120, followed by decreases in the number at d160 and 200. In contrast, we found a steady increase in the CTBP2+ area size between d80 and 200. The temporal changes in the ribbon size and shape were confirmed by expansion microscopy. Additionally, we observed partial colocalization of CTBP2+ puncta and Bassoon, suggesting recruitment of ribbons to the active zone in hair cells.
Conclusions: These results suggest that the number of ribbon synapses in organoid hair cells increases during hair cell differentiation, followed by a decrease due to refinement. Additionally, the size and shape of ribbons change during hair cell maturation. Our results are consistent with the temporal morphological changes during ribbon synapse maturation in mouse cochlear and vestibular hair cells, and suggest that organoid hair cells undergo ribbon assembly and refinement, similar to presynaptic maturation in native hair cells of the inner ear.

M70. Temporal Changes in Neuronal Innervation During Human Inner Ear Organoid Development

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Category: Development: Cellular/Systems
Background: Ribbon synapses are specialized synapses in sensory systems, crucial for tonic neurotransmitter release. Neuronal refinement, involving synaptic elimination and strengthening, is essential for nervous system development. While vestibular neuronal refinement is not well understood, cochlear ribbon synapse maturation in mice provides a reference for investigation. This study aims to test whether neuronal refinement and pruning, similar to those seen in the mouse inner ear, take place in human pluripotent stem cell-derived inner ear organoids during development.
Methods: POU4F3-2a-ntdTomato human embryonic stem cells were differentiated into inner ear organoids, and at differentiation day 80, 120, 160 or 200, samples were fixed and subjected to wholemount immunofluorescence for PCP4, TUJ1 and tdTomato, followed by tissue clearing based on the AbScale protocol. Confocal microscopy was used to capture 3D volumetric images of hair cells and neurons. The number of neurite processes contacting a hair cell was quantified by manually rotating 3D reconstructed images and counting direct contact points between TUJ1+ neurires and PCP4+ hair cells. Using Nikon NIS Elements software "3D Measurement", only hair cells that were complete and distinguishable were tagged and counted within a sample set. This annotation process ensured accurate counting of hair cells and prevented double-counting of neurons.

Results: The mean number of neurons contacting each hair cell significantly decreases from day 80 to day 200 ($\bar{x}$ = 2.56 to 1.60). Additionally, the variability in these contacts reduces, as indicated by the standard deviations (σ = 1.131 to 0.7107). Despite this, there is persistent non-normal distribution of neuronal contacts. Quantification of day 160 data is currently underway and will be included. The early peak of the number of neurite contacting a hair cell is in contrast with a delayed peak of the number of CTBP2+ puncta, representing ribbon synapses, at day 120.
Conclusions: This study demonstrates temporal changes in neuronal contacts with hair cells in a human in vitro model of inner ear development. Observations include decreased neuronal contacts after differentiation day 80, branching refinement at day 120, and stabilization until day 200. During mouse cochlear development, type 1 spiral ganglion neurons refine branching from E15.5 to P8+, while glutamate receptors and ribbon bodies mature around P0 to P8+. Synaptic pruning occurs between P5-P14, eliminating about 50% of ribbon synapses. Thus, the temporal order of neuronal refinement and synaptic pruning observed in human inner ear organoids is consistent with that in the mouse cochlea. These results suggest that human inner ear organoids can be used to recapitulate normal and pathological development of ribbon synapses and neuronal innervation, essential components for sensory transduction in the human inner ear.

M71. Requirement of SMOC1, an Extracellular Protein, in Morphogenesis of the Middle and Inner Ear

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Category: Development: Cellular/Systems
Background: Inner ear bony labyrinth has two openings, which act as the intersection between the air-filled middle ear and the fluid-filled inner ear. The oval window is contacted by the stapes while the round window is sealed by round window membrane. Opposing movement of the stapes footplate and round window membrane ensures the fluid motion in the cochlea for sound perception. The failure of the windows and/or stapes development causes conductive hearing loss, but the etiology of these malformations and developmental mechanisms of the bony arrangements remain to be understood.
Sparc-related modular calcium binding 1 (Smoc1) encoding an extracellular protein is a major causative gene for Waardenburg Anophthalmia Syndrome (WAS), characterized by eye and limbs defects. Since there are some cases that patients with WAS suffer severe hearing loss, we examined the roles of Smoc1 in ear function.

Methods: Smoc1 knockout mice were generated as previously described (Takahata et al., 2021 Commun). Biol.). Auditory brainstem response (ABR) was measured at 3-4 week old.
Results: We found that Smoc1 is widely expressed in the developing ear and deletion of Smoc1 caused moderate/severe hearing loss in mice. In Smoc1-null mice, round window was occasionally constricted or occluded and these defects were often concomitant with stapes malformation. In case of the mouse with round window atresia, round window membrane could be adhered to ectopic bone, suggesting normal formation of the membrane. The phenotypic variability could be attributed to mixed strain background.
Conclusions: Smoc1 plays key roles in morphogenesis of the bones in the middle and inner ear.

M72. PKM2 Controls Cochlear Development Through Lactate-Dependent Transcriptional Regulation

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Category: Development: Cellular/Systems

Background: Metabolic dynamics have emerged as a critical determinant in the cellular fate transition, playing an essential role in the development and regeneration of organs. The delineation of metabolic signatures within the embryonic cochlea promises to uncover innovative avenues for hair cell (HC) regeneration. While glycolysis is commonly regarded as the principal source of energy to sustain cell proliferation, the comprehensive metabolic profile of the developing cochlea and the specific impact of glycolysis on cochlear development and regeneration are yet to be fully understood.

Methods: We utilized RNA-seq on proliferating cochlear organoids to identify genetic changes associated with metabolic status. Cellular respiration tests and liquid chromatography-mass spectrometry (LC-MS) were employed to determine metabolic targets. We then generated transgenic mice with conditional knockout (CKO) of the glycolytic enzyme pyruvate kinase M2 (PKM2) to assess its influence in cochlear epithelium formation. Additionally, we applied targeted cleavage and transposition enzyme technology (CUT and TAG) combined with RNA-seq on PKM2 CKO cochlear organoids to map epigenetic changes within the genome and uncovered crucial regulatory targets. Moreover, we constructed AAV-2/DJ vectors carrying full-length PKM2 gene for round window injection into the mouse cochlea to overexpress PKM2 and explore its capacity to promote HC regeneration.

Results: We found enhanced canonical glycolysis in cochlear organoids expansion, evidenced by an increased extracellular acidification rate (ECAR), diminished mitochondrial oxidative phosphorylation, and accumulation of glycolytic intermediates such as phosphoenolpyruvate (PEP), pyruvate (PYR), and the end product of anaerobic glycolysis, lactate (LAC). The key glycolytic enzyme PKM2 was significantly upregulated in correlation with the proliferative activity of the organoids. Conditional knockout (CKO) of PKM2 substantially impeded cochlear organoid proliferation. Deletion of PKM2 in vivo led to significant developmental malformations in the cochlea, including a reduction in otic vesicle volume, shortened cochlear epithelium, and a decrease in hair cell count. Additionally, aberrant stereocilia morphology and orientation were observed. PKM2-dependent lactate accumulation promoted histone 3 lysine 9 lactylation (H3K9la), thereby modulating the expression of various genes. In vivo overexpression of PKM2 facilitated by AAV-2/DJ vector was found to stimulate the proliferation of cochlear progenitor cells, potentially initiating hair cell regeneration.

Conclusions: Glycolysis is a critical metabolic pathway in the formation of the cochlear sensory epithelium, with the enzyme PKM2 serving as a pivotal regulator. The PKM2-dependent modification of histone 3 lysine 9 lactylation (H3K9la) orchestrates gene expression within the cochlea, thereby facilitating the proliferation of cochlear progenitor cells. The in vivo overexpression of PKM2 augments the regeneration of cochlear HCs, offering a novel metabolic strategy for the regeneration of inner ear HCs.

M73. Effect of ITGA8 Inactivation During Inner Ear Development

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Category: Development: Cellular/Systems
Background: We previously identified an association between the Usher protein, protocadherin-15 (Pcdh15) and integrin alpha 8 (Itga8) in zebrafish and, in vitro, in stably transfected cell lines. In the inner ear both proteins are co-expressed between embryonic day 16 and post-natal day 6, with mutations in Pcdh15 or Itga8 genes affecting hair cell bundle development. To gain further inside into the possible functional role(s) of Pcdh15-Itga8 complex, we developed and characterized an Itga8 knockout (KO) epithelial cell line and found that this integrin is involved in the cytoskeletal rearrangement through the regulation ezrin-radixin-moesin (ERM) proteins and in the activation of Yap. Because Itga8 is involved in the signaling cascade that occurs between the extracellular matrix (ECM) and the intracellular environment, we are now presenting novel data showing the effect of different ECM stiffnesses in Yap/ERM activation in the absence of Itga8. We have also developed an Itga8 conditional knockout (Itga8 cKO), to study the effect of Itga8 absence in inner ear morphology, signaling cascade activation and Pcdh15 distribution
Methods: Culture cells grown in different stiffness in the presence of collagen I (non-Itga8's ligand) or fibronectin (Itga8's ligand). Analysis of these cells by immunohistochemistry and immunoblot techniques. Generation of the Sox2-CreER::Itga8-LoxP to study inner ear development in the absence of Itga8.
Results: In the Itga8 KO cells, there is a dysregulation of Yap/ERM signaling pathway compared to controls. Moreover, cytoskeletal structures are also affected by the lack of Itga8 and the growth in the different stiffnesses and in the presence/absence of Itga8's natural ligand. We are currently performing experiments with the Itga8 cKO line to see whether we can recapitulate some of the results observed in vitro. Given the cytoskeletal alterations observed in the cell lines and dysregulation of Yap activation, we are expecting to find structural abnormalities among the supporting and hair cells and an impairment of Yap and ERM activities.
Conclusions: Itga8 might be an important key molecule during the late stages of inner ear development.

M74. POU3F4 is a Critical Factor in Auditory and Vestibuar Synaptic Development

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Category: Development: Cellular/Systems
Background: POU3F4 is a transcription factor expressed by mesenchyme cells throughout the inner ear and is associated DFNX2, which includes middle ear malformations, profound hearing loss and vestibular dysfunction. It is known that Pou3f4 loss leads to reduced endocochlear

potential, and spiral ganglion neuron (SGN) developmental defects. A role for POU3F4 in vestibular development has not yet been determined. Here, we report on new findings on the role of POU3F4 in the auditory and vestibular systems. In particular, we used Pou3f4 knockout mice and Ca2+ imaging to test the hypothesis that POU3F4 is necessary for the development of spontaneous activity, which is critical for auditory wiring. In addition, we tested the hypothesis that POU3F4 is necessary for vestibular canal and otolithic function by measuring rotational and translational vestibulo-ocular reflexes (VORs) and vestibular afferent activity in Pou3f4 knockout mice.

Methods: Using Snap25GCaMP6s, Ca2+ imaging was performed to evaluate spontaneous activity in SGNs in Pou3f4 knockout mice and littermate controls at P1. Fluo4-AM was used with both genotypes to evaluate Ca2+ events in inner supporting cells. In addition, inner ears were dissected and used for RNAseq, immunohistochemistry, and western blotting to evaluate the insulin-like growth factor (IGF) pathway and factors associated with mitochondria. Using Pou3f4 knockout and littermate controls at 3-4 months of age, horizontal eye movement responses to head rotation (rVOR) and translation (tVOR) were recorded to assess canal and otolith function, respectively. Mice were subjected to sinusoidal head rotation (0.2-4Hz) and translation (0.2-2Hz) while eye movements were recorded using an ISCAN system. Gains and phases of the rVOR and tVOR were calculated by performing a fast Fourier transform (FFT) on the de-saccaded eye velocity and head velocity signals. Peripheral vestibular function was assessed by single unit recordings of afferent activities.

Results: Loss of Pou3f4 conferred defects in spontaneous activity in SGNs and inner supporting cells in the cochlea. We also found altered levels of IGF binding proteins, suggesting POU3F4 may regulate IGF signaling, a pathway known to be critical for auditory development. Interestingly, loss of Pou3f4 also led to altered profiles of cytochrome c in the cochlea, which may indicate altered mitochondria structure or function. With respect to the vestibular system, the Pou3f4 knockout mice showed gain decreases in the rVORs but gain increases in the tVORs at high frequency (2Hz) compared to the wildtype mice. The Pou3f4 knockout mice also demonstrated phase leads in the rVOR compared to controls. In current studies, we are performing additional single unit recordings and examining ribbon synapse formation in the vestibular system of Pou3f4 knockout mice.

Conclusions: These results support the hypothesis that POU3F4 plays important roles in both auditory and vestibular development and function.

PZ and RH, equal contributors; WZ, HZ, RH, and TC, co-senior authors.

M75. Investigation of the Gene Regulatory Network That Determines the Timing of Cell Cycle Exit and Developmental Patterning in the Organ of Corti

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Category: Development: Cellular/Systems
Background: During development of most tissues, progenitor cell cycle exit and differentiation are coupled during development. This pattern is also seen in the vestibular sensory organs, where hair cells are generated in the growing macula throughout organ development. In contrast, in the organ of Corti, the progenitor cell cycle exit and differentiation are uncoupled. A rapid apical-to-basal wave of transcriptional upregulation of Cdkn1b, which encodes the cell cycle inhibitor p27kip1, arrests progenitor cell proliferation between E12.5 and E14.0 in the cochlea. Hair cell specification is then initiated in the opposite basal-to-apical wave to create a unique pattern of hair and supporting cell organization. Despite its significance, the transcriptional regulation of Cdkn1b uniquely utilized in the developing cochlea is not well-understood.
Methods: Here, we propose that distinct transcriptional regulators are utilized in the organ of Corti to enforce Cdkn1b upregulation causing abrupt cell cycle exit. To test this idea, we compared the gene regulatory networks (GRNs) employed in the mouse vestibular and auditory systems during development (E12.0, E13.5, and P1). To construct the GRNs, we simultaneously profiled gene expression and chromatin accessibility at a single cell resolution using 10x Chromium Single-Cell Multiome platform. We then utilized SCENIC+, which allows the identification of putative tissue-specific transcription factors (TF), enhancers, and their downstream targets. To assay their activity, the predicted enhancers were cloned together with a minimal promoter and tested for their ability to drive GFP-reporter expression in the cell type of interest in vivo. To investigate if direct interaction of the predicted TF and enhancer is necessary, we mutated the predicted TF motifs in the enhancers.
Results: We identified two Cdkn1b enhancers that are located approximately 25kb away from Cdkn1b gene. The activity of the identified enhancers was confirmed to be restricted to the cochlear floor epithelium and not observed in the utricular macula. Additionally, published CUT and RUN data confirmed that these putative regulatory elements are enriched for both H3K4me1 and H3K27ac active enhancer marks in the P1 cochlear supporting cells. We are currently validating the transcription factors predicted to interact with putative Cdkn1b enhancers to drive its expression and, more broadly, understanding their effects on the development of the organ of Corti.
Conclusions: The timing and pattern of cell cycle exit in the organ of Corti are crucial for proper auditory system development and, ultimately, for normal hearing. Despite its significance, the mechanisms governing this developmental process remain unknown. This study seeks to uncover the transcription factors and gene regulatory elements specifically employed in the developing cochlea to establish the postmitotic prosensory domain and ensure lifelong mitotic quiescence in the organ of Corti.

M76. Creer Recombination Rate in Murine Intermediate Cells: A Comparison Between Three Different Models

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Category: Development: Cellular/Systems
Background: Stria vascularis, a multilayered highly vascularized epithelium located on the lateral wall of the cochlea, is primarily comprised of three types of cells: marginal cells,

intermediate cells, and basal cells, each derived from distinct embryonic origin. These cells function collectively in a synergistic and interdependent manner to pump potassium ions into the scala media, generating a positive endocochlear potential. A defect in any of these cell types leads to the dysregulation of the ionic composition of the endolymph, ultimately resulting in deafness. Therefore, it is important to study the roles of these cells in endolymph production. Surprisingly, to date, there is an absence of Cre driver mouse lines that effectively facilitate the study of individual cell functions within the stria vascularis, limiting our ability to understand strial deafness. Furthermore, the use of CreER transgenic mice runs the risk of poor recombination or non-specific expression. Therefore, the present study aims to determine the most appropriate line that targets the intermediate cells based on the development of melanoblasts, which are crucial for pumping potassium ion and maintaining ionic homeostasis of the endolymph.

Methods: We examined three different CreER transgenic mice under melanocyte specific promoters, namely, Pax 3-CreER, DCT-CreER and Tyr-CreER crossed with reporter mice Ai9/ Ai14. Our study investigated the efficiency of tamoxifen induced recombination, the recombination specificity, and the leakiness of each line.
We tested those 3 lines at 3 stages (E11, P0 and P28) and then fixed the sample at P0, P2 and P30 respectively to be processed for histology. Intermediate cells were labelled with CD44 to allow us to analyze the percentage of recombination and specificity.

Results: We observed that Pax3-CreER, DCT-CreER and Tyr-CreER successfully target the intermediate cells at three different stages: E11.5 (early development of otocyst), P0 (maturation of cochlea) and P28 (adult stage). However, the recombination was non-specific at P0 of Tyr-CreER mice line as the modiolus region showed some recombination. A consistent recombination was observed in DCT and Pax3 Cre ER x Ai9 mice line in all three developmental stages.
Conclusions: Our study reveals that both Pax3-CreER and Dct-CreER mouse lines exhibit efficient recombination in intermediate cells. Among these, the Pax3-CreER line demonstrated particularly high specificity and reliability for tamoxifen-induced recombination, making it the preferred model for spatiotemporally targeting the intermediate cell populations.

M77. Avian Cochlear Nucleus Neurons Exhibit Tonotopic Specializations Across Development

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Category: Development: Cellular/Systems
Background: From auditory periphery to cortex, tonotopy is a hallmark organizational pattern of auditory structures. Tonotopic patterns develop using extracellular signaling proteins that differ across time (i.e., development) and space (i.e., region). In the cochlear nucleus of mammals and the analogous nucleus magnocellularis (NM) of birds, high-frequency regions develop synaptic connections earlier than low-frequency regions. While we know that cochlear nucleus neurons express some intrinsic differences across the tonotopic axis, it is unclear

whether these differences are caused simply by a developmental delay or if the differences are independent of development.
Methods: Using whole-cell patch clamp electrophysiology, we recorded from embryonic chicken neurons within NM before hearing onset (E13) and after hearing onset (E20-21), separating between low- and high-frequency regions. We used current and voltage clamp to test the active and underlying ion channel properties that contribute to functional differences.
Results: At both early and late stages in development, high-frequency NM neurons demonstrate faster action potential properties than low-frequency neurons of the same age, indicating variations to potassium channel expression across tonotopy. In voltage clamp, we found high-frequency neurons exhibited larger potassium currents than low-frequency neurons at low depolarizing voltages. This demonstrates that low-voltage activated (e.g., Kv1) potassium channel expression differs between high- and low-frequency neurons across development, even at embryonic maturity. This suggests that a developmental delay across the tonotopic axis does not simply cause these differences. Interestingly, we also found an increase in sodium currents and sodium-dependent action potential properties in early-developing high-frequency neurons compared to low-frequency; later in development, there was no such tonotopic difference. This is likely caused by a developmental delay, where high-frequency neurons express optimal amounts of sodium channels earlier than low-frequency neurons. Overall, this suggests that NM neurons exhibit distinct phenotypes across the tonotopic axis and development, mediated by differences in sodium and potassium channels.
Conclusions: While NM neurons are defined as one single cell type, our results clearly indicate cellular tonotopic variation that is independent of synaptic input. While some intrinsic properties are tonotopically different due to a developmental delay, other properties remain distinct across development, suggesting unique functional phenotypes. Identifying both the synaptic and intrinsic differences across the tonotopic axis is an essential step to understanding how tonotopic maps develop in extremely temporally precise auditory structures.

M78. Spatial Transcriptomics and Its Application to the Mouse Cochlea

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Category: Genomics A: Genomics and Gene Regulation
Background: The cochlea is a highly heterogenous organ with many functionally distinct cell types. Fully describing the cell-type specific, transcriptional profiles within the cochlea provides major contributions to our understanding of genetic drivers of inner ear development, cell type specialization, and correlating molecular composition with function. However, the cellular diversity of the cochlea presents challenges when applying single-cell methodologies. Single-cell RNA sequencing (scRNAseq) currently dominates transcriptional profiling techniques.

Unfortunately, given the cellular heterogeneity of the cochlea, it requires a large number of cells to achieve sufficient resolution. This is particularly challenging as each sequencing reaction is normally limited to approximately 20,000 cells. Furthermore, single-cell sequencing cannot assign a spatial location to a cell – thus cellular identity is inferred by gene expression. Importantly, cochlear cell types contain location-based gradients of gene expression from base to apex, which correlate with their physical properties.
Methods: Spatial transcriptomics maps transcriptional data onto high-resolution images, allowing for the specificity of scRNAseq with location-based mapping. We applied two new spatial transcriptomics platforms, the Xenium In Situ platform and the Visium HD by 10x Genomics, to the mouse inner ear. We sectioned paraffin-embedded inner ears from postnatal mice and followed the standard Xenium In Situ and Visium HD protocols. We then performed downstream analysis using Xenium Explorer, Loupe Browser, and Seurat.
Results: Both spatial platforms accurately mapped representative marker genes to their respective cell types. Visium HD's resolution was limited to 8-microns, however, nearly 800 unique transcripts were detected per bin as compared to only 118 median transcripts per cell using the Xenium platform. Additionally, Visium HD's detection of over 19,000 different genes provided better performance when applying differential gene expression analysis. The wider transcript detection by the Visium HD also permitted the identification of gene expression gradients in same cell types from the base to the apex of the cochlea. Lastly, the Xenium platform exhibited low correlation of gene expression when compared to single cell and bulk RNASeq datasets.
Conclusions: When choosing a spatial transcriptomic platform, it is important to consider the overall motives for a particular study. The Xenium In Situ platform uses fluorescent based technologies that provides subcellular visualization of transcript detection, while the Visium HD platform allows for greater overall transcript detection limited by 8 micron binning. The high number of unique genes detected by the Visium HD primes this platform as a useful tool for discovery, while the Xenium may be more valuable for validation of known, highly expressed transcripts. Moving forward, we plan to continue optimizing the Xenium and Visium HD pipelines for inner ear tissues. We will also expand our analysis to a range of developmental timepoints to describe the transcriptional changes that occur in the inner ear from embryonic to adult stages.

M79. The Ticking Clock of Hearing: Precision in Gene Expression Timing for Optimal Rescue

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Category: Genomics A: Genomics and Gene Regulation
Background: Congenital hearing loss affects millions worldwide, with gene therapy showing promise as a treatment. However, its efficacy is limited by critical developmental windows, beyond which interventions become ineffective. In mouse models like the Eps8 knockout, hearing can only be restored if gene therapy is administered by postnatal day 2. The challenge lies in determining the precise developmental stage when gene expression becomes essential for hearing rescue.

Methods: The variability in gene expression levels and the lag time between AAV vector administration and actual gene expression complicate identifying critical timepoints for intervention. To overcome this, we used two inducible gene expression systems in Eps8 knockout mice:
1. Doxycycline-inducible system

2. Light-inducible system based on plant phytochrome B (PhyB)

These systems enable precise temporal control of Eps8 gene expression.

Results: The inducible models allowed us to modulate gene expression with spatiotemporal precision. The PhyB system, in particular, provided unprecedented control over the timing and localization of gene expression, aiding in the identification of crucial timepoints for hearing restoration.
Conclusions: This study provides a novel approach to understanding the relationship between gene expression timing and hearing rescue. By pinpointing critical timepoints for gene therapy, we aim to optimize interventions for congenital hearing loss, potentially transforming gene therapy approaches.

M80. The Transcription Factor Helios is Necessary for Both Outer Hair Cell Maturation and Functional Maintenance

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Category: Genomics A: Genomics and Gene Regulation
Background: In 2018, our laboratories demonstrated the transcription factor Helios is necessary for outer hair cell (OHC) maturation. The Ikzf2 mRNA, which encodes Helios, was found to be enriched and actively transcribed in OHCs of mice from P5 to adulthood. Our mouse model, which had dysfunctional Helios, exhibited reduced OHC electromotility as well as early-onset hearing loss. Ectopic Helios expression within inner hair cells (IHCs) resulted in a transcriptional shift towards an OHC-like state. However, the direct binding sites of Helios were not identified, and the necessity of Helios in adult OHCs was not determined. Here, we examine whether Helios also plays a role in maintaining OHC function after the onset of hearing using an Ikzf2 conditional knockout (cKO) mouse. Both transcriptomic and epigenomic analysis was performed to characterize the disrupted regulatory networks associated with Ikzf2 deletion.
Methods: Exon eight of Ikzf2 was conditionally deleted by crossing Ikzf2 floxed mice with Gfi1-Cre (deletion at ~E16.5) or prestin-CreERT2 (tamoxifen-induced at P12) mice. Auditory function of these Ikzf2 cKO mice was evaluated at 6-weeks of age by distortion product otoacoustic emission (DPOAE) and auditory brainstem response (ABR) threshold measurements. To evaluate OHC loss, cytocochleograms were performed on cochlear preparations from 6-week-old mice, stained with phalloidin, DAPI and an anti-prestin antibody. Inner ears from P8 Ikzf2fl/fl;Gfi1-Cre mice were harvested, OHCs were isolated using flow cytometry and transcriptionally analyzed through bulk and single cell RNA-seq. Transcriptomic results were

validated using spatial transcriptomics and RNAScope. Helios-deficient OHCs were investigated for epigenetic changes using single nucleus ATAC-seq on extracted cochleae of P8 Ikzf2fl/fl;Gfi1-Cre mice.
Results: Both Ikzf2 cKO mouse lines demonstrated a decrease in auditory function as assessed by ABR and DPOAE testing. Histological analyses showed significant OHC loss throughout the cochlear duct in the Ikzf2fl/fl;Gfi1-Cre mice, however, no OHC loss was seen in the Ikzf2fl/fl;prestin-Cre line despite elevated hearing thresholds. Transcriptomic analysis revealed significant alterations in gene expression within OHCs following Helios dysfunction. Notably, there was a downregulation of OHC-specific marker genes (e.g. Ocm, Slc26a5) alongside an upregulation of IHC-specific marker genes (e.g. Slc17a8, Otof). The biological processes significantly impacted include those related to sensory perception of sound as well as the activation of axon development pathways. Motif analysis of promoter and enhancer regions of dysregulated genes identified multiple transcription factors involved in the development of cochlear cell types as potential co-factors of Helios.
Conclusions: Our data reveal that Helios is critical not only for OHC development but also for maintaining OHC function. Mirroring our 2018 study, Ikzf2 deleted OHCs began shifting their transcriptional identity towards an IHC-like state. However, new pathways identified suggest Helios may prevent maturing OHCs from also taking a neuronal-like state. Lastly, epigenetic analysis demonstrates Helios' ability to reorganize chromatin to coerce an OHC fate.

M81. ESRP1 and ESRP2 Regulate Hair Cell Function by Affecting mRNA Stability in Zebrafish

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Category: Genomics A: Genomics and Gene Regulation
Background: RNA-binding proteins (RBPs) are involved in post-transcriptional RNA processing, such as pre-mRNA alternative splicing, mRNA stability and translation. Several RBPs have been shown to play important roles in the hair cells, whose dysfunction usually leads to auditory and/or balance deficits. Mutations in the gene encoding for epithelial splicing-regulatory protein 1 (ESRP1) have been associated with sensorineural hearing loss in humans, and Esrp1 knockout leads to cochlear development deficits through affecting alternative splicing of Fgfr2 mRNA in mice. However, Esrp1 knockout mice die soon after birth, preventing further analysis at later postnatal ages.
Methods: In the present work, the role of ESRP1 and its paralog ESRP2 in the hair cells was investigated using zebrafish as a model. Expression patterns of esrp1 and esrp2 transcripts were examined by performing in situ hybridization. Then esrp1 and esrp2 knockout zebrafish were established using CRISPR/Cas9 technique. Inner ear structure of the knockout zebrafish larvae was examined using a light microscope. Hair cell morphology and function were evaluated by whole-mount immunostaining and FM dye uptake. Downstream target mRNAs were identified by RNA-seq and validated by RT-qPCR.
Results: The transcripts of esrp1 and esrp2 were detected in the inner ear and anterior lateral line neuromasts. Moreover, esrp1 and esrp2 knockout affects inner ear development as well as hair cell function. The level of several transcripts is significantly decreased in esrp1/2 double

knockout larvae. Further investigation showed that ESRP1/2 could directly bind to their target mRNAs and affect their stability.
Conclusions: Our present work showed that ESRP1 and ESRP2 are required for the function of zebrafish sensory hair cells. Furthermore, they regulate the stability of the target mRNAs that are important for hair cell function.

M82. Promotion of New Connexin Gene Expression in the Cochlea after Deletion of Cx26 (GJB2)

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Category: Genomics A: Genomics and Gene Regulation
Background: Connexin 26 (Cx26, GJB2) mutations induce a high incidence of hearing loss, responsible for 70-80% of nonsyndromic hearing loss in children. However, the genetic changes after deficiency of Cx26 remail largely unclear, which hampers to fully understand the underlying deafness mechanisms and develop therapeutic interventions. In this study, we investigated the genetic changes in the cochlea after deletion of Cx26.
Methods: Cx26 knockout (KO) transgenic mice were used. The mouse cochlea was collected. Bulk Poly(A) RNA Sequencing and immunofluorescent staining were performed to assess gene expression changes in the cochlea after Cx26 deletion. Hearing function tests were also examined by ABR and DPOAE recordings.
Results: RNA-Seq examination demonstrated that deletion of Cx26 caused lots of genes up-regulation and down-regulation in the cochlea. In particularly, Cx46 (GJA3), which is an “eye” connexin gene and normally expresses in the eye but not in the ear, had a remarkable upregulation and was newly expressed in the cochlea after deletion of Cx26. Immunofluorescent staining confirmed that Cx46 had expression in the cochlea and occurred at the original places of Cx26 expression. Such new expression of Cx46 also occurred in the Cx26 heterozygous deficient mice. Moreover, this promotion of new connexin gene expression is Cx26-specific. Deletion of Cx30 (GJB6), which co-expresses with Cx26 in the cochlea, could not promote Cx46 expression in the cochlea.
Conclusions: These data demonstrated that Cx26 deficiency could promote new connexin gene expression for compensation. This may provide a useful cue for developing a therapeutic approach for this common hereditary deafness.
Supported by NIH R01 DC019687 to HBZ

M83. Mapping Chromatin Interactions in Cochlear Cells Using Micro-C Technology

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Category: Genomics A: Genomics and Gene Regulation

Background: Hearing loss affects millions worldwide, with various causes including noise, drugs, aging, and genetics impacting the integrity and functionality of cochlear cells. A major challenge in the ongoing research to delay, reverse, or treat hearing loss is our incomplete understanding of the complex molecular networks that regulate gene expression in the inner ear. Recent advances in transcriptomic and epigenetic analyses, at the bulk and the single cell levels, have made it possible to infer the transcription regulatory networks within cochlear cells using specialized bioinformatic tools. However, the accuracy and completeness of these networks are compromised due to the lack of information on spatial chromatin organization, particularly the interactions between distal enhancers and promoters.
Methods: We employ the cutting-edge Micro-C technique to map chromatin interactions in the cochlea. This high-resolution approach provides detailed insights into 3D chromatin architecture, reflecting chromatin compaction, folding, and spatial organization details. The results of the technique reveal distal regions of the genome that have been physically brought closer together to interact. While Micro-C captures genome-wide interactions, we have a particular focus on enhancer-promoter interactions within this chromatin organization. We integrate the Micro-C chromatin interaction map with existing transcriptomic, epigenetic, and transcription factor binding data to construct a comprehensive cochlea-specific gene expression model.
Results: Our preliminary findings reveal uncharacterized chromatin interactions in cochlear cells, including enhancer-promoter interactions, providing a more detailed understanding of the gene regulatory networks. We have identified novel long-range interactions that may play crucial roles in cell type-specific gene expression in the cochlea. Additionally, our integrated analysis revealed potential regulatory networks involving key transcription factors essential for cochlear development and function.
Conclusions: Here, we present the first Micro-C data acquired for the inner ear. Our high-resolution chromatin interaction map offers new insights into the complex transcriptional regulatory networks in cochlear cells. Future research will focus on validating these interactions and exploring how genetic variants within non-coding regions may disrupt enhancer-promoter interactions, potentially leading to hearing loss. These results will advance our understanding of the transcriptional regulation of cochlear cells and help to guide the search for novel therapeutic targets for cochlear cell regeneration and treating hereditary deafness.

M84. Molecular Genetic Testing for Usher Syndrome in a Diverse South Florida Population

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Category: Genomics B: General
Background: Usher syndrome (USH) is the most common genetic cause of combined deafness and blindness in humans. Patients with USH are classified as having one of the 3 distinct clinical subtypes- USH1, USH2 and USH3 based on the severity, progression, age at onset of HL and the presence or absence of vestibular dysfunction. So far, 12 loci have been described for USH. Here, we analyzed mutation spectrum of USH in an ethnically diverse South Florida patients.

Methods: This study includes 154 patients selected from a comprehensive database of patients with USH syndrome enrolled within The University of Miami Health system at Bascom Palmer Eye Institute and the Department of Otolaryngology. Demographic information and clinical evaluations were obtained from the electronic medical record. Race and ethnicity were self-reported by the patient. Genetic testing of the 12 target genes was performed by commercial laboratories. All variants were classified based on the American College of Medical Genetics and Genomics (ACMG) and Association for Molecular Pathology (AMP) guidelines.
Results: Of the 154 patients recruited for this study, 86 were female and 68 were male, ranging in age from 7 to 83 years old. In this population, 8 identified as African-American (AA), 6 as Asian (AS), 43 as Hispanic (H), and 92 as White, non-Hispanic (WNH); 5 patients had no race/ethnicity information. The subtype distribution among the diverse groups was as follows: AA: 4 USH1, 4 USH2; AS: 2 USH1, 2 USH2, 1 USH3, 1 not known (NK); H: 13 USH1, 24 USH2, 2 USH3, 4 NK; and WNH: 13 USH1, 40 USH2, 3 USH3, 7 NK. The distribution of subtypes was similar between the H and WNH groups, with USH2 accounting for 45% and 43% of cases, respectively. Only 61(40%) of these patients had a molecular genetic diagnosis:1 AA (25%), 2 AS (33%), 16 H (37%), and 27 WNH (29%). Of the 13 patients with USH1, 77% had pathogenic variants in MYO7A (1 AA, 1 AS, 3 H, 2 WNH, 3 NK). Of the 46 with USH2, USH2A was the most common gene (86.8%, 1 AS, 10 H, 20 WNH, 9 NK). The 2 patients with USH3 (both WNH) had a variant in CLRN1 in a homozygous state.
Conclusions: Overall, our findings highlight the gap in USH studies in US minority groups and the critical need for increased inclusion of racial/ethnic minorities in genetic HL studies to eliminate disparities in access to care and healthcare quality and preventive screening.

M85. Precision Medicine of Hereditary Hearing Loss – The Diagnostic Rate of Whole-Exome Sequencing

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Category: Genomics B: General
Background: Hereditary hearing loss (HHL) is the most common inherited sensory deficit in newborns and children. The genetic causes of HHL can be identified efficiently through next-generation sequencing (NGS). Given that the phenotype of HHL varies widely and the efficacy of cochlear implant (CI) depends on the causative gene of hearing loss, the genotype-phenotype mapping enables physicians to predict disease progression and to plan treatment. Here we aim to establish a multidisciplinary genetic testing platform for patients with HHL.
Methods: Whole exome sequencing (WES) were conducted to identify the causative genes of HHL cosegregated with the disease phenotype in non-consanguineous families. After conducting variant calling of bioinformatics, the results were validated with Sanger sequencing and in silico analyses for functional prediction. The diagnostic rate of WES between prelingual and postlingual HHL were also compared.
Results: From November 2020 to July 2024, 62 pathogenic variants were identified in 48 probands and their relatives from 35 families, including novel variants in MYO15A, CDH23 and TMC1. 29 probands were prelingually deafened and 19 developed hearing loss postlingually. 12

patients who received CI had all discovered their pathogenic genes, and all showed good efficacy of CI related to their etiology. The most common type of mutation was missense (72.9%), followed by frameshift (10.0%) and splicing (10.0%), and nonsense (7.1%). 16 patients had compound heterozygous mutations, while 8 patients carried homozygous and 21 carried heterozygous mutations. Autosomal recessive inherited gene variants accounted for 84.6% of prelingual HHL, while most of postlingual HHL were inherited autosomal dominantly (73.7%). The overall diagnostic rate was 81.3%, and prelingual HHL yielded higher diagnostic rate compared to postlingual HHL (89.7% and 68.4%, p LESS THAN 0.001). Some patients with postlingual HHL had more than one mutant variant discovered. All the mutant variants identified via WES had been confirmed by Sanger sequencing.
Conclusions: WES provides the advantage in improving the diagnostic rate of HHL, especially for children with congenital deafness. Such genetic investigating tools should be integrated into the selection for CI candidacy.

M86. Genetic Hearing Loss: Molecular Diagnostic Challenges and Solutions

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Category: Genomics B: General
Background: Hearing loss is a genetically and clinically heterogeneous condition, with pathogenic variants in over 150 genes identified to date. Given the complexity of the hearing process, it is predicted that there are more genes yet to be discovered. The recent advances in next-generation sequencing over the past decade have facilitated rapid gene discovery; however, it has unveiled a multitude of variants of uncertain significance (VUS), which pose challenges in risk assessment and clinical management. It is also estimated that about half of inherited hearing loss cases remain unsolved, therefore, we hypothesize that noncoding variants in hearing loss associated genes or in regulatory regions would likely explain the hearing loss cause in some of these cases.
Methods: We analyzed genetic variants from whole exome sequencing (WES) data performed on 1038 adult deaf probands from the Tipa BiobankTM and eight trio families with inherited sensorineural hearing loss. An artificial intelligence (AI)-based methodology, PredHL, is being developed for the prediction of variants' pathogenicity at the protein level. We are also performing H3K27ac enrichment genome-wide analysis using mouse ChIP-seq data.
Results: The causal variant was identified in about 10% of the biobank cases using our bioinformatics meta-analysis pipeline. In an additional 20% of cases, VUS were identified in hearing loss-related genes. In the trio cases, the genetic cause was identified in two families; a heterozygous pathogenic variant was identified in a third family with no second allele. The genetic cause in the remaining families was not identified. By analyzing H3K27ac enrichment in the genomic vicinity of known deafness genes, we have identified putative regulatory elements

in the non-coding genome of the mouse. We will integrate these elements with the sequencing data of unsolved cases in the hopes of identifying the causative variants.
Conclusions: Using a hearing loss-specific variant prediction tool can improve variant prioritization and classification. Additionally, exploring the noncoding regions of the genome will help us better understand the role of regulatory elements in auditory function and increase our diagnostic yield.
Research Support: The ISF Precision Medicine Partnership Program 3499/2019 (IPMP), the ISF Breakthrough Grant 2711/2022 and the National Institutes of Health/NIDCD R01DC011835.

M87. Genomic Foundation of Sensorineural Hearing Loss

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Category: Genomics B: General
Background: Sensorineural hearing loss (SNHL) is a prevalent sensory disorder with a high Mendelian genetic contribution. However, approximately 50% of cases remain genetically elusive even after comprehensive testing, such as exome sequencing. The diagnostic and clinical value of whole-genome sequencing (WGS), particulary in cases undiagnosed after previous genetic testing, remains unknown in the context of SNHL.
Methods: To evaluate the additional diagnostic yield of WGS in SNHL and to identify factors associated with WGS diagnostics in cases where targeted and exome sequencing have not yielded a diagnosis. To elucidate a comprehensive genome-phenome landscape linked to inner ear targeted therapies.
Results: We explored the genetic variants of 394 SNHL families and 746 individuals including probands (183 [46.2%] male; median age at genetic testing, 13 [0-76]) and their family members. Collectively, we achieved a gradual improvement of diagnostic yield in the cohort, and complete genetic diagnosis (a pathogenic or likely pathogenic variant that explains the phenotype) was made in 220 families (55.86%). Among patients who remained undiagnosed after exome sequencing, all syndromic SNHL patients and a representative subset of nonsyndromic SNHL patients were selected for WGS using a sample size estimation with a stratified sampling approach (n = 120). Among these, WGS identified causal variants in 24 families (20.0%). In particular, 14 of them (11.7%) , including deep intronic variants, small structure variants, and complex genomic rearrangements, were otherwise challenging to detect through previous genetic testing, despite their medical implications including inner ear targeted therapies using splice-switching antisense oligonucleotides (ASOs). Furthermore, our comprehensive genomic landscape refined the mutational landscape, revealed previously undefined genotype-phenotype correlations or clinical factors for genetic completion, and presented a newframework for understanding genetic hearing loss at the level of the inner ear molecular functions and inner ear spatiotemporal expression of the mutated deafness genes.
Conclusions: This is the first study of the systematic application of WGS in a SNHL cohort, confirming an additional diagnostic yield of approximately 12% that had not previously been detected by exome sequencing and other techniques. This study suggests the clinical utility of WGS in real-world SNHL practice towards the precision medicine to come, and offers a comprehensive genome-phenome landscape possibly linked to inner ear targeted therapies.

M88. Cep250 in Atypical Usher Syndrome

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Category: Genomics B: General

Background: Usher Syndrome (USH) is the foremost cause of inherited deaf-blindness presenting a combination of retinitis pigmentosa (RP) and sensorineural hearing loss (SNHL). USH is genetically and phenotypically heterogeneous, with three subtypes (type 1, 2, and 3) of differing severity and age of onset. Cep250, known to encode for a centrosome linker protein (C-Nap1), was recently identified as a causative gene of atypical USH, presenting with severe SNHL and mild RP. Although Cep250 is known to regulate centriole cohesion, its function in normal hearing and vision remains uncharacterized, leaving the pathophysiology of atypical USH caused by Cep250 mutations poorly understood.

Methods: Using a Cep250 reporter-tagged knockout mouse line (Cep250tm1a(EUCOMM)Wtsi), we're investigating the expression pattern and the mutant phenotype of Cep250 in the cochlea. We utilize the LacZ reporter gene driven by the endogenous Cep250 promoter and immunostaining of Cep250 to investigate the Cep250 expression pattern in the developing and adult mouse cochlea. We perform immunohistochemistry of hair cell and supporting cell cytoskeletal proteins, SEM and ABR to assess hair cell development and hearing function in Cep250tm1a/tm1a mutants. To determine whether Cep250 is required in hair cells for normal hearing, we have crossed Cep250tm1a/+ mice with a Atoh1-FLPo mouse line to remove the FRT-flanked gene targeting cassette in Cep250tm1a, thereby restoring Cep250 gene function in hair cells.

Results: Although single-cell RNA sequencing data(1) suggests Cep250 is enriched in hair cells at early postnatal ages, our LacZ staining results indicate Cep250 is broadly expressed in both sensory and non-sensory cells in the cochlea at P0 and becomes more enriched in the hair cells at P6. Our preliminary immunostaining results showed that the Cep250 protein is localized to punctate structures around the basal body in hair cells and supporting cells, suggesting a role in microtubule organization. Interestingly, Cep250 also showed an enrichment in the hair bundle at early postnatal ages. Cep250tm1a/tm1a adult mutants had high-frequency hearing loss by ABR testing. However, preliminary light microscopy and SEM analysis did not reveal gross defects in hair bundle development and maintenance.

Conclusions: Our preliminary results suggest that Cep250 is required for hearing of high-frequency sound but is dispensable for hair cell development. Ongoing experiments aim to elucidate the cell-type requirement and cellular functions of Cep250. Our work will provide a deeper understanding of SNHL by investigating the disease mechanisms of atypical USH caused by Cep250 mutations.

Reference:

1. Orvis J, Gottfried B, Kancherla J, et al. gEAR: Gene Expression Analysis Resource portal for community-driven, multi-omic data exploration. Nat Methods. 2021;18(8):843-844. doi:10.1038/s41592-021-01200-9

M89. Genotype-Phenotype Correlations in DFNA5-Related Hearing Loss

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Category: Genomics B: General

Background: Variants in the gene DFNA5 (also known as GSDME) are associated with autosomal dominant non-syndromic hearing loss (ADNSHL). The hearing loss is typically progressive and down sloping. All reported cases of DFNA5-related HL involve the skipping of exon 8, which results in the expression of a constitutively active but truncated protein that induces apoptosis of cochlear hair cells. We have observed variant-dependent differences in the levels of aberrant splicing, leading us to hypothesize that partial loss of splicing will result in a milder HL phenotype as compared to complete loss of splicing.
Methods: A literature review of reported pathogenic DFNA5 variants was completed to identify variants resulting in complete or partial loss of splicing. In addition, seven pathogenic variants from patients we have tested for genetic deafness were assessed using a minigene splicing assay to determine the impact on splicing. Audiometric analysis was performed by generating regression models to compare the level of hearing impairment across age for the complete and partial loss of splicing groups.
Results: Five of seven pathogenic DFNA5 variants showed partial loss of splicing on a minigene assay. Three variants with extensive audiometric data were studied in detail: two variants (NM_004403.3:c.991-15_991-13delTTC and NM_004403.3:c.991–6 C GREATER THAN G) that result in partial loss of splicing and one variant (NM_004403.3:c.990+503_990+1691delins132) that results in complete loss of splicing. A censored random effects linear model comparing the hearing loss associated with these variants identified statistically significant differences at 1000, 2000, 4000, and 8000 Hz (p LESS THAN 0.001). Complete loss of splicing was associated with greater initial and more quickly progressive HL as compared to partial loss of splicing.
Conclusions: The phenotype-genotype correlation seen with DFNA5-related HL reflects the amount of active but truncated protein that is expressed. Greater amounts of mutant protein result in more severe and more rapidly progressive HL in the mid to high frequencies.

M90. Deciphering the Role of Rfx1/3 in Cochlear Hair Cell Development and Function

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Category: Genomics B: General
Background: Our laboratory previously demonstrated that conditional deletion of both Rfx1 and Rfx3 (Rfx1/3;Gfi1-Cre) in cochlear hair cells at embryonic (E) day 16.5 results in profound hearing loss and rapid outer hair cell (OHC) degeneration around the onset of hearing. This finding underscores the importance of Rfx1/3 during embryonic development. However, the critical expression window of Rfx1/3 in the auditory system and the underlying molecular mechanisms through which they regulate hair cell maintenance remain unclear.
Methods: To characterize the spatiotemporal expression of Rfx1/3, fluorescent in situ hybridization (RNAscopeTM) was performed on C57BL/6 mice at embryonic and postnatal timepoints. To further investigate whether RFX1/3 are necessary beyond embryonic time points for hair cell development and/or mature hair cell maintenance, we created two Rfx1/3 conditional knockout (cKO) mouse models. Rfx1/3;Myo15-Cre, to conditionally delete Rfx1/3 at postnatal (P) day 0, and Rfx1/3;Prestin-CreERT2, to conditionally delete Rfx1/3 at P4 and P8 by tamoxifen (TAM) injections. To measure auditory function, Rfx1/3;Myo15-Cre and Rfx1/3;Prestin-CreERT2 homozygous mutant and wild type mice underwent auditory brainstem response testing (ABR) and distortion product otoacoustic emissions (DPOAEs), followed by histological analyses. To investigate the underlying regulatory network of Rfx1/3, scRNA-sequencing was performed on cochlear sensory epithelia from P2 and P7 Rfx1/3;Myo15-Cre mice.
Results: Rfx1 and Rfx3 are expressed in hair cells during both embryonic and postnatal stages. In Rfx1/3;Myo15-Cre homozygous mutant mice, significantly elevated ABR and DPOAE thresholds were observed as early as P16 compared to wildtype littermates, with OHC loss initiating at P12 following a basal-to-apical gradient. In Rfx1/3;Prestin-CreERT2 homozygous mutant mice, TAM administration from P4 resulted in statistically significant difference in ABR and DPOAE thresholds at high frequency region of the cochlea compared to wildtype littermates, alongside corresponding OHC loss. However, in Rfx1/3;Prestin-CreERT2 homozygous mutant mice injected with TAM from P8, no statistically significant difference in ABR and DPOAE thresholds or OHC loss were observed, suggesting a narrow window of Rfx1/3 necessity for cochlear function and maintenance. Furthermore, 177 differentially expressed genes were identified between wildtype and conditional knockout Rfx1/3;Myo15-Cre mice. Pathway analysis of those genes revealed that Rfx1/3 are involved in intrinsic apoptotic signaling pathways and neurodegeneration, supporting their role in regulating critical pathways necessary for hair cell survival and function during early cochlear development.
Conclusions: Our findings establish that the expression of Rfx1/3 is necessary for OHC development and maintenance, with a critical expression window extending from the embryonic period to early postnatal stages, ending before P8. This highlights that Rfx1/3 activity is essential for cell maintenance only until this stage. Future directions will focus on determining the involvement of Rfx1/3 in hair bundle formation during earlier embryonic stages and validating the key downstream targets of RFX1/3 for hair cell survival.

M91. Reduced Level of Kcne1, Kcnj10 and Col4a3 are Sufficient to Maintain Hearing

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Category: Genomics B: General

Background: KCNE1 and KCNJ10 encode potassium channels expressed in marginal and intermediate cells of the stria vascularis, respectively, where they regulate the movement of potassium into the endolymph to maintain the endocochlear potential (EP). Mutations in KCNE1 are associated with Jervell and Lange-Neilson Syndrome which causes profound bilateral deafness that begins in early childhood, and prolongation of the cardiac QT interval. Mutations in KCNJ10 are associated with epilepsy, ataxia, sensorineural deafness and renal tubulopathy (EAST) syndrome. COL4A3 encodes a collagen chain comprising a major component of the strial capillary basement membrane which balances cochlear homeostasis. Mutations in this gene are associated with human autosomal recessive Alport syndrome. It is currently unclear whether it is possible to restore the hearing loss associated with KCNE1, KCNJ10 or COL4A3 using gene therapy or other treatments. Here, we aim to characterise the auditory phenotype in three mutant mice carrying novel alleles in these genes (Kcne1tm1a, Kcnj10tm1a and Col4a3tm1a).
Methods: Mice are on a C57BL/6NTac background in which the Cdh23ahl variant was repaired to ensure good hearing as mice age. All mutant alleles carry a large cassette of exogenous DNA designed to interrupt normal gene transcription. A longitudinal set of auditory brainstem response (ABR) recordings was used to characterise hearing impairment in Kcne1tm1a/tm1a, Kcnj10tm1a/tm1a and Col4a3tm1a/tm1a mice compared to littermate controls (N = 6 per genotype). EP recordings were performed at 8 weeks of age in all mutant mice compared to littermate controls to investigate function of the stria vascularis in vivo (N = 4 per genotype). Whole mount preparations of the stria vascularis were used to investigate changes to the structure and arrangement of marginal cells in all mutant mice compared to littermate controls (N = 4 per genotype). Whole inner ear tissue was collected at 4 weeks of age to quantify any changes to the level of expression of Kcne1 or Kcnj10 or Col4a3 using digital droplet PCR (N = 6 per genotype).
Results: Surprisingly, all mutant mice had normal hearing between 4 weeks and 6 months of age in terms of ABR thresholds and EP. Marginal cell arrangement in the stria vascularis was normal for Col4a3tm1a/tm1a mice but showed evidence of early cellular degeneration in Kcnj10tm1a/tm1a and Kcne1tm1a/tm1a mice at 8 weeks old. Subsequently, digital droplet PCR revealed that around 25% of the normal level of gene expression of Kcne1, Kcnj10 and Col4a3 remained in Kcne1tm1a/tm1a, Kcnj10tm1a/tm1a, Col4a3tm1a/tm1a mutants respectively.
Conclusions: Together, these data suggest that these three mutants are not suitable to test for hearing loss reversal. However, results suggest that partial restoration of KCNE1, KCNJ10 and COL4A3 expression in patients affected by mutations in these genes leading to hearing loss may be sufficient to preserve auditory function.

M92. Unveiling the Genetic Architecture of Hearing Loss in Populations With African Ancestry

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Category: Genomics B: General

Background: Hearing loss identification often addresses the symptom, not its underlying cause. While genetic variants are known in nearly 50% of patients with hearing loss (Shearer, Hildebrand, Schaefer, 2023), current clinical genetic testing panels identify a cause in anywhere from 10% to 83% of cases (Shearer and Smith, 2015). This inconsistency is largely due to selection bias, with higher diagnostic rates observed in European populations. Consequently, existing genetic panels and research are biased, resulting in individuals of African ancestry experiencing higher rates of variants of unknown significance (VUS) due to their underrepresentation in genetic studies (Florentine et al., 2022; Yan et al., 2016). With Europeans and Asians making up 96.4% of study populations, this lack of diversity impedes accurate diagnosis for individuals of African ancestry (Rouse et al., 2022). There is a critical need to investigate the genetic basis of hearing loss in this underrepresented group.
Methods: We will utilize whole genome sequencing (WGS) data from BioVU, the Vanderbilt DNA biorepository, to conduct a genome-wide association study (GWAS) focusing on patients of African ancestry. For consistency, cases and controls were defined based on PheKB (Wei-Qi and Denny, n.d.), but only included those with race reported as African-American. For cases (N = 2,500), inclusion criteria utilized ICD9/ICD10 diagnosis and procedure codes related to sensorineural or mixed conductive and sensorineural hearing loss, hearing aids and cochlear implantation. For controls (N = 22,940), exclusion criteria utilized ICD9/ICD10 diagnosis and procedure codes related to sensorineural, conductive, or mixed conductive and sensorineural hearing loss, tinnitus, hearing aids, and cochlear implantation. Once sequencing data is complete, genotyping data will follow standard QC for GWAS and all analyses will be conducted using REGENIE (Mbatchou et al., 2021). Following the GWAS, we will perform functional annotation of genome-wide significant associations to interpret the biological significance of identified variants.
Results: Expected outcomes include identifying genomic regions that overlap with those found in studies of other populations, which will help assess the transferability of genetic influences. Additionally, we aim to pinpoint any novel variants specifically associated with hearing loss in this underrepresented group, contributing to a better understanding of the genetic architecture of sensorineural hearing loss.
Conclusions: This study may reveal new genetic loci and variants associated with hearing loss in individuals of African ancestry, addressing current research gaps and improving our understanding of genetic influences across diverse populations. The preliminary data will provide insights into the genetic basis of sensorineural hearing loss and serve as a comparison for a future study using measured hearing thresholds as a quantitative marker of hearing loss severity for more precise phenotyping.

M93. Analyzing Mitochondrial Heteroplasmy and DNA Copies in the HEI-OC1 Cell Line Treated With Hydrogen Peroxide and Cochlear Samples of CBA/CaJ Mice

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Category: Aging

Background: Sensorineural hearing loss (SNHL) is often linked to mitochondrial diseases, which involve complex changes in mitochondrial DNA (mtDNA). One key aspect of this is mtDNA heteroplasmy, where multiple variants of mtDNA coexist within a single cell. This heteroplasmy may result from a mixture of normal and mutated mtDNA or from the presence of different healthy variants. However, it remains unclear whether age-related heteroplasmy levels correlate with the onset of age-related hearing loss (ARHL). This study examines the relationships between mtDNA heteroplasmy levels and ARHL.

Methods: Method: The study utilized both in vivo and in vitro models. In vivo experiments utilized young adult (n=3, 3-month-old) and aged (n=3, 30-month-old) CBA/CaJ mice. The auditory function of these mice was evaluated using auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE) before they were sacrificed. In parallel, an in vitro oxidative stress model was employed using HEI-OC1 cells, which were treated with hydrogen peroxide (H2O2) at two concentrations (50μM and 100μM) for 24 hours. Mitochondrial DNA was extracted from the cochleae of the mice as well as from the HEI-OC1 cells using the QIAGEN Miniprep Kit. To ensure accurate NGS analysis, PCR amplification was conducted to prevent the co-amplification of nuclear mitochondrial insertion sequences, assess the relative copy numbers of genomic DNA and mtDNA.

Results: The aged mice (30 months old) displayed significant hearing loss compared to the young adult mice (3 months old), as measured by ABR and DPOAE thresholds and amplitudes. Genebank BLAST analysis of the mtDNA sequences revealed that the identified sequences were distributed across 21 chromosomes, so, a single primer pair was used to enrich the entire mitochondrial genome, providing uniform coverage while avoiding nuclear DNA interference. PCR analysis showed that mtDNA copy numbers relative to nuclear DNA were approximately 340 in both young adult mouse cochlear samples and HEI-OC1 samples. A heteroplasmy frequency threshold of 0.003% was used to detect alternate variants. Nine significant heteroplasmic point mutations were identified (P LESS THAN 0.05), some in coding regions like cytochrome c subunit 1 and cytochrome b, and others in non-coding regions such as tRNA and the D-loop. Two high-frequency mutations were found in H2O2-treated HEI-OC1 cells and were also present in the mouse models. Aged mice also showed a marked reduction in mtDNA content in the cochlea, with a similar decrease observed in H2O2-treated HEI-OC1 cells

Conclusions: This study provides a detailed catalogue of mtDNA mutations related to heteroplasmy and mtDNA copy number changes in the aging mouse cochlea. The findings suggest that age-related alterations in heteroplasmy and mtDNA copy number could impair mitochondrial function, contributing to age-associated hearing loss. Further research is required to clarify the molecular mechanisms through which mtDNA heteroplasmy affects mitochondrial function as part of ARHL.

M94. Big Brown Bats (Eptesicus Fuscus) are Resistant to Age-Related Hearing Loss

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Category: Aging

Background: Hearing mediates many behaviors critical for survival in echolocating bats, including foraging and navigation. Although most mammals are susceptible to progressive age-related hearing loss, the evolution of biosonar, which requires the ability to hear low-intensity echoes from outgoing sonar signals, may have selected against the development of hearing deficits in bats. Many echolocating bats exhibit exceptional longevity and rely on acoustic behaviors for survival to old age; however, relatively little is known about the aging bat auditory system. We hypothesized that long-lived bats may show resistance to age-related hearing loss (ARHL), indicated as comparable peripheral auditory sensitivity among young and aging bats to facilitate effective echolocation-based behaviors throughout their natural lifespan.

Methods: In this study, we used DNA methylation to estimate the ages of wild-caught big brown bats (Eptesicus fuscus) and measured hearing sensitivity in young and aging bats using auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs). We used immunohistochemistry to label cochlear hair cells and afferent and efferent auditory nerve terminals at 8 locations along the total length of the cochlea to evaluate structural evidence for aging in bats. We used linear mixed-effects modeling to assess the effects of age on physiological hearing sensitivity (ABR and DPOAE metrics) and cochlear histological variation in young and aging bats.

Results: We found no evidence for hearing deficits in bats up to 12.5 years of age, demonstrated by comparable ABR-derived thresholds, similar ABR wave morphology, and similar DPOAE amplitudes across age groups. Although aging bats had slightly smaller ABR wave 1 amplitudes compared to young bats, the amplitudes and latencies of ABR waves 1-5 were not statistically different across age groups. We additionally found no significant histological evidence for cochlear aging, with similar hair cell and afferent terminal counts observed in young and aging bats. Efferent innervation patterns differed with age in which aging bats showed greater terminal density per inner hair cell, potentially indicating changes to the lateral olivocochlear system with age and/or accrued sound exposure experience in bats.

Conclusions: Here we demonstrate that big brown bats show minimal physiological and structural evidence for ARHL. Relative to young bats, aging bats retain comparable auditory sensitivity from 4-84 kHz, robust outer hair cell function, and cochlear structural integrity. We present echolocating bats as informative models for investigating mechanisms that may preserve hearing function over a long lifetime.

M95. Gender Differences in the FBN Rat Model of Aging: Investigation of ABR Waveforms and Ribbon Synapse Changes

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Category: Aging

Background: Age-related hearing loss (ARHL) affects at least one-third of individuals over 65 years of age, negatively impacting cognition and social interaction. ARHL impacts men and women differently, with large epidemiological studies showing smaller age-related threshold shifts in females. Recent research suggests that these gender differences may partly result from cumulative noise exposure over a lifetime. Historically, the correlation between ARHL, speech understanding loss, and cochlear hair cell loss has been inconsistent. In male Fischer Brown Norway (FBN) rats, an extensively studied NIA model of aging, we previously investigated the relationship between age-related changes in inner hair cell (IHC) ribbon synapses, auditory thresholds and amplitudes of the auditory brainstem response (ABR) (Cai et al., 2018).
Methods: In this study, we examined changes in IHCs and outer hair cells (OHCs), and ribbon synapses between IHCs and spiral ganglion neurons (SGNs), alongside ABR thresholds, and wave I and V amplitudes, across four age groups of female FBN rats (4, 12, 24, and 28 months). Preliminary data include 4-8 female FBN rats per group.
Results: Similar to male FBN rats, female FBN rats exhibited significant age-related OHC loss in the apical turn at 2 kHz by 24 months and at 4 kHz by 29 months, with additional OHC losses in the basal turn. While male FBN rats previously showed no significant age-related IHC losses, preliminary data from female FBNs suggests significant apical IHC losses with aging. Unlike males, female IHC-SGN ribbon synapses showed no significant age-related changes at 2 kHz or 4 kHz. Preliminary quantitative comparison of ABR wave I and wave V changes between genders reveal larger wave I amplitudes in female FBN rats than males, with similar proportional declines in amplitude with age, across tested frequencies. Male/female ABR threshold comparisons await additional animals across groups.
Conclusions: Preliminary ABR and ribbon synapse findings suggest gender differences in wave I peak amplitudes of young rats, with qualitatively similar but quantitatively different ABR and synapse age-related changes. Gender differences in the FBN rat model are generally consistent with those observed by Balagova et al. (2018) in the F344 rat model, a rat strain with a different pattern of peripheral aging.

M96. Neural Synchrony is a Sensitive Measure of Early Age-Related Auditory Deficits in Mice

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Category: Aging
Background: Aging is associated with deficits in auditory structure and function. Recognizing age-related changes that emerge in middle-age may be crucial for characterizing the initial functional impairments that occur with age, and the temporal and spatial progression of age-related pathophysiology in the auditory pathway. There is increasing evidence that suprathreshold measures of auditory nerve (AN) function may be able to detect age-related auditory deficits earlier than reduced pure-tone thresholds. These age-related AN deficits may be exacerbated in the presence of background noise. We hypothesize that early age-related auditory deficits will be more pronounced when analyzing auditory evoked responses recorded in noise compared to quiet. Specifically, we predict that in noisy conditions, middle-aged mice will

exhibit decreased response amplitude and weaker neural synchrony (measured by phase-locking value; PLV) than younger mice.
Methods: To test our hypothesis, we collected auditory brainstem responses (ABRs) from young (2-4 months, n=19), middle-aged (12-15 months, n=20), and aged (+24 months, n=23) mice. ABRs were collected in silence and in broad-band background noise (-5 to +5 dB SNR) in response to 11.3 kHz tones. ABR wave amplitude, to measure aggregated neural activity, and PLV, to measure neural synchrony, were collected from wave I and V for the quiet and noise conditions. The number of synapses per inner hair cells for each age group were quantified to examine if an early age-related synapse loss predicted age-related functional changes.
Results: We compared the PLV, amplitude and latency of waves I and V to examine age-related functional changes in noise and quiet conditions. We found significant interactions between age, noise, and wave (I and V). We found that in quiet, AN PLV from middle-aged mice was significantly weaker than younger mice. In contrast, AN amplitudes were similar in middle-aged and younger mice. In the presence of background noise, AN responses from middle-aged mice become more similar to aged mice, with significant decreases in amplitude and weaker synchrony in middle-aged relative to younger mice. We found that PLV at the midbrain response (wave V) in middle-aged mice was similar to young mice in quiet and similar to old mice in noise. There were no significant differences in wave V amplitude between any age group. AN and midbrain response latencies in middle-aged mice were not significantly different from young mice in either condition.
Conclusions: These data show the importance of measures such as PLV as a sensitive tool for the early detection of age-related auditory functional decline and suggest that AN pathological alterations that affect neural synchrony may exhibit the earliest age-related deficits. Effects of noise demonstrate that using noise as a challenge to auditory functioning can reveal early age-related deficits. The possible contribution of synaptopathy to age-related synchrony deficits will be further analyzed and discussed.

M97. Biomarkers of Alzheimer's Disease (AD) Expression Levels Increase With Aging in the CBA/CaJ Mouse Auditory System

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Category: Aging
Background: The majority of our elderly population worldwide faces the challenges of age-related hearing loss (ARHL-presbycusis). ARHL includes changes in the ear and brain that can coexist with dementias, including Alzheimer's disease (AD), and has been identified by NIH as one of the most modifiable risk factors for AD. The primary elements of the initial pathology of AD involve deposition of amyloid protein plaques [major component with amyloid beta (Aβ) formed from amyloid precursor protein (APP)], which may be an associated factor also linked to brain pathologies of ARHL
Methods: Brain samples from CBA/CaJ mice in different age groups [N=3; 2, 14, 24, 28 and 36 months (mon)], 5XFDA transgenic mice (8 mon) and non-transgenic (NTG-control) mice. Cryosection immunohistochemistry staining with the APP antibody (6E10) and β amyloid antibody (MOAB-2), and imaging with confocal laser scanning microscopy were performed.

Young and middle-aged CBA/CaJ (2 and 14 mon), 5XFDA, NGT mice auditory cortex tissues were processed for western blot analysis of the Aβ and APP protein expression levels.
Results: Intracellular and extracellular APP and Aβ expression changes were detected in the auditory cortex in CBA/CaJ mice as a function of age. Intracellular APP had a significantly higher expression by 14 months of age in the CBAs compared to 2 month old CBAs and NGT referenced by 5XFDA; however, no differences were seen for intracellular Aβ for CBAs from 2 to 14 mon and for the NTG. Also, western blots showed that aggregated Aβ was not detected in the auditory cortex of NTG and young adult CBA mice. Additionally, intracellular Aβ appeared significantly in 24 mon CBAs; and extracellular Aβ expression appeared in 28 mon CBAs similar to the younger 5XFDA. These results indicate that aging may play some role in the amyloid protein accumulation in older CBAs -a pathologic mechanism somewhat independent of genetic AD mechanisms.
Conclusions: The primary initial elements of the pathology of AD involve amyloid protein plaque deposition, which may be an associated factor linked to brain pathology of ARHL. The upregulation of APP and Aβ in these non-AD mouse models supports the idea that common underlying brain area pathogenesis mechanisms may exist between the progression of ARHL and AD. These results suggest that older CBAs may exhibit some form of AD type pathology that would make this strain a valuable model to study late-stage AD.

M98. Genome-Wide Association Study for Age-Related Hearing Loss in CFW Mice

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Category: Aging
Background: The most common form of hearing loss is age-related hearing loss (ARHL), one of the most prevalent conditions affecting the elderly. As mouse and human inner ears are functionally and genetically homologous, we investigated the genetic basis of ARHL in an outbred mouse population. The goal of this study was to identify genetic loci involved in regulating ARHL and lead to a better understanding of the molecular mechanisms of this condition.
Methods: We used Carworth Farms White (CFW) outbred mice, due to this strain having variation in the onset and severity of ARHL and being an outbred population, which allows mapping complex traits to small genomic regions. Auditory Brainstem Response (ABR) was measured at 6 different frequencies between 4-32kHz in 946 male and female CFW mice at the ages of 1, 6, and 10 months. We performed genetic analysis for the ABR thresholds for each frequency at each age, and for the time of onset of deafness for each frequency.
Results: Genome-wide association analysis identified several regions associated with ARHL that contained potential candidate genes. We obtained genotypes at 4.18 million single nucleotide polymorphisms. This work is ongoing, our final target sample size is 2,000 CFW mice.
Conclusions: We performed GWAS for ARHL in CFW outbred mice and identified several loci, containing multiple candidate genes. This work helps to identify genetic risk factors for ARHL and to define novel therapeutic targets for the treatment and prevention of ARHL.

M99. Association Between Ethnicity/Race and Extended High Frequency Hearing: Implications for Understanding Early Signs of Auditory Aging

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Category: Aging

Background: Humans can perceive sounds up to 20 kHz. Research indicates that age-related hearing loss often begins at extended high frequencies (EHFs: GREATER THAN 8 kHz). EHFs are also believed to be crucial for speech perception in noisy environments. Despite its importance, there is limited research exploring how demographic factors such as ethnicity, race, and sex influence EHF hearing sensitivity. Understanding these relationships is essential to identify potential disparities in auditory health that may arise from genetic, environmental, or socioeconomic factors. Additionally, EHF hearing loss is a marker of preclinical auditory damage, suggesting that understanding these connections could help explain the relationship between ethnicity/race and individual hearing loss risk. This study aimed to examine the effects of ethnicity, race, and sex on EHF hearing thresholds to better understand demographic influences on hearing health.

Methods: Hearing thresholds at standard audiometric frequencies (0.25 to 8 kHz) and EHFs (10, 12.5, and 16 kHz) were obtained from 103 adult participants (ages 18-35). Race and ethnicity were determined by self-report from each participant in an intake questionnaire. Ethnicity was defined as Hispanic or Latino or Non-Hispanic or Latino, while Race was categorized based on U.S. Census Bureau classifications (e.g., White, Black or African American, Asian). Statistical analyses were used to evaluate the effects of test frequency, ethnicity or race, sex, and potential interactions among the variables.

Results: Preliminary analyses showed a statistically significant effect of race on EHF thresholds at two tested frequencies (10 and 16 kHz) but not at 12.5 kHz. There was also a statistically significant effect of ethnicity on EHF thresholds at 10 kHz. Preliminary analysis of sex suggests that males have higher EHF thresholds than females, regardless of race or ethnicity groups. Additional analyses will be performed to control the effect of sex and age on EHF thresholds and possible interactions among variables to better understand if certain races or ethnicities are more likely to exhibit EHF loss.

Conclusions: Initial results suggest that race and ethnicity may add to individual risk profile for EHF loss, and subsequently, clinical auditory damage. Further, consistent with previous work, there appears to be an established effect of sex on EHF loss. The differences across different demographic groups underscores the importance of individual risk evaluation and consideration in auditory research and clinical evaluations. Future studies will include a more diverse and representative sample across all racial and ethnic groups to provide a more comprehensive understanding of EHF hearing loss and its underlying determinants.

M100. The Association of Diabetes With the Rate of Hearing Decline in Aging

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Category: Aging

Background: Previous cross-sectional epidemiological studies have reported that diabetes is associated with hearing loss, but there is less, and inconclusive, evidence from longitudinal studies regarding associations of diabetes with the incidence or progression of hearing loss. The purpose of this study, conducted in a community-based cohort study of individuals from the general population, was to determine the association of diabetes with the rate of pure-tone threshold change per year.

Methods: Participants were adults from the Medical University of South Carolina Longitudinal Cohort Study of Age-related Hearing Loss, an ongoing (1988-current) community-based cohort study based in Charleston, SC. Diabetes was self-reported (yes/no) at participants' baseline examination. Outcome measures were audiometric thresholds (0.25-8.0 kHz), which are measured annually, and pure-tone average (PTA) of thresholds at 0.5, 1.0, 2.0 and 4.0 kHz, averaged bilaterally. Linear mixed regression models were used to estimate the effect of age (for every 1-year increase) on the rate of threshold and PTA change for participants with and without self-reported diabetes at the baseline examination. The effect of diabetes on the rate of annual change was determined by an interaction term of age and diabetes. Results from preliminary unadjusted models are described.

Results: This study included 1371 participants, with a mean baseline age of 63.7 (SD 14.3) years and a mean PTA of 24.5 (SD 15.1) dB HL; 58.0% were female and 18.4% were racial Minority (17.6% of the sample were Black/African American). The prevalence of self-reported diabetes was 11.2%. Participants with and without self-reported diabetes had similar age-adjusted baseline hearing thresholds at most frequencies, although participants who reported diabetes had higher (poorer) thresholds at 0.25 and 0.5 kHz. In unadjusted linear mixed effects regression models, self-reported diabetes (versus none) was associated with a higher rate of hearing change (decline) at 0.25 kHz, but not other frequencies or PTA.

Conclusions: Results from unadjusted regression models suggest that self-reported diabetes was not associated with the rate of hearing change per year in a community-based sample of the general population. Planned analyses will adjust for confounding variables, such as demographic and health related factors. More research from longitudinal studies is needed to determine the impacts of diabetes on changes to hearing over time, and to determine the reasons behind the reported associations between diabetes and the prevalence of hearing loss.

M101. Healthy Aging Increases the Neural Reliance on Higher-Level Processing in Competing Speech Comprehension

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Category: Aging

Background: In everyday life, spoken speech streams are often masked by noise or competing speech streams in the surrounding, creating challenging listening situations, particularly for older adults. To navigate these situations, listeners reconstruct the continuous speech signal from incomplete sensory input using higher-level processing. In an EEG study, we investigated how acoustic and higher-level linguistic processing contribute to competing speech comprehension across the adult life span. Previous results on the link between the behavioral and the neural reliance on acoustic and higher-level information in language comprehension across the adult life span have been inconsistent. Our pre-registered hypothesis was that older adults compensate for declines in lower-level sensory processing by relying more on higher-level information on the behavioral and the neural levels to maintain speech comprehension.
Methods: 63 normally hearing participants (age range = 18 – 70 years) heard 240 trials of two sentences presented simultaneously and were instructed to follow one speaker while ignoring the other one. They subsequently repeated the target sentence. The individual task difficulty was adjusted using an adaptive staircase procedure to account for peripheral hearing differences. Additionally, they listened to 20 trials, in which target sentences were replaced by word lists composed of three words with low semantic similarity. The hypothesis was assessed using a generalized linear mixed effects model predicting word comprehension performance from the interactions of age with acoustic and linguistic word-level variables. Additionally, we assessed the extent of neural representation of acoustic and linguistic features of the target and distractor sentences using the trial-wise, cross-validated fits of multivariate temporal response functions.
Results: Consistent with our hypothesis, comprehension differences between word lists and target sentences were positively predicted by age, indicating that older adults benefited more from the context provided in the target sentences (p LESS THAN .001). However, the influence of word surprisal on sentence comprehension was not influenced by age ($p = .31$). Neural results revealed an increased neural representation of linguistic word-level features over centro-parietal sensors with increasing age ($p = .003$), as well as an increased influence of the neural representation of these features on comprehension performance in older adults ($p = .014$). This indicates that older adults' comprehension performance was more strongly influenced by the neural representation of higher-level target information.
Conclusions: The results provide a link between a stronger behavioral and neural reliance on higher-level processing supporting speech comprehension with increasing age, which may provide an important compensatory strategy in well-functioning older adults.

M102. Effects of Presbycusis on the Production of Voice and Enjoyment of Amateur Choral Singing

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Category: Aging
Background: Many people in the age range pertinent to age-related hearing loss (presbycusis) participate in choral singing and it has been documented that participation in choral singing is beneficial for the aging auditory system (Dubinsky et al. 2019). But it has not been documented how the aging auditory system affects the enjoyment of and participation in singing for people who have been singing for a majority of their lives.

This paper explores the effects of presbycusis on the production of voice and the enjoyment of amateur choral singing. Focusing on choral societies and chamber choirs in the Oxfordshire area to control for the qualities of genre that would differentiate the singing experience, this project crosses audiometric examination and acoustic voice assessment with current musicological theories to provide a multifaceted view of the interaction between auditory perception and vocal production, and own-voice perception as a marker of identity.

Methods: The study simulated presbycusis (high frequency attenuation on the order of ±25 dB SPL) to measure the immediate acoustic effects of change in auditory context (pitch, resonance and dynamics). The production and control of resonance above 4kHz is an important aspect of vocal timbre for this style of singing. Thus, singers (8, 3M, mean age 24) underwent audiometric examination, and then sang through vocal tasks under three conditions (solo singing, singing with an ensemble backing track, and singing with attenuation of frequencies GREATER THAN 4kHz) to assess whether the perception of high frequencies affects the production of pitch in that frequency range for choral singing. Qualitative data was also collected, using rating scales and open-ended questions to assess own-voice satisfaction as a correlate with socialization and participation.
Results: The experimental data found that the production of frequencies above 4kHz was significantly lowered for ensemble singing conditions (One way ANOVA, $p = 0.027$), and ratings of satisfaction did not decrease with the attenuation of high frequencies. The immediate change in auditory context affected vocal output when singing with others, but a reduction in high frequencies had no significant effect, thus suggesting that singing participation would be unaffected with simulated presbycusis.
Conclusions: Further research explores this finding with a population of 200 participants over the age of 50, looking at the relationship between singing experience, satisfaction, and own-voice perception, as both an immediate (laboratory induced) auditory change, and the socio-emotional long-term effects (questionnaire evaluated). This research studies the individual experience of presbycusis and its effects on singing participation to expand the knowledge base about presbycusis as a major modifiable risk factor for cognitive impairment.

M103. Role of Prenatal Stress in Accelerating Age-Related Hearing Loss

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Category: Aging
Background: Age-related hearing loss (ARHL) is a progressive, bilateral, symmetrical hearing loss, which is associated with significant psychological and medical morbidity. Preventive medicine for the disease is essential since there are no approved cures. Preventive medicines for other conditions have been established based on studies of the association between epigenetic changes caused by prenatal stress and the development of diseases in adult offspring. Environmental stresses during the prenatal period, endured during pandemics, genocide, famines, and natural disasters are linked in large population studies to diseases in adults who were

exposed to prenatal stress. Recently, studies of prenatal stress related to the COVID-19 pandemic warn that the psychological stress experienced during the pandemic could impact the disease risk of adult offspring later in life. An association between prenatal stress and hearing loss has been reported in young adult animals, however, it is not known whether prenatal stress impacts susceptibility to ARHL. We hypothesize that prenatal stress leads to development and premature progression of ARHL. To test this hypothesis, we used our established restriction prenatal stress protocol and performed audiological and histopathological analyses in a mouse strain, C57BL/6, known to develop progressive hearing loss in mid-adulthood.
Methods: Pregnant female C57BL/6 mice were assigned to the prenatal stress and control group. The prenatal stress group were given restraint stress in a pill tube for 2 h for 5 days during their pregnancy. One male and one female offspring were assigned for testing from each litter. To determine the audiological impact of prenatal stress in the offspring, auditory brainstem responses (ABRs) were repeatedly recorded from 1 to 6 months old to clicks and tone bursts (4, 8, 12, 16, 24, and 32kHz, 10-90 dB SPL) until 6 months old. To evaluate the outer and inner hair cell loss in the offspring at the age of 1 and 6 months, phalloidin labeled outer and inner hair cells were manually counted in cochlear whole mounts.
Results: The ABR thresholds were not significantly different at the age of 1 month. However, at 5 and 6 months of age, ABR thresholds at 12, 16, and 24kHz were significantly elevated in the prenatal stress group compared to controls. Similarly, the incidence of hair cell loss was not significantly different at the age of 1 month, however, at 6 months of ageouter hair cell loss in 8, 12, 16, 24, and 32kHz was significantly higher in the prenatal stress group than that in the control.
Conclusions: This study provides evidence that ARHL is accelerated by prenatal stress in a mouse model. Outer hair cell loss may be one of the mechanisms underlying the auditory impact of prenatal steress on ARHL.

M104. Gap Detection Ability Declines With Central Auditory Neurodegeneration Following Age-Related Cochlear Synaptopathy

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Category: Aging
Background: Age-related hearing impairment (ARHI) is commonly associated with decreased auditory temporal resolution caused by auditory neurodegeneration. Age-related deterioration in gap detection ability, resulting in poor temporal auditory processing, is often attributed to pathophysiological changes in both the peripheral and central auditory systems. This study aimed to investigate whether the gap detection ability declines in the early stages of aging and to determine its usefulness in detecting peripheral and central auditory degeneration.
Methods: 1-month-old (1M), 6-month-old (6M), and 12-month-old (12M) CBA/J mice were used. Auditory brainstem response (ABR) and distortion product otoacoustic emission were measured as auditory function. Then, the acoustic startle refrex (ASR), prepulse inhibition of ASR, and gap prepulse inhibition of ASR were investigated to evaluate auditory processing performance and tinnitus perception. Finally, cochleae were examined to confirm cochlear pathology including the loss of hair cells, spiral ganglion neurons, and their synapses, and

transverse sections of the brainstem and brain, through the cochlear nucleus to the auditory cortex, were examined for the expression of synaptic markers.
Results: Although hearing thresholds did not significantly differ between the groups, the amplitude of ABR wave I decreased significantly in an age-dependent manner, consistent with age-related cochlear synaptopathy. The relative ABR amplitude ratio of waves 2 and 5 to wave 1 was significantly increased in 12M mice, indicating that the central auditory system had increased in relative neuroactivity. A significant increase in gap detection thresholds was observed in 12M mice compared to 1M mice. Although cochlear synaptopathy and central hyperactivity were positively correlated with gap detection thresholds, central hyperactivity strongly influenced gap detection ability. In the cochlear nucleus and auditory cortex, the inhibitory synaptic expression of GAD65 and the expression of parvalbumin were significantly decreased in 12M mice, consistent with central hyperactivity.
Conclusions: In middle-aged mice with no increase in the hearing threshold, we observed a decrease in gap detection ability and cochlear and central synaptic dysfunction, which are considered the initial pathology and symptoms of ARHI. As there is no simple clinical test to identify cochlear and central synaptic dysfunctions that cause poor speech discrimination ability, this study suggests that the evaluation of the gap detection ability may be promising in identifying the onset of ARHI.

M105. Behavioral Validation of Salicylate-Induced Hyperacusis in CBA/CaJ Mice Using an Active Avoidance Paradigm

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Category: Tinnitus
Background: Hyperacusis, a condition marked by heightened sensitivity to everyday sounds, affects a substantial portion of the population and can severely impair individuals' ability to function in noisy environments. Despite its clinical significance, treatment options for hyperacusis remain limited, and there is a critical need for effective methods to evaluate and understand its mechanisms. Mouse models are invaluable for gaining insights into the pathophysiology of hyperacusis and exploring potential therapeutic strategies due to their anatomical and physiological similarities in auditory processing to humans.
Methods: This study examines the utility of the active avoidance (AA) task as a behavioral tool for assessing hyperacusis in CBA/CaJ mice. The AA task relies on operant conditioning, where mice learn to avoid an aversive stimulus by moving to a different compartment in response to an auditory cue. Failure to move within 5 seconds resulted in a mild foot shock. We hypothesized that sodium salicylate (SS), a compound known to induce hyperacusis-like symptoms in mice, would enhance task performance due to their increased responsiveness to sound. All mice met study inclusion criteria by achieving ≥75% accuracy across 32 randomly presented frequencies (8-36 kHz) at 70 dB SPL. After initial baseline testing, mice were injected with SS and tested for 3 sessions at both 50 dB and 70 dB SPL. After a washout period, they were retested under the same conditions and with saline as a control. The acoustic startle reflex (ASR) input/output function, considered the gold standard for hyperacusis assessment in rodents, was also measured to validate the behavioral outcomes of SS administration.

Results: Of the 14 mice tested, 9 exhibited an increase in conditioned response rate and a decrease in response latency across all frequencies compared to baseline. At 50 and 70 dB SPL, hyperacusis-positive mice demonstrated an average conditioned response rate of 95% and 96%, with an average escape latency of 2.14 seconds and 2.00 seconds, respectively. Baseline response averaged 90% with an escape latency of 2.66 seconds. At 50 dB SPL, escape latency effects were more pronounced at higher frequencies, while at 70 dB SPL, the effect was greater at lower frequencies (≤ 16 kHz). We also observed a strong negative correlation between conditioned response rate and escape latency for hyperacusis-positive and non-responding mice.
Conclusions: Our findings demonstrate that this novel AA task is a reliable and effective tool for measuring hyperacusis-like symptoms in rodent models. The majority of mice administered with SS exhibited hyperacusis, reflected in their improved task performance and faster escape latency to auditory stimuli. This study validates the use of operant conditioning paradigms in hyperacusis research and supports utilization of the AA task in the development of future treatments for sound sensitivity disorders.

M106. Combining Psychoeducation, Sound Exposure, and Counseling: A New Therapeutic Approach for Hyperacusis

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Category: Tinnitus
Background: Hyperacusis is a heightened sensitivity to normal environmental sounds, leading to significant distress and impairment in daily life. Current treatments for hyperacusis vary in effectiveness, and new therapeutic approaches are needed to address both the auditory and psychological aspects of the condition. This study aimed to evaluate the short- and long-term efficacy of a novel Cognitive Sound Exposure Therapy (CSET) in reducing hyperacusis symptoms. CSET incorporates sound exposure techniques and psycho-education, combined with relaxation and cognitive strategies drawn from both Acceptance and Commitment Therapy (ACT) and Cognitive Behavioral Therapy (CBT). The main goal was to determine whether CSET could help increase patients' tolerance to sounds and decrease their hinderance of hyperacusis over time.
Methods: This pre-post study involved patients aged 18 years or older who were diagnosed with hyperacusis and had either no or mild hearing loss. Participants were recruited from Speech and Hearing Centers in Hengelo and Zwolle, the Netherlands. The therapy, delivered by an experienced clinical audiologist and a social worker, involved bi-weekly sessions aimed at gradually increasing patients' tolerance to sound exposure, with a target exposure level of 70 to 80 dB Sound Pressure Level (SPL). Each session also incorporated breathing and relaxation techniques. The short-term effects were measured at the start and end of therapy using the Hyperacusis Questionnaire (HQ), the tolerable sound exposure level, and the subjective hindrance caused by hyperacusis. The long-term effects were assessed using the HQ six months after therapy completion. Statistical analyses, including linear mixed effects models and regression analyses, were used to examine the change in outcomes over time.

Results: A total of 30 patients (15 males, 15 females), aged 24 to 76 years, participated in the study. The number of sessions per patient ranged from four to eight, with a mean of six sessions. Patients had experienced hyperacusis for periods ranging from six months to 20 years at the start of therapy. Short-term outcomes indicated a significant improvement in patients' sound tolerance, with an average increase of 23.7 dB SPL (SD = 7.9, p LESS THAN 0.001). Sensitivity to sound also decreased, as shown by a mean change in HQ score of -9.8 (SD = 4.9, p LESS THAN 0.001). Six months after therapy, there was no significant change in HQ scores (mean change = 0.2, SD = 4.3, p=0.81), suggesting a sustained long-term benefit.
Conclusions: CSET significantly improved both short- and long-term auditory tolerance in patients with hyperacusis. The therapy led to a meaningful reduction in hyperacusis symptoms and had a positive impact on patients' daily lives by increasing their tolerance to environmental sounds. These results suggest that CSET is a promising treatment for hyperacusis and highlight the need for further research into its broader applicability, including its potential use in patients with hearing loss.

M107. Auditory Processing Deficits Following Exposure to Open-Field Blasts in a Non-Human Animal Model

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Category: Hearing Loss: Consequences and Adaptation
Background: Over 5.3 million individuals in the U.S. have been diagnosed with traumatic brain injury (TBI) and are living with related disabilities making TBI a major cause of long-term disability (CDC, 2019; Dams-O'Connor et al., 2020). Civilian TBI cases are primarily due to falls, motor vehicle accidents and sports related injuries (Stopa et al., 2021; Theadom et al., 2020). However, CDC estimates do not include military service members, and as of 2024 Q1, 505,896 military personnel have been diagnosed with TBI (DoD, 2024), resulting primarily from blast-induced trauma associated with increased use of improvised explosive devices, but also including exposure to more traditional explosives and high-power weaponry.
In this investigation, efforts to determine the effects of open field blast exposure on auditory processing were undertaken to generate a model of explosion-induced central nervous system (CNS) trauma in a non-human species, the CBA/CaJ mouse. An array of noninvasive neurophysiological tests was employed to probe various levels of the system and animals were studied longitudinally to assess acute and chronic effects of blast exposure on auditory processing.

Methods: Anesthetized mice were exposed to the explosive force of 350 grams of the plastic explosive Composition C-4 at a distance of 3 m in an open field setting. The incident peak pressure associated with the explosions was 6.94±0.14 psi (~47.8 kPa) and reflected peak pressure was ~19.15 psi at the position of the animal's head. Head movement was not observed during the blast based on high-speed video analysis.
A well-defined suite of electrophysiological tests designed to assess function at specific regions of the central auditory pathway, as well as the auditory periphery, served as the operational platform of the study. Tests were based on auditory evoked responses, including the auditory

brainstem response (ABR), as well as later occurring responses, to assess the functional integrity of brainstem, thalamocortical and cortical pathways. Envelope following responses were also acquired to evaluate temporal processing capabilities. This presentation will center on brainstem responses representing the functionality of peripheral and central elements of the auditory pathway. The effects of stimulus repetition rate and the effects of background noise will also be considered.

Results: During the acute and sub-acute post-blast time period, biomarker attributes reflecting CNS and peripheral function were consistent with partial recovery from trauma; initially elevated response thresholds decreased, and reduced peak amplitudes and phase-locking indices increased. However, during the chronic phase of the investigation, recovery stalled and performance progressively declined.
Conclusions: Based on auditory electrophysiological responses, early findings suggest that the progression of blast-induced CNS outcomes in mice is similar to that observed in human patients diagnosed with TBI, and, consequently, this model will serve as a platform for extended investigation, including prospective treatment strategies.

M108. Mitochondrial Dysfunction and Metabolic Maladaptation in the Stria Vascularis of Alport Syndrome Mice: Insights From Noise-Induced Metabolic Stress Testing

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Category: Hearing Loss: Consequences and Adaptation
Background: This study aims to reveal how Alport Syndrome (COL4a3 knockout, KO) mice exhibit maladaptive metabolic responses to noise-induced stress compared to their wild-type (WT) counterparts. We investigated mitochondrial dysfunction and metabolic reprogramming in the stria vascularis (SV) under quiet (Q) and noise conditions, highlighting the relevance of these findings to cochlear dysfunction involving noise induced metabolic stress.
Methods: 129Sv WT and KO mice, 9 wks of age (n=8/condition) were exposed to either quiet or noise-induced stress (10kHz OBN, 106 dB SPL, 10H). Data were collected from quiet-reared mice and at two time points post-noise: Immediately (D0) and five days (D5) post-exposure. SV tissues were isolated, flash frozen and stored (-80oC) until assay. Glucose, lactate, pyruvate (cellular energy status), and glutamate with α-ketoglutarate (Krebs Cycle anaplerotic flux) were measured. ELISA assays quantified phosphorylated vs. total AKT, AMPK, and mTOR, as well as HIF1a, VEGF, Cytochrome C, GLUT1, and inflammatory markers (p53, p21, TNFα). Adenylates, AMPK (TRFRET) and Na+-K+-ATPase activities were also assessed.
Results: Compared to WTQ, KOQ mice exhibited significant metabolic dysregulation, with heightened AMPK activity, increased GLUT1 expression, and elevated lactate levels, indicating a shift toward glycolysis, possibly due to impaired mitochondrial oxidative phosphorylation. KOQ mice showed disrupted AKT/AMPK/mTOR signaling, highlighting an elevated energy stress response absent in WTQ mice, which displayed a balanced metabolic profiles. KO-D0 mice demonstrated immediate metabolic disruption, with maladaptive increases in ATP/ADP ratios, increased glucose levels and decreased Na+-K+-ATPase activity, indicating mitochondrial

dysfunction with impaired ion homeostasis. By D5, KO mice showed very limited recovery in energy metabolism, evidenced by normalizing ATP/ADP ratios probably secondary to continuing Na+-K+-ATPase activity suppression. This limited ability of KO mice to recover from noise-induced stress aligns with persistent mitochondrial dysfunction and a preference for an alternative energy substrates. In contrast, WT-D5 mice demonstrated an efficient full metabolic recovery.
Conclusions: KO mice exhibit maladaptive metabolic shifts under both quiet and noise conditions, characterized by increased reliance on glycolysis and impaired mitochondrial function compared to WTQ mice. The progression from KO-D0 to KO-D5 highlights persistent mitochondrial dysfunction and reduced metabolic flexibility, indicating that KO mice struggle to recover from noise-induced stress. These findings underscore the importance of developing therapies that target mitochondrial resilience and metabolic flexibility to protect cochlear function in Alport Syndrome. Future research will extend beyond the SV to include the organ of Corti, to provide a more comprehensive understanding of cochlear metabolic dynamics during noise-induced metabolic stress. Targeting mitochondrial resilience is crucial for preventing progressive cochlear dysfunction in Alport Syndrome.

M109. Cortical Responses in the Primary and Higher-Order Auditory Cortex of Cochlear Implant-Stimulated Unilaterally Deaf Cats

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Category: Hearing Loss: Consequences and Adaptation
Background: Congenital unilateral deafness refers to profound hearing loss affecting one ear only. The asymmetric auditory input leads to plastic reorganization of the auditory system. Congenitally deaf cats are an animal model of bilateral (congenitally deaf cats, CDC) or single-sided deafness (SSD). In this study, we have assessed the reorganization of cortical activity by comparing local field potential (LFP) and multiunit activity in the primary and the higher-order auditory cortex using cochlear implant (CI) stimulation.
Methods: Recordings were performed on four SSD animals, six bilaterally hearing cats (HCs), and five CDCs. Stimulation was with three biphasic charge-balanced electric pulses (200 µs/phase) applied through bilateral cochlear implants. Neuronal activities from the primary auditory cortex (A1) and the posterior auditory field (PAF) were recorded simultaneously with Neuronexus 16-channel multielectrode arrays. The intergroup analyses compared amplitude and latency of cortical responses of the hearing and deaf ear in SSDs (CI stimulation) with hearing controls (HCs), and bilateral CDCs.
Results: As a rule, LFP amplitudes and unit firing rates were in most comparisons significantly higher and response latencies shorter in A1 compared to PAF in all animal groups, and never the opposite, confirming recordings in primary and secondary auditory cortex. The cortical responses expressed a hemispheric preference for contralateral stimulation in HCs, which was

reduced in CDCs. In SSDs, the cortical responses favored the previously hearing ear. When comparing the effects of SSD in A1 to PAF, we observed a near-absence of responses to the deaf ear in PAF.
Conclusions: This suggests that while the effect of SSD is observed already in the field A1 (Kral et al., 2013, Brain), the effect size is progressively amplified along the cortical hierarchy. This leads to perceptual weakening of the representation of the previously deaf ear, significantly contributing to the aural preference syndrome. This reduces the clinical options for implanting the deaf ear later in life.

M110. Differential Sensitivity of Speech-In-Noise Instruments to Central Auditory Deficits

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Category: Hearing Loss: Consequences and Adaptation
Background: Many blast-exposed Veterans report listening difficulties despite normal or near-normal audiometric hearing. Prior work suggests these blast-exposed Veterans have deficits in speech recognition in noise (SIN), auditory temporal processing, and working memory (WM). Similar deficits have been observed in aging listeners, suggesting an interaction between aging and blast exposure. The goal of the present study was to examine the effects of blast exposure and aging on SIN performance across a battery of tests. The SIN battery included tests that might nominally be described as "auditory dominant" (word recognition in babble, sentence recognition in speech-shaped noise) or "cognition dominant" (sentence recognition in babble, matrix style competing speech). We hypothesized that blast exposure and age would primarily affect performance on "cognition dominant" tests that stress interactions between auditory and cognitive processing mechanisms.
Methods: Eighty-one Veterans (aged 25-60) with variable degrees of blast exposure and other mild head trauma were recruited. All had pure-tone average thresholds (0.5, 1, 2, 4 kHz) of 35 dB HL or better. The Blast and Blunt Trauma Index (BBTI), which a continuous measure of lifetime blast exposure and mild head trauma, was determined from responses on the Quantification of Cumulative Blast Exposure. Participants completed a battery of six SIN tests: the Words in Noise (WIN) test, the Quick Speech in Noise (QuickSIN) test, and four conditions of the Theo-Victor-Michael (TVM) matrix-style speech test. For the TVM, the four conditions varied by type of background interference (all at 0 dB target-to-masker ratio): (1) steady state speech-shaped noise; (2) speech-shaped noise modulated by the envelope of two-talker speech; (3) one competing talker; and (4) two competing talkers. Finally, participants completed a battery of WM tests including the digit span (forward, backward, sequencing) and the NIH Toolbox List Sorting test.
Results: Bayesian multivariate regression was performed with the six SIN test scores as the multivariate outcome, BBTI, age, and composite WM score as predictors of interest, and audiometric thresholds, PTSD symptom severity, and premorbid verbal intelligence as control covariates. Contrary to our hypothesis, performance on "cognition dominant" SIN tests (QuickSIN, TVM 3 and 4) was primarily driven by WM scores, whereas BBTI and age were

strongly associated with performance on "auditory dominant" SIN tests (WIN, TVM 1 and 2) and were less strongly associated with WM.
Conclusions: One possible explanation of the results is that blast exposure and age impact SIN performance by decreasing the fidelity of ascending auditory signals rather than disrupting top-down (i.e., cognitive) mediation of auditory processing. Another explanation is that "cognition dominant" tests compress inter-listener variance that might otherwise be explained by aging and blast exposure. In any event, appropriate selection of SIN tests is necessary for detection of (often subtle) impacts of blast exposure and/or early aging.

M111. Evaluation of Model- and Neural-Network-Based Augmented Hearing Algorithms

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Category: Hearing Loss: Consequences and Adaptation
Background: A general difficulty in compensating for specific aspects of sensorineural hearing loss (SNHL) relates to the nonlinearities of hair-cell and auditory-nerve-fiber properties and how these are altered to impair our speech processing. Standard hearing-aid signal processing mostly focuses on compensating the loss of hearing sensitivity and compression, while omitting aspects related to cochlear synaptopathy (CS) because there are neither diagnostics available, nor a clear view on how to improve the signal-processing for CS. However, the pursuit for sound-processing algorithms that compensate for all aspects of SNHL is important as CS is expected to affect a large part of the population, and novel diagnostic methods for CS quantification in humans are becoming available.
Methods: This study uses a model- and machine-learning-based approach to design individual hearing-impaired (HI) auditory models that are personalized based on the audiogram and an envelope-following-response (EFR) marker of CS. Afterwards, the HI model is placed in a closed-loop network with a normal-hearing (NH) model as the reference and uses the difference signal between NH and HI speech processing as a loss function to train a DNN-based signal-processing algorithm. The resulting audio processing algorithm runs end-to-end with latencies less than 10ms, and are based on the best mathematical compensation, rather than on constraining the signal processing up front (e.g., band-filtering, gain rules, wideband compression, loudness limits etc.).
Results: We recruited 60 patients in this study (NCT06114680 – clinicaltrials.gov), who had more than 30% reduced 4-kHz 110-Hz RAM EFR markers compared to a young normal-hearing control group, indicative of CS. Among these listeners, 30 had normal audiograms and 30 impaired audiograms. We trained algorithms that compensated for individual outer-hair-cell (OHC) loss, CS and combined losses and first evaluated the transfer functions of these algorithms, to better understand which speech features the processing focused on. Afterwards, we conducted a Matrix sentence test in stationary noise to evaluate whether the SRT in noise improved after processing.

Conclusions: We report SRT data from 16 subjects and six compensation algorithms to conclude that neural-network-based augmented hearing algorithms are a promising tool to develop end-to-end algorithms that can compensate for the OHC and CS aspect of SNHL.

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M112. Moderate Noise Exposure Influences the Cochlear Nucleus Function in a Frequency-Related Pattern

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Category: Hearing Loss: Consequences and Adaptation
Background: Different from exposure to loud noise that causes observable impairment of the inner ear and elevates the hearing threshold, long exposure to moderate noise (MN) rarely changes the hearing threshold significantly. Recent studies indicate that MN exposure can result in the impairment of the inner ear such as ribbon synapses and the functional reorganization of the central auditory system such as input-specific plasticity in the auditory cortex. To date, little is known about the functional alteration in the cochlear nucleus, a gate from the periphery to the central auditory system. This study investigated the immediate changes in neuronal receptive fields in the mouse cochlear nucleus following long exposure to pure tone.
Methods: Under anesthesia (ketamine and xylazine), 41 C57 mice, aged 4-7 weeks old, were unilaterally exposed to 1 hour of continuous pure tone (characteristic frequency, CF of recorded cochlear nucleus neurons) at 65 dB SPL. Before and after exposure, unit recordings were applied to assess the changes in neuronal auditory response in the cochlear nucleus. A paired t-test was used to compare the difference between the cochlear nucleus receptive field before and after the exposure.
Results: After the noise exposure, significant threshold increases were found in response to the frequencies from the exposure frequency to 0.8 octaves above (p LESS THAN 0.01). As a result, 23 out of 41 neurons show CF shifted towards frequencies lower than CF. At the exposed frequency, the average elevation of the minimum threshold was 4.15 ± 3.11 dB. Similarly to the changes in threshold, the decreases in spike number in response to frequencies higher than CF were also observed (p LESS THAN 0.05). However, the frequency tuning width was only narrowed at 10 dB above the minimum threshold, but not at higher levels. The dynamic range measured at CF illustrated that the decrease in spike number was significant at the intensity level below 26.73 ± 12.82 dB SPL.
Conclusions: Our results suggest the impact of 1-hour exposure to pure tone with 65 dB SPL on the neuronal function of the cochlear nucleus is significant and highly specific to the exposed tone. The increase in response threshold and the decrease in auditory response magnitude might be implicated by the functional impairment in the inner ear.

M113. Clinical Assessments of Functional Auditory Performance Better Expose the Impact of Hearing Loss on Operational Performance Than Audiometry Alone

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Category: Hearing Loss: Consequences and Adaptation

Background: Hearing loss can impair the performance of Army aviators, compromising safety, reducing situational awareness, and increasing mental workload and listening effort. However, current hearing standards in Army aviation are primarily based on pure tone testing and speech recognition in quiet, which do not necessarily predict the functional impact of hearing loss. In 2019 the Department of the Army Pamphlet (DA-PAM) 40-502 and Army Regulation (AR) 40-502, which governs all Service Members in the Army, were updated to include a functional assessment of auditory performance known as the clinically adapted Modified Rhyme Test (MRT80). The current study examined the impact various degrees of simulated hearing loss have on MRT80 scores, simulated flight performance and cognitive workload in aviators.

Methods: Twenty-one Army aviators underwent clinical audiological testing, including the MRT80 and flight performance assessments. Independent variables of simulated hearing loss and task workload were manipulated to investigate their effects on speech recognition, flight performance, and cognitive workload. Laboratory testing was conducted in a sound-treated audiometric booth using a tablet-based system and aviation communication earplugs. Audiometric performance measures included percent correct on monaural word recognition scores at 80 dB HL, binaural word recognition scores at a preferred listening level and the MRT80. Simulated flight performance data were collected in a full-motion UH-60 Black Hawk flight simulator at the U.S. Army Aeromedical Research Laboratory. Flight simulator routes included a normal hearing and simulated hearing loss condition for both high and low auditory workloads. For subjective workload assessments, National Aeronautics and Space Administration Task Load Index (NASA-TLX) were administered and compared across conditions.

Results: Speech recognition scores declined with increasing levels of hearing loss compared to the normal hearing condition. Degradation in speech intelligibility caused by the hearing loss simulator was seen in the flight simulator as well indicating that the larger the hearing deficit, the more missed or incorrect calls subjects had on average. High workload conditions resulted in significantly degraded flight performance (p LESS THAN 0.001), exacerbated by simulated hearing loss ($p = 0.006$). NASA-TLX scores validated increased workload with simulated hearing loss raising perceived effort, frustration, and temporal demands. No significant findings were observed for the hearing assessment.

Conclusions: The Army has recently adopted a new Military Operational Hearing Test (MOHT) to assess the functional impact of hearing loss. The current study examined various degrees of hearing loss, how it interferes with communication signal quality and its impact on flight performance. Increased workload has been shown to decrease flight performance. Findings suggest hearing loss also negatively impacts speech recognition and flight performance,

especially under high workloads. Further research is needed to determine if the clinically adapted MRT can reflect the impact of hearing loss on operational performance for other jobs in the military.

M114. Amygdalar Hyperactivity and Non-Discriminate Auditory Threat Evaluation After Noise-Induced Sensorineural Hearing Loss

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Category: Hearing Loss: Consequences and Adaptation

Background: Noise-induced sensorineural hearing loss (SNHL) triggers compensatory plasticity in the central auditory pathway that often overshoots the mark, culminating in neural hyperactivity, hyperacusis and tinnitus. The neural and perceptual sequelae of SNHL are typically studied through the lens of disordered sensory processing, yet the clinical presentation of hyperacusis and tinnitus often features an affective dimension, whereby patients report that sounds elicit aversion, anxiety, or discomfort. We propose that the affective sequelae of SNHL can be modeled in mice and understood through a simple extension of the Excess Central Gain model, wherein hyperactive projection neurons in the auditory cortex induce hyperactivity and dysregulated sensory processing among their postsynaptic targets in limbic brain areas. Here, we investigated changes in sound processing before and after SNHL in the lateral amygdala (LA), a prominent sensory gateway to the limbic system.

Methods: We expressed GCaMP in LA excitatory neurons and performed daily monitoring of sound-evoked calcium activity over a several-week period using fiber photometry in awake, passively listening mice. After a baseline period, mice either underwent exposure to an innocuous noise (sham) or acoustic trauma that induced a restricted high-frequency SNHL. We monitored LA bulk calcium activity before and after noise exposure and throughout a subsequent 7-day Pavlovian discriminative auditory threat conditioning protocol.

Results: LA neurons were initially responsive to a wide range of novel, neutral sounds but exhibited strong neural habituation over the ensuing days before and after sham exposure. In mice experiencing acoustic trauma, LA responses to spared, low-frequency inputs never habituated and, in fact, grew to exceed levels observed at baseline. Mice in the sham and acoustic trauma groups exhibited a marked increase in sound-evoked freezing in the recall session compared to baseline. In mice that had previously undergone a sham noise exposure, freezing was selective to the sound associated with foot shock (CS+) and was progressively reduced over subsequent extinction sessions. Mice with SNHL exhibited non-selective freezing to CS+, and control sounds that did not extinguish over time. Neural recordings paralleled behavioral measurements in both groups.

Conclusions: Our study reveals that cochlear sensorineural damage profoundly affects amygdala sound processing and discriminative threat memory recall and extinction. These results offer new insights into the affective dimensions of hearing loss and auditory hypersensitivity, highlighting the need for a more comprehensive approach when modeling the neural basis of hearing disorders triggered by cochlear SNHL. Ongoing experiments focus on the role of auditory cortex hyperactivity in LA plasticity, exploring potential interventions to reinstate normal LA sound processing and auditory threat evaluation. Understanding the consequences of excess central gain

beyond the central auditory pathway will be crucial for developing targeted therapies for hyperacusis and tinnitus.

M115. Development of Human Inner Ear Organoid Platforms for Human Auditory/Vestibular Disorders

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Category: Hearing Loss: Consequences and Adaptation
Background: Human induced pluripotent stem cell (hiPSC) has been used in studying human diseases and showed its potential to serve as a model system. Unlike animal models and other transformed immortal cell lines, hiPSCs practically have unlimited supply, are no ethical concerns, and, the most importantly, maintain human genetic architectures. Moreover, hiPSCs carrying various genetic variants can be easily introduced using CRISPR-based editing technology or reprogrammed from patient samples, e.g., fibroblasts or Peripheral blood mononuclear cells (PBMCs). As a part of effort to advance inner ear research, we aim to establish a hiPSC bank for hearing loss, or more general, inner ear research. Then we will utilize these hiPSC lines to develop a platform by generation of human inner ear organoids from hiPSCs as a model system to test inner ear gene therapies and introduction of advanced genome editing strategies to silence dominant mutations or repair recessive mutations that cause dysfunction in human sensory hair cells.
Methods: Once blood sample is collected from a patient, PBMCs will be isolated within 24 hours. PBMCs will then be reprogrammed using the Cyto-Tune kit. Isolated colonies will undergo quality controls to ensure they are pluripotent, contain normal chromosome structure, and free from mycoplasma contamination. Subsequently, the isogenic control line will be generated by correcting the mutation using CRISPR-based gene editing technology.
Results: We have generated nine hiPSC lines carrying different genetic variant associated with hearing loss, as well as their isogenic control lines. Moreover, we showed inner ear organoids derived from some of these hiPSC lines, e.g., P2RX2 and USH2A.
Conclusions: We demonstrated our collection of hiPSC lines that are associated with genetic-related hearing loss. This hiPSC bank will serve as a power resource for the field to advance our knowledge in human inner ear. Moreover, the human inner ear organoids will serve as a power tool for researchers. The long-term impact will be to establish a new paradigm for personalized genetic models of human inner ear organoids affected in hearing loss for in vitro screening of available therapeutics and multi-omic identification of disease and biomarkers for treatment responses.

M116. The Role of Extremely Long-Lived Proteins in Acquired Hearing Loss

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Category: Hearing Loss: Consequences and Adaptation
Background: The orchestration of protein production and degradation and the regulation of protein lifetimes play a central role in many fundamental biological processes. Nearly all mammalian proteins are replenished by protein turnover in alternating waves of synthesis and degradation. Protein lifetimes in vivo are typically measured in days, but a small number of extremely long-lived proteins (ELLPs) persist for months or even years. ELLPs are rare in all tissues but are enriched in tissues containing terminally differentiated post-mitotic cells and extracellular matrix. Emerging evidence from our lab suggests that the cochlea is particularly enriched in ELLPs. Damage to ELLPs in specialized cell types, such as crystallin in the lens cells of the eye, causes organ failure such as cataracts. In a similar way, damage to cochlear ELLPs is likely to occur because of many insults, including acoustic overstimulation, drugs, anoxia, antibiotics, aging, and may play an underappreciated role in hearing loss.
Methods: Stable isotope metabolic labeling with 15N spirulina was accomplished as described (Savas JN et al., 2012). P30 FVB mice were placed on ad libitum 15N diet for 4 months and were sacrificed. The cochlea was micro-dissected and the proteins were extracted, digested into peptides, and analyzed by tandem mass spectrometry (MS)-based proteomic analysis. Protein identification/quantification were performed with the Integrated Proteomics Pipeline–IP2. Multi-isotope imaging MS (MIMS) was performed with a NanoSIMS 50L as described (Steinhauser M et al., 2012). Briefly, LR white embedded tissue samples were sectioned, mounted on silicon wafers, and gold coated. Samples were analyzed in automated chain analysis mode and quantified using the OpenMIMS plugin for ImageJ.
Results: Proteomic analysis revealed that ~7% of cochlear proteins are long-lived, with lifetimes exceeding 4 months. This is the largest pool of ELLPs we have identified and exceeds the brain, heart, female reproductive system, spleen, pancreas, liver, lung, and blood. Many cochlear ELLPs play structural roles and include collagens, fibronectin, connexin 29, and fibrillin2, among others. We also identified a panel of nuclear ELLPs, including components of the nuclear membrane (e.g., NUPs and lamins) and chromatin (e.g., histones and hmgs). Several more surprising ELLPs involved in axon guidance and signaling were also discovered. In addition, several tectorial membrane (TM) proteins also have very long lifetimes. Finally, MIMS analysis confirmed that the TM is loaded with long-lived molecules and revealed several additional long-lived cochlear structures.
Conclusions: Our results indicate that cochlea contains an exceedingly large number of intra- and extracellular ELLPs that are maintained for months or even years without being replaced. Seeing that proper hearing requires several long-lived structures and terminally differentiated cells, the identification of cochlear ELLPs represents a key step towards a comprehensive understanding of cochlear protein homeostasis in healthy hearing and during hearing loss.

M117. Perception of Auditory and Visual Emotions in Children With Hearing Aids

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Category: Hearing Loss: Consequences and Adaptation

Background: Emotion recognition is an important part of human interaction and this ability contributes to children's social-emotional development. Children with hearing loss who use hearing aids may have difficulties perceiving relevant acoustic cues conveying emotions, but the combined effects of neuroplasticity, physiological effects from hearing loss, and the compensatory features of hearing aids are not fully understood. As a result, it is also unclear if difficulties in vocal emotion perception are mostly due to a reduced access to relevant acoustic cues, an acute effect, or also because emotion processing skills develop differently in children with hearing loss, an accumulated effect over a longer period of time that may be less directly related to hearing difficulties. In the latter case, children with hearing loss may experience difficulties with emotion perception outside of the auditory domain, such as with visual emotion recognition or general emotion understanding. In this research, we assessed both auditory and visual emotion recognition in children with hearing aids to study the development of these skills throughout childhood.

Methods: Vocal emotion recognition and facial emotion recognition was tested in children with hearing aids (age range 6–18 yr), along with an age-matched control group of children without hearing loss. The EmoHI test was used to assess vocal emotion recognition, using non-language specific pseudospeech sentences expressing three basic emotions: happiness, sadness, and anger. Facial emotion recognition was assessed by presenting photographs from the Amsterdam Dynamic Facial Expression Set (ADFES) of individuals expressing the same three basic emotions.

Results: Preliminary results from children with hearing aids show developmental effects on vocal emotion recognition, but not on facial emotion recognition. These children performed close to ceiling on the facial emotion recognition test across the full age range. In the vocal emotion recognition test, there was a general group difference between children with hearing aids and age-matched children with normal hearing.

Conclusions: While vocal emotion recognition continues to develop in children with hearing aids, these children do not seem to have difficulties recognizing facial expressions of basic emotions. For children with hearing aids, emotion perception difficulties seem to be specific to the auditory domain. We will discuss potentially relevant contributing factors in the development of emotion perception in children with hearing loss.

M118. Quantifying Hidden Hearing Loss Through the Efficient Coding Hypothesis

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Category: Hearing Loss: Consequences and Adaptation

Background: Even though auditory deafferentiation is likely to be a primary cause of noise-induced hidden hearing loss (NIHHL), the specific link between observed physiological changes and perceptual effects of subtle acoustic trauma continues to be an open problem in hearing research. This work addresses this gap by viewing spike-trains recorded from the inferior colliculus of small mammals through a framework that implements the efficient coding hypothesis, with the aim of quantifying differences in the optimality of neural coding of changing acoustic environments, among several groups of animals versus a NIHHL group.
Methods: The developed framework takes rate-intensity functions (RIFs) of single-neurons as input, and infers the parameters of the optimal solutions spaces to which the set of RIFs might belong to, locating every group into a continuum of optimality given statistical characteristics of the stimulus input. This way, we make contrasts of the dynamics within three dimensions involved in the construction of the model (1) neural coding utility (2) neural gain regulation and (3) entropy of the solution spaces.
Results: Observations supported by this model reproduce and unify recently reported adaptation changes of cochlear synaptopathy and NIHHL, specifically, that neural gain is up-regulated by the loss of afferent inputs and moreover, that gain up-regulation is detrimental in general to neural coding utility, which systematically increases the associated entropy as well. Conversely, increased inhibition in circuits involving fibers that encode quiet acoustic contexts explain, for the NIHHL group, lower spiking probability, less entropy and enhanced coding utility only for quiet environments.
Conclusions: The consistency of these results suggest that the employed framework might be a starting point to construct a measure of hearing and hearing dysfunction stemming from computational neuroscience principles that refers to the quality and information content of the neural representations, rather than energetic thresholds.

M119. Integrating Physiological and Perceptual Assays to Resolve the Effects of Sensorineural Hearing Loss on Neural Place and Time Cues for Pitch

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Category: Hearing Loss: Consequences and Adaptation
Background: Prior research indicates that hearing loss due to aging and/or noise exposure negatively impacts pitch perception even when sounds are audible. Physiological changes in the cochlea may disrupt the auditory cues necessary for robust pitch perception, but the relative contribution of place and time degradations remain unclear. Our previous studies in chinchillas demonstrate that ototoxic and noise-induced hearing loss lead to deficits in temporal and tonotopic representations of tone complexes that vary based on the type of cochlear damage. We are further exploring how variations in the fidelity of place and time coding from diverse profiles of cochlear dysfunction impact pitch perception through physiological and perceptual assays of pitch processing in young to middle-age participants, with and without hearing loss.
Methods: Six tone-complex stimuli (F0 = 103 Hz, six harmonics) were used to assess neural and perceptual pitch representation. The stimuli varied in harmonic rank and were presented with alternating phases to enhance phase-locking-value energy at the envelope frequency (2*F0) when harmonics were unresolved. High harmonic rank stimuli have more unresolved harmonics,

resulting in quantifiable perceptual (Fundamental Frequency Difference Limen, F0DL) and electrophysiological (EFR) resolved-to-unresolved transition points. We also recorded acoustic change complexes (ACCs) in response to pitch shifts and psychophysical tuning curves at 1- and 4-kHz center frequencies, alongside a peripherally-focused diagnostic battery (audiometry, OAEs, MEMR). Our ongoing dataset includes 25 subjects varying in age (19-65) and degree of hearing loss.
Results: Our participants exhibit diverse auditory physiology and pitch discrimination thresholds. By grouping subjects based on age (under/over 35) and hearing status, we found that middle-aged participants and those with hearing loss had broader tuning, reduced tip-to-tail ratios, and lower EFR and ACC amplitudes. These results predict perceptual F0DLs, which indicate middle-aged participants and those with hearing loss have transition points at lower harmonic ranks than younger listeners with normal hearing, i.e., show less resolvability. However, cochlear tuning deficits alone would predict stronger envelope coding (elevated EFRs) in the absence of temporal coding deficits. Our chinchilla findings clarify these results; disrupted place coding from noise exposure led to over-representation of envelope signals at low harmonic ranks, while carboplatin-induced inner-hair-cell damage decreased EFR amplitudes. Preliminary data imply that people experience a combination of place and temporal coding deficits, which we are exploring using our diagnostic assays.
Conclusions: We have begun to elucidate how the severity of sensorineural hearing loss and underlying cochlear pathologies influence fundamental-frequency representation. Our initial findings suggest that although frequency selectivity is vital for pitch discrimination, reductions in temporal envelope coding due to inner-hair-cell dysfunction can also impact pitch processing and warrant further investigation. Future work will leverage our cochlear diagnostics to disentangle the mixture of peripheral place and time deficits we observe and how they impact pitch discrimination.

M120. Peritraumatic Near-Infrared Treatment Attenuates the Severity of Permanent Hearing Loss

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Category: Hearing Loss: Consequences and Adaptation
Background: Noise is the second most common cause of hearing loss after age. Previous studies have shown that multiple post-traumatic irradiation of the cochlea via the external auditory canal (12 days, 60 min daily) with near-infrared light (NIR) can reduce permanent hearing loss pantonally by approximately 20 dB in animal experiments. Furthermore a single, short NIR pre-treatment (10-20 min) was shown to have the same effect. In the presented study, the effect of a combination of pre- and post-treatment was investigated to gain more detailed insights into the NIR mechanisms of action in the inner ear.
Methods: Frequency-specific auditory brainstem potential thresholds (fABR thresholds) of 24 young adult mice with normal hearing were determined at 5, 15, 25 and 35 kHz. Mice were equally allocated to the experimental groups. The treatment group underwent irradiation of the cochlea with 120 mW near-infrared light (NIR) for 15 minutes, followed by a noise trauma using broad band noise (5-20 kHz) for 30 minutes at 115 dB. A post-treatment was administered for a

period of 30 minutes (120 mW) immediately following the noise trauma. The trauma-only group was subjected exclusively to the noise trauma, without any irradiation and the control group was not subjected to either noise trauma or irradiation. Following a 14-day period, the fABR thresholds were ascertained for all animals, and the resulting degree of hearing loss and the according hearing protection was calculated. Subsequently, histological examinations were conducted to investigate the density of spiral ganglion neurons.
Results: A combined pre- and post-treatment resulted in a significantly lower threshold increase compared to the trauma only group. Hearing protection in these animals exceeded the effect of an exclusive pre-treatment by about 8.7 dB (± 0.6) across all frequencies. Histologic examinations showed a loss of spiral ganglion neurons in the trauma only group which was significantly rescued by the combination of pre- and post-NIR treatment. Conclusion: The results show a significantly increased effect of combined NIR pre- and post-treatment on the preservation of the hearing threshold after noise trauma.
Conclusions: The possibility of intervening to reduce permanent hearing loss using the side-effect-free NIR application could also be an interesting approach for tratment of noise induced hearing loss but also in other models of damage to inner ear function.

M121. Stimulus Optimization and Cross-Sectional Analysis of Frequency-Following Responses in Individuals With Sensorineural Hearing Loss

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Category: Hearing Loss: Consequences and Adaptation
Background: Scalp-recorded frequency-following responses (FFRs) are sustained neuro-electrical potentials representing the periodicity of acoustic stimuli and have proven to be an effective tool for studying the mechanisms underlying sound processing and speech recognition. Our recent systematic review on FFRs in individuals with sensorineural hearing loss (SNHL) suggested that these patients struggle to track the fundamental frequency (F0) of (speech) stimuli, particularly with longer stimuli (≥ 170 ms). However, the review also highlighted some important limitations in previous research, such as small sample sizes and inconsistent methodologies. To address these issues and further investigate SNHL's impact on FFRs, we designed a cross-sectional study.
Methods: The first phase of this study aimed to optimize the stimulus, as the commonly used /da/ stimulus has limitations in tracking the temporal fine structure. In collaboration with the University of Barcelona, this phase involved recording FFRs from 34 normal-hearing subjects using four different stimuli (/dao/, /doa/, /oa/, and /ao/). Results indicated that the /dao/ stimulus was the most suitable. With this stimulus, FFRs are being obtained from 80 subjects with varying degrees of SNHL and normal hearing. Subjects are matched as closely as possible by age and gender across different hearing level groups. To control for audibility, stimuli are presented at 60 dB SPL above the pure-tone average (PTA) or at a maximum comfortable loudness level if the initial intensity is uncomfortable. The stimuli are delivered monaurally in three blocks of 1,000 presentations per ear.

Results: FFRs have been acquired in 56 subjects (mean age 48.9 years). Preliminary analyses reveal a trend of decreasing F0 response amplitudes with increasing PTA's. This trend is evident in both the /o/ and /a/ sections of the stimulus and the overall response, with the most pronounced effect observed in the /o/ section. Analysis of F0 response amplitudes across four PTA-based groups reveals significant differences for the /o/ section (p LESS THAN 0.001), the /a/ section (p LESS THAN 0.001), and the entire stimulus (p = 0.001). Post-hoc tests for the /o/ section show significant differences between the normal hearing group and those with moderately severe to severe SNHL, as well as between the normal hearing group and the profound SNHL group. Current analyses do not show significant differences in neural lag or root-mean-square of the pre-stimulus interval across the groups.
Conclusions: Preliminary findings suggest that subjects with SNHL have difficulty tracking the F0 of the stimulus, as evidenced by reduced F0 response amplitudes, which is more pronounced with increasing SNHL. We anticipate presenting the results of our study involving 80 subjects at the conference. By that time, we will also conduct more detailed analyses, including exploring the correlation with PTA and assessing the impact of SNHL on the temporal fine structure of the FFR.

M122. Surgical Planning for Implantable Middle Ear Microphone in Sheep Using Temporal Bone Micro-CT

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Category: Auditory Prostheses
Background: Totally-implantable cochlear implant development, which would address drawbacks from external microphones, is limited by a well-functioning implantable microphone. The umbo Microphone (UMic), a dual-layer PVDF sensor under development by our team, detects umbo motion and converts it into electrical charge. To support the UMic's development, we are preparing for a live sheep study by refining the surgical approach for placement and fixation of the UMic using cadaveric sheep temporal bones. In this study, we aim to use micro-CT imaging to evaluate sheep temporal bone anatomical variations (e.g. facial nerve course) that influence development of a universal implantable device, to guide the surgical process, and to confirm correct placement of the UMic.
Methods: Four cadaveric Hampshire sheep temporal bones were evaluated by pre- and post-surgical micro-CT scans. Bone structures were segmented from pre-surgery micro-CT scans and down-sampled to ensure smooth simulation in an open-source image processing software (3D Slicer). Critical anatomical landmarks were segmented, and drilling was simulated with the eraser tool. The simulated drilled bone was then exported to a CAD virtual design program (SolidWorks). To secure the UMic sensor in place, a 3D printed metal fixation device was designed with a ball-and-socket mechanism. The socket arm was available in different lengths to accommodate variations in the distance between the umbo and the mastoid cortex; the length was chosen preoperatively using micro-CT. Fixation devices of various lengths were virtually tested to ensure fit. We then surgically prepared the cadaveric specimen for facial recess access to the middle ear cavity. Subsequently, one specimen was implanted with the UMic. To secure the

UMic, the fixation device was screwed into the mastoid cortex. Placement of the UMic was checked by microscopic visualization and post-operative micro-CT.
Results: All four specimens were surgically prepared. Micro-CT imaging and actual surgical drilling revealed a small or absent antrum, abundant middle ear mucosa, and poorly pneumatized mastoid in all four specimens. The round window was fully visualized in both the simulated and all the actual post-surgical specimens. The socket arm length for each temporal bone was determined by virtually fitting the fixation device into the simulated drilled bone. Implantation of the fixation device and UMic in one specimen confirmed that the pre-selected version provided the best fit. Post-surgical micro-CT confirmed that the sensor tip contacted the umbo.
Conclusions: Definition of the sheep middle ear anatomy via micro-CT analysis will aid in future translational studies. In addition, this study demonstrates a feasible approach for simulating surgery using open-source software without the need for specialized equipment. This enabled surgical planning and device selection for the UMic and could have wider applications for other implantable prostheses.

M123. Revisiting Analog Stimulation in a Guinea Pig Model of Cochlear Implant

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Category: Auditory Prostheses
Background: The goal of cochlear implants (CI) is to provide speech comprehension in individuals with severe hearing loss. Since the 1990s, encoding of the speech waveform has relied almost exclusively on envelope extraction and non-simultaneous, interleaved pulse stimulation. Alternative coding strategies based on feature extractions (F0 and formants frequencies) were also proposed but, unfortunately, they were coupled with simultaneous sinusoidal analog stimulation and monopolar or bipolar stimulation modes leading to elevated channel interactions due to current summation spread. Progresses in methods for focusing electrical fields with tripolar stimulation are leading us to revisit analog stimulation and feature extraction-based in CIs. In this study we used advanced current focusing to compare analog with pulsatile stimulation in a guinea pig model.
Methods: Ten pigmented guinea pigs were studied. We used an 8-electrode cochlear implant array inserted into scala tympani. Multiunit responses were recorded in the central nucleus of the inferior colliculus with a 16-channel probe (Neuronexus). First, probe placement along the tonotopic axis was confirmed by acoustic stimulation with pure tones from 8 to 40 kHz. Next, deafness was induced via 10% neomycin infusion. CI stimulation consisted of tripolar mode, using an intracochlear active electrode with flanking electrodes sharing half of the return current. We measured the IC spread of excitation (SOE) obtained with analog stimulation, single biphasic pulses, low rates pulse trains (50-100Hz) and clinical rates pulse rates (1kHz). Finally, we stimulated two channels simultaneously, each having a specific rate (19 vs. 37 Hz or 100 vs. 337Hz) to quantify channel interactions in the temporal domain.

Results: Overall, tripolar analog stimulation mode evoked very focal IC activations as previously described with single pulses and pulse trains. IC activations were tonotopically organized and consistent with the apical-to-basal position of the stimulating electrode. Responses were strongly phase locked in accordance with the stimulation pulse rate and/or the sinusoidal period. In general, the SOE in the IC tended to be narrower with analog stimulation compared with biphasic pulse trains. Analyses of adaptation and synchronization index are ongoing to further characterize the viability of tripolar analog stimulation.
Conclusions: Results showing focal and tonotopic responses demonstrate that place cues were well-conveyed by focused analog stimulation presented in tripolar mode. Results showing strong phase locking demonstrate that temporal cues can also be provided by focused analog stimulation. Future studies, though, will be needed to characterize and minimize channel interactions in analog stimulation. Overall, tripolar analog stimulation shows promise for conveying important sound features like temporal fine structure that should improve hearing restoration in humans.

M124. Cochlea Implants in Meniere's Disease – Altered Response Capacity of the Spiral Ganglion Cells and the Influence of Prior Intratympanic Gentamicin and Corticosteroids Therapy on Speech Understanding

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Category: Auditory Prostheses
Background: Intratympanic injection of corticosteroids (ITC) and gentamicin (ITG) are widely used treatments for vertigo in Meniere's disease (MD). Even though studies show good results after cochlea implantation (CI) in MD patients when compared to non-MD groups, there is no indication on the effect of ITC and ITG prior to CI on hearing after CI. This study compares the post-operative hearing of CI patients with and without MD and patients who have received ITG or ITC prior to CI and analyses cochlear nerve function.
Methods: Patients with MD (n=28) who received CI from 2002 until 2021 were compared to a matched control group without MD (n=33). Patients with prior ITC (n=6)/ITG (n=9) were identified. Pre-operative audiological results were evaluated and trends across post-operative monosyllabic word recognition score at 65 decibels (WRS65CI) at switch-on, 3-6 months, 1 year and last yearly value were analyzed across all groups. Additionally the intraoperative electric compoundaction potentials (eCAPs) measurements and converted to amplitude growth functions (eCAP AGF). Their slopes and thresholds were compared between MD and non-MD patients.
Results: WRS65CI increased significantly with time within MD and control groups, but no difference in WRS65CI was found between ITG and ITC. ITG ears showed fluctuating WRS65CI after CI, while ITC ears showed significant increase in trend of WRS65CI. Additionally the slope of the eCAP AGF was statistically significantly higher in the MD patient. Non-MD patients required higher thresholds to generate eCAPs. The electrical charge required by the non-MD group was significantly higher in the medioapical electrodes.
Conclusions: MD and non-MD patients showed comparable hearing results after CI. Prior ITC might positively influence hearing with CI whereas ITG group showed fluctuating hearing. The

lower threshold and steeper slopes of the eCAP AGF in MD patients indicate an altered response capacity of the spiral ganglion cells.

M125. Optical Imaging of Auditory Cortex Responses in the Awake Common Marmoset (Callithrix Jacchus) With Unilateral Cochlear Implants

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Category: Auditory Prostheses
Background: Our laboratory has recently developed a through-skull wide-field optical imaging method in awake common marmosets (Callithrix jacchus) (Song et al, 2022). This non-invasive imaging method has shown to be an efficient tool for identifying tonotopic gradients in the auditory cortex. It also serves as a localizer for directing the parcellation of the auditory cortex, separating the primary auditory cortex (area A1) and belt subregions. Although this approach has been extensively investigated under acoustic stimuli, it has never been examined in the context of electrical stimulation from cochlear implant (CI) devices.
Methods: In this study, we implemented this optical imaging method in the awake marmoset models with unilateral CI, a highly vocal non-human primate with a similar hearing range as humans, to investigate cortical response patterns under CI stimulation. Imaging sessions were recorded in three awake marmosets from both hemispheres while varying current levels across all CI electrodes using three different CI configurations (monopolar/MP, partial tripolar/pTP, and tripolar/TP) in random order.
Results: Results showed the feasibility of this imaging technique to demonstrate tonotopic gradients across the hemispheres under CI stimuli. In general, for all paradigms tested, increasing current level corresponded with increased activation amplitudes and areas. However, we also observed non-monotonic responses in the left hemisphere under TP and pTP stimulation. In comparison between left and right hemispheres, we found that CI stimulation was more effective at activating neuronal responses in the left hemisphere (contralateral to the CI ear). Between MP and TP stimulation, MP induced greater activation areas, whereas TP activation areas were more focused.
Conclusions: This through-skull mapping approach provides an alternate perspective for understanding how the auditory cortex processes the electrical stimulation from the CI device and may help address some deficits observed in the performance of CI users in perceptual tasks.

M126. Effects of Aging and Processing Speed on Temporal Gap Detection in Cochlear Implant Users

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Category: Auditory Prostheses
Background: Encoding of silent gaps is an important cue for speech perception. Gap detection in cochlear implant (CI) users is quite good, and often better than non-implanted listeners. Gap

detection often improves in CI users when increasing pulse rate, but underlying factors to encoding of gaps at high rates is poorly understood. In non-CI users, gap detection generally declines with increased age, and both auditory nerve health and cognitive processing speed help to predict senescent declines in gap detection sensitivity. Given that the majority of adult CI users are over the age of 65 it is important to determine contributing factors to temporal precision in CI users. The overall aim of the current study was to determine how peripheral and cognitive factors contribute to gap detection sensitivity in CI users when using low and high pulse rates. Further, we examined if gap detection sensitivity is related to speech recognition in the same listeners.
Methods: 17 cochlear implant recipients with adult-onset hearing loss (Ages 43-87) have been examined thus far. Auditory nerve health was assessed using electrically-evoked compound action potential (ECAP) measures and electrodes with better and poorer neural health were selected for each listener. Psychophysical gap detection thresholds were assessed using a 4AFC approach and were measured on selected electrodes for each listener using a 500 and 3500 pps stimulus. The Connections Test was used to evaluate processing speed for each listener. Speech recognition in noise (AzBio Sentences, +10 dB SNR) were presented at 60 dBA, and scored as percent correct for each listener.
Results: Preliminary data suggest that gap detection worsened with increased age, but for all participants thresholds were better when using a higher pulse rate; there was a significant interaction between age and pulse rate, with younger listeners showing greater improvement in gap detection thresholds when increasing the pulse rate. Contrary to our predictions, in this preliminary data set, AN health was not a significant predictor of gap detection thresholds. Slower processing speed was independently associated with poorer gap detection thresholds and speech understanding in noise, regardless of pulse rate, AN status or listener age.
Conclusions: Preliminary results suggest that decreased processing speed is a primary contributing factor to age-related declines in encoding of silent temporal gaps in CI users.

M127. The Effects of Dexamethasone-Eluting Cochlear Implant Arrays on Post-Operative Longitudinal Impedance Measurements and Intracochlear Computed Tomography Changes in Humans

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Category: Auditory Prostheses
Background: Intracochlear injury during surgical placement of a cochlear implant (CI) array can result in fibrotic scar formation and neo-ossification. Scar formation decreases the efficiency of electrical stimulation and can inhibit residual acoustic processing. Electrode impedance is sensitive to intracochlear fibrosis and increases over time post implantation. Our center is evaluating subjects who have received dexamethasone-eluting CIs (Dex-CIs), which are intended to reduce the foreign body response to the CI. This study aims to evaluate both electrode impedance and intracochlear changes over time within recipients of Dex- and non-Dex-CIs. We hypothesize that longitudinal impedance measures, particularly the subset of measures that estimate the near-field local environment, will be reduced in Dex-CI implanted ears. We also hypothesize that these impedance measures will positively correlate with the percentage and

location of longitudinal neo-ossification changes as seen on photon counting computed tomography (PCCT).
Methods: Two groups of subjects have participated in this research. Eight of nine CI recipients who participated in a multicenter randomized, double-blinded study received either a Dex-CI or a comparable, commercially available CI632 electrode array also participated in our P50 grant. 15 additional subjects have been implanted with a similar Dex-CI in a non-randomized arm with recruitment currently underway for 15 control subjects.
Electrode impedance measures were performed serially: at the time of surgery, and at each post-operative visit (i.e. Initial activation, 0.5-, 1-, 3-, 6- and 12-months post activation). Transimpedance matrix measurements were made using multiple stimulation modes and at multiple time-points across the duration of the stimulation current pulse. The measurements allowed for a time-based approach to separating access resistance and polarization impedance to estimate the near-field/local environment. The serial measurements allowed for assessing changes across time. PCCT was performed pre-operatively, around initial activation, and around 12-months post-operatively. Electrode position relative to intracochlear structures and local longitudinal changes in neo-ossification were calculated. Relationships between impedance and CT measurements will be evaluated using correlation analysis.

Results: Preliminary analyses reveal that impedance values fall into one of two distinct ranges postoperatively. Impedance differences across groups were smallest at the time of surgery and increase post-operatively; this was true of total impedance as well as access resistance and polarization impedance. In the control group, access resistance increases over time, particularly in the basal region while polarization impedance may tend to decrease or remain more stable over time. In the Dex-CI group, both access resistance and polarization impedance tend to decrease over time, particularly in the apical regions. Analysis of PCCT images is currently ongoing. Data collection is ongoing.
Conclusions: Dexamethasone elution appears a promising strategy to reduce electrode impedance following cochlear implant surgery. It is of interest whether these effects are associated with intracochlear changes on PCCT.

M128. Influence of Boundary Conditions on Bone Conduction Experiments: A Finite Element Study

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Category: Auditory Prostheses
Background: Research on bone conduction (BC) mechanisms has traditionally relied on human subjects, temporal bone studies, and cadaver head experiments. In cadaver studies, parameters such as promontory velocity, transcranial attenuation (TA), and intracochlear pressure are frequently measured. However, the methods used to secure or position cadaver heads during these experiments vary significantly among researchers. These methods include placing the head on vibration damping pads, laying it down, or fixing it with clamps or pins. Despite these variations, there has been little systematic analysis of how these setup methods affect BC

responses, such as promontory velocities. To address this gap, this study aims to evaluate the influence of different experimental setups on BC responses using finite element simulations, providing insights that may help standardize experimental conditions and improve the reliability of BC research.
Methods: A comprehensive full-head finite element model, including the auditory periphery, was utilized to assess the effects of fixation strength, fixation location, and contact condition on BC responses. The model incorporated soft tissue, cortical and cancellous bone, cerebrospinal fluid, brain, cartilage, and cochlear structures. The primary metrics analyzed were TA, defined as the ratio of velocities between the ipsilateral and contralateral promontories, and the promontory velocities themselves.
Results: The simulation results demonstrated that TA exhibited a variation of less than 5 dB across different boundary conditions. For promontory velocities, variations in fixation location and contact condition also led to differences of less than 5 dB. However, changes in fixation strength produced differences of up to 20 dB at frequencies below 350 Hz. These findings suggest that fixation strength significantly influences the transmission of vibrational energy to the cochlea, having a more pronounced effect on promontory velocity than changes in fixation location or contact condition.
Conclusions: This study shows that fixation strength is a key factor influencing bone conduction responses, especially in promontory velocity, while transcranial attenuation remains mostly unaffected by changes in boundary conditions. The findings highlight the importance of standardizing fixation methods to improve the accuracy and consistency of bone conduction experiments. By bringing attention to the previously overlooked effects of boundary conditions, this work provides valuable guidance for improving experimental protocols in future BC research.

M129. Measuring the Effective Polarity of Electrical Excitation Across the Auditory-Nerve Array to Enhance Spectral Resolution by Cochlear-Implant Listeners

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Category: Auditory Prostheses
Background: Speech perception by cochlear implant (CI) listeners is limited by the broad spread of excitation produced by electrical stimulation. Current-focussing methods, whereby the polarity of the injected current differs between a central electrode and two or more flanking electrodes, can produce sharp voltage profiles as measured along the electrode array. To produce a perceptual benefit we need to sharpen the profile of excitation at the AN and prevent current injected at the flanking electrodes from creating sidelobes of excitation. To do this, we need to know both the amplitude and effective polarity of stimulation at the neural level.
Methods: Users of the Advanced Bionics CI detected a probe pulse train in isolation and in the presence of two different background stimuli. The background was a □99 pulse-per second (pps) train of symmetric biphasic pulses, with pulse phases in Anodic-Cathodic (AC) or Cathodic-Anodic (CA) order. The stimulation mode was either monopolar (MP, experiments 1 and 2),

wide bipolar ("BP+10", e.g. electrode 2 and 11; experiment 3) or wide tripolar mode (TP+5; experiment 4). Each probe pulse immediately followed a background pulse with zero gap. These "pseudo-monophasic" pulses had the same rate as the background and consisted of a short high-amplitude anodic phase followed by an 8-times-longer and one-eighth-amplitude cathodic phase. This shape was chosen so that thresholds would be primarily determined by charge interactions between the 2nd phase of the background pulses and the first (anodic) phase of the probes. Pulses were presented in focussed mode (tripolar, partial tripolar, or quadrupolar-virtual-channel).
Results: In experiment 1 the monopolar CA background produced a very large (10-20 dB) threshold reduction relative to no background. We attribute this substantial unmasking to the anodic-leading phase of the probe being integrated at the nerve membrane with the 2nd phase of the CA background. The AC background produced modest amounts of positive masking. Moving the background electrode progressively further from the probe (experiment 2) gradually reduced these effects. Experiment 3 revealed an interaction between the polarity of the BP background and the probe electrode, with greatest unmasking when the probe was presented to the electrode where the 2nd phase of the background was anodic. This method is currently being applied to measure variations in the effective polarity of TP backgrounds at different probe locations (experiment 4).
Conclusions: Substantial unmasking occurs when the second anodic phase of a background pulse immediately precedes the initial anodic phase of a probe. This leads to very low detection thresholds allowing us to use focussed probes and to measure how the effective polarity and excitation level of a given stimulus varies across the AN. We are employing this method to develop new stimuli that aim to provide more-selective stimulation by CIs.

M130. Effects of Stimulation Parameters on the Phase Locking Value in Postlingually Deafened Adult Cochlear Implant Users

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Category: Auditory Prostheses
Background: In our previous study (He et al., 2024a), we developed a new tool to assess neural synchrony in the cochlear nerve (CN) in cochlear implant (CI) users using an index named the phase locking value (PLV). The PLV quantifies the trial-to-trial phase coherence of the electrically evoked compound action potential (eCAP) and can be measured in both adult and pediatric CI users (He et al., 2024a, 2024b). In this study, we conducted two experiments that evaluated the effects of stimulation rate and stimulation level on the PLV in postlingually deafened adult CI users.
Methods: Study participants included 11 postlingually deafened adult CI users with Cochlear® Nucleus™ devices. Each participant was tested for both experiments. For each participant, the PLV was measured at four electrode locations (i.e., electrodes 3, 9, 15 and 21) across the electrode array. To evaluate the stimulation rate effect on the PLV, a cathodic-leading biphasic pulse was presented at the maximum comfortable level with rates of 1, 15, 30, 60 and 120 Hz. To evaluate the stimulation level effect on the PLV, a cathodic-leading biphasic pulse was presented at 15 Hz across five different stimulation levels. These levels included the eCAP threshold, the

25%, 50%, and 75% of the dynamic range between the eCAP threshold and the maximum comfortable level, as well as the maximum comfortable level. Statistical analysis was conducted using a Linear Mixed effects Model (LMM) with electrode location, rate and stimulation level as fixed effects and an intercept for individual participant.
Results: The result of the LMM showed a significant effect of stimulation level and electrode location on the PLV. The results of pairwise comparisons showed that the PLV measured at electrode 15 was significantly larger than those measured at other electrode locations. The PLV measured at electrode 3 was significantly smaller than those measured at other electrode locations. No statistically significant difference was found between PLVs measured at electrodes 9 and 15. PLVs significantly increased with stimulation level. There was no statistically significant effect of stimulation rate on the PLV.
Conclusions: Our preliminary results indicate that the PLV is affected by stimulation level and electrode location but not by stimulation rate within the 1-120 Hz range in postlingually deafened adult CI users. Further studies, with larger participant groups or other patient populations, are warranted to confirm these preliminary results.

M131. Evaluating the Spread of Excitation With Red Light Optogenetic Stimulation of the Auditory Nerve Through Computer Simulations and In-Vivo Electrophysiology

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Category: Auditory Prostheses
Background: The future optical cochlear implant (oCI) utilizes optogenetic stimulation of the spiral ganglion neurons (SGNs) in the cochlea to improve hearing restoration beyond the state of the art. Preclinical data has shown that this may improve bionic hearing in CI users by more spectrally confined SGN activation compared to electrical stimulation. This study investigates the influence of the light emitter characteristics (one/two laser-coupled glass fibers) on the stimulation precision in red light optogenetic stimulation.
Methods: For an initial estimation of the excited cochlear volume, we used 3D modelling based on x-ray data of nine Mongolian gerbil cochleae with sham fiber insertions to simulate light spread and explore relationships between insertion parameters and the irradiance experienced by the SGNs. We then performed in-vivo multiunit recordings from the inferior colliculus activity in anesthetized Mongolian gerbils that postnatally received AAV mediated gene therapy to render their SGNs light sensitive via expression of the red-light activated channelrhodopsin f-Chrimson. During the in-vivo recordings, we systematically varied stimulus intensity, fiber diameter, position within the cochlea, insertion angle, and, the inter-fiber distance, and analysed the cochlear spread of excitation (SoE) in the inferior colliculus. Afterwards we determined the density and f-Chrimson-EYFP expression of SGNs using confocal microscopy.
Results: We collected recordings from 13 animals. We observed that light stimulation through an apical cochleostomy activates neurons in low-frequency regions while basal stimulation activates high- frequency regions, in agreement with previous studies. Using f-Chrimson allowed for stimulation with repetition rates up to 350 Hz. Smaller fiber diameters can have a more

confined excitation pattern than larger diameters when they point directly at the Rosenthal's canal. This ideal fiber placement is harder to achieve for smaller diameters. The SoE was comparable to acoustic stimulation around the intensity threshold that is needed to detect a significant change in spike rate (sometimes below 0.5 mW). For high stimulation intensities the SoE was larger for light stimulation than for the acoustic control with comparable spike rates. For high intensity red light stimulation we sometimes observed two activation bands which was more rare with blue light stimulation in previous studies.
Conclusions: The aim of this dataset is to help in establishing the design of the future optical cochlear implant and the optogenetic sound coding strategy. We can use the observed results on the activation threshold and spread of excitation for large stimulation intensities to formulate requirements on the light emitter intensity range for the medical device. We also saw the importance of a good emitter positioning which needs to be taken into account in finalizing the implant design and possible insertion tools. In the next steps the goal is to look in more detail on the interaction between neighbouring emitters to investigate possible channel interactions.

M132. Developing Novel Electrical Stimulation Strategies for Cochlear Implant Users Based on a Model of the Healthy Human Cochlea

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Category: Auditory Prostheses
Background: Many individuals with cochlear implants (CIs) face difficulties in understanding speech in noisy environments and often express frustration with the quality of music they hear. This may be partly due to the simplified filter banks used in current CI technology, which don't fully replicate the natural processes of the cochlea. This project aims to improve cochlear implant strategies by more accurately mimicking the responses of the auditory nerve, potentially addressing these limitations. A sophisticated auditory model, the Cascade of Asymmetric Resonators with Fast-Acting Compression (CARFAC) is used.
Methods: A deep neural network (DNN) was trained to produce 22 electrode stimulation currents from audio inputs. These currents were then passed through an electrical hearing (EH) model that accounts for the current spread, neural adaptation, and refractoriness. The DNN was trained using the difference between the output of the electrical and acoustic hearing models. The DNN and EH models were developed in JAX.
Results: We trained the models on sentences from the TIMIT database and show that the DNN-EH architecture achieves a higher similarity (i.e., a higher structural similarity index and a lower mean squared error) to CARFAC outputs compared to Neurograms obtained from a standard CI coding strategy - Continuous Interleaved Sampling (CIS)
Conclusions: Our method of producing electrode stimulation currents results in neurograms that resemble the simulated neural activity of a healthy cochlea, more than those produced by CIS. The perceptual significance of this method compared to CIS is being investigated.

M133. Neural Network Models of Hearing Clarify Factors Limiting Cochlear Implant Outcomes

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Category: Auditory Prostheses

Background: Current cochlear implants (CI) fail to restore fully normal hearing. These shortcomings could arise from factors such as suboptimal encoding strategies, degeneration in the auditory system and/or limits on the brain's ability to adapt to CI input. To clarify how these different factors shape CI outcomes, we developed artificial neural network models of hearing that recognize speech and localize sound from CI input.

Methods: We modeled normal hearing by training a feedforward convolutional neural network to recognize speech or localize sounds in noise given simulated auditory nerve input from an intact cochlea. We modeled CI hearing by testing this trained network on simulated nerve input from CI stimulation. To simulate learning to hear through a CI, we re-optimized the network for CI input. To simulate the best case (complete plasticity), all the network weights were re-optimized. To simulate potential limits on plasticity, only late-stage network weights were re-optimized. Additionally, we modeled peripheral nerve degeneration by silencing some nerve fibers in the input representation. To model central nerve degeneration, we randomly silenced particular frequencies in each channel for each stage of the neural network.

Results: Models trained with CIs exhibited impaired speech recognition and sound localization relative to the normal hearing model. When the entire network was optimized for CI input, speech recognition was substantially better than that of typical CI users, even with substantial simulated peripheral and central degeneration. Speech recognition on par with typical CI users was achieved only when just late model stages were optimized. However, model localization performance remained much worse than normal even when the entire network was optimized for CI input.

Conclusions: This work provides initial validation of machine-learning-based models of CI-mediated perception. Our results point to central plasticity as limiting CI outcomes while also identifying limitations in existing stimulation strategies.

M134. Effect of Piezoelectric Thickness on Dual-Bandwidth Accelerometer Design for Totally Implantable Auditory Prostheses Applications

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Category: Auditory Prostheses

Background: Hearing aids and cochlear implants are beneficial devices that aid their users to ameliorate their hearing loss. Both devices positively impact patients' lives, but their adoption rates are low. Several key limitations associated with both HAs and CIs have been identified as the culprits including appearance, cost, perceived ineffectiveness, and ease-of-use. Some of the limitations are linked to the external elements (e.g., microphones) of these devices that are removable, can easily be misplaced, damaged, or stolen, and can limit the range of activities users can partake in while wearing them (e.g. sleeping or swimming). A totally implantable

auditory prosthesis (TIAP) would help to address issues associated with these external elements by eliminating them. A major barrier to progress toward a commercially available TIAP is the lack of a completely implantable acoustic sensor capable of matching or exceeding the performance of commercial external microphones. Our previous study has indicated that a dual-bandwidth piezoelectric microelectromechanical systems (MEMS) accelerometer has the potential to function as an implantable sensor within the middle ear meeting a 20-phon noise floor over a 100Hz – 8kHz range. In the current study, we optimize this dual-bandwidth design by investigating the effect of the piezoelectric thickness on its sensor area. Understanding this effect can lead to even smaller designs with equivalent performance.
Methods: The dual-bandwidth sensor is comprised of two piezoelectric cantilever bimorph plates (sensing elements) tip-loaded by silicon proof masses, each operating at different sub-bandwidths (together adding up to the 100Hz – 8kHz). Each sensing element has the resonance occur outside the designated sub-bandwidth, which allows for a nearly constant sensitivity response over that sub-bandwidth. The original combinatorial problem looked for the best combination of cross-over frequency (transition frequency between sensing elements) and other geometric parameters (excluding the piezoelectric thickness) that minimized the overall sensor area. In this new investigation, we will include the piezoelectric thickness in our combinatorial problem. All dual-bandwidth designs identified through this new optimization have the same performance, meeting the 20-phon noise floor over 100 Hz – 8 kHz.
Results: Results show that the piezoelectric thickness has a direct effect on the sensor area of the dual-bandwidth accelerometer. Thinner piezoelectric thicknesses do not always lead to smaller sensor areas. This is contrary to what we observe for canonical cantilever sensors under uniform pressure.
Conclusions: Through our optimization process, we found that there exists an optimal piezoelectric thickness that minimizes the sensor area of a dual-bandwidth sensor while balancing the constraints of 20-phon noise floor over the 100 Hz – 8 kHz frequency bandwidth.
We would like to acknowledge the NIH training grant (T32 DC00011) and (R01 DC021596) that funded this research.

M135. Atypical ECAP Measures and Auditory Outcomes in Cochlear Implant Users With Vestibular Schwannoma: A Case Series Study

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Category: Auditory Prostheses
Background: Vestibular Schwannomas (VS) are benign tumors that primarily affect the vestibulocochlear nerve. The growth of VS tumors, along with associated treatments, can result in severe hearing loss or complete deafness. Cochlear implants (CI) may restore hearing in these patients by directly stimulating the auditory nerve, bypassing the cochlea. However, the effectiveness of CIs is reduced in VS patients compared to non-tumor patients. VS tumors usually grow adjacent to the auditory nerve, often at a distance from the cochlea. Despite this, studies in animal models have shown that VS can compromise the health of peripheral nerve cells in the cochlea. The impact of VS tumors on the auditory nerve and CI outcomes is not well understood in CI patients.

Methods: We studied the peripheral auditory nerve function and behavioral CI outcomes in three VS patients who had poor speech perception with their CI. Auditory nerve function was assessed by recording electrically-evoked compound action potentials (ECAP) from the CI electrodes. Behavioral auditory outcomes were assessed by measuring speech perception, gap and amplitude modulation detection at single CI electrodes, and pitch ranking across electrodes. Single-electrode and pitch ranking results were used to identify 5 best-performing electrodes in each subject and create a modified program with these electrodes. The new modified program was evaluated after a one-month acclimatization period by comparing phoneme identification scores to the scores obtained with the subject's clinical program.
Results: ECAP waveforms were either absent or showed atypical morphology and refractory recovery times in the majority of the electrodes tested in these subjects. Gap and amplitude modulation detection thresholds were within the normal range for most of the tested electrodes, but modulation detection thresholds were considerably elevated for some of the electrodes in one subject. Electrode pitch ranking results were rather atypical and showed the same pitch percept for many of the electrodes in each subject's implant. Interestingly, the pitch pattern across cochlea was non-monotonic in one of the subjects. Vowel and consonant identification scores were very poor with both clinical and modified programs.
Conclusions: The results from these VS patients were mostly atypical compared to non-tumor CI patients. In particular, ECAP waveforms showed abnormal morphologies and, in some cases, very long refractory recovery times (GREATER THAN 20ms). Anomalous ECAP results support the findings of animal studies that VS tumors can compromise peripheral auditory nerve cells in the cochlea. None of the three patients in this study received benefit from our modified CI program, which included a subset of pitch-ordered electrodes. The results suggest that deficits in the peripheral auditory nerve may underlie poor speech perception in these patients. Further research is required to better understand the reasons underlying poor speech perception in CI patients with tumors and to find strategies for improving patient outcomes.

M136. Ultra-High Resolution Models of Neural Activity in the Human Inner Ear

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Category: Auditory Prostheses
Background: Several factors that influence CI performance can only be evaluated with the help of computational models. To develop the most realistic models, base our models on high-resolution µCT scans of human temporal bones and analyze the importance of anatomical differences between the cochleae.
Methods: We reconstructed anatomically realistic models from high-resolution µCT scans of eight human temporal bone specimens contrasted with osmium tetroxide. CI electrode arrays were virtually inserted, and the electrical current spread was calculated using the finite element (FE) method. We then reconstructed the path of 500 spiral ganglion neurons and determined the extracellular potential at each node of Ranvier. With a biophysically motivated multi-

compartment model, which included the most important voltage-activated ion channels, we were able to derive excitation patterns of the auditory nerve fibers along the cochlea and visualize how action potentials are generated and conducted to the brainstem.
In the next step, we re-scanned 11 of our samples at the Munich Compact Light Source (MuCLS), a phase contrast CT system with a low-divergence, partially coherent, quasi-monochromatic X-ray source. We obtained scans with a voxel resolution of 6 µm, which allowed us to segment the cochlea and to identify the auditory nerve in its details.

Results: The stained temporal bone scans obtained with the MuCLS showed enhanced contrast compared to the µCT scans. The modiolar bone structure, Reissner's membrane, basilar membrane and especially the auditory nerve was clearly recognizable for segmentation. Electrically evoked excitation patterns were not smooth but rough with irregularities like cross-term stimulation. Moreover, the models predicted realistic variations between specimens, which are comparable, for example, in the thresholds of CI users.
Conclusions: Our findings are a major breakthrough for our understanding of the electrical excitation of large neuron populations. They are relevant for all types of electrical and optogenetic stimulation paradigms. With these predictions, we can anticipate the development of the next generation of CIs and coding strategies. In addition, our spectacular visualizations provide detailed and illustrative insights into the function of the most delicate sensory organ.

M137. Development of a Novel Pitch Discrimination Test for Cochlear Implant Users

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Category: Auditory Prostheses
Background: Music perception in cochlear implant (CI) users remains poor, despite advances in speech perception. Music presents a challenging task for cochlear implants, which requires an integration of rhythm, melody, harmony, and timbral elements of sound. One element that contributes to CI users' poor music perception is decreased pitch discrimination. However, there does not yet exist a screening test for CI users to assess pitch discrimination.
Methods: A musical stimuli composed of repeated notes of a C4 major scale was developed. The first semitone played was C4, and pitches were incrementally increased by one semitone until C5. The stimuli consisted of a pseudo-randomized number of repeats of each semitone (ranging between 5-10 repeats of each semitone) before proceeding to the next semitone. Each semitone was separated from the succeeding semitone by silence with a duration equal to the note itself. Stimuli were composed of pure tones, and were volume-roved using Fletcher-Munson curves. The test was distributed online through a Qualtrics survey. Performance metrics were used to assess the user's responses, including precision, recall, accuracy, and F1 score.
Results: Forty-four participants completed this survey, with 24 CI users and 20 normal hearing (NH) users. CI users tended to click the response button more often than NH users (20 vs. 13 clicks), though the range of number of responses for CI users was much higher than NH users (75 vs. 16). CI users had lower precision (0.49 vs. 0.86, p LESS THAN 0.001), recall (0.66 vs.

0.91, p LESS THAN 0.01), accuracy (0.84 vs. 0.97, p LESS THAN 0.001) and F1 score (0.51 vs. 0.88, p LESS THAN 0.001) compared to NH users. Average semitone resolution across a C4 scale was poorer for CI users compared to NH users (1.41 vs. 1.08 semitones). Amongst CI users, no significant differences in F1 score were seen between users with and without musical backgrounds (0.48 vs. 0.54, p=0.80). Additionally, no significant differences in the Pearson's correlation coefficient for F1 score by age of onset of deafness, duration of CI use, or length of deafness without CI use were observed (r= -0.39, p=0.08; r= -0.01, p=0.95; r=0.27, p=0.23).
Conclusions: In this study, we present the findings of a novel pitch discrimination test that can be administered in-clinic for screening evaluation of pitch discrimination. While NH users have superior pitch discrimination compared to CI users, there exists a wide spectrum of pitch discrimination ability amongst CI users. Future studies may explore what factors impact improved pitch discrimination in CI users. Our findings support the clinical application of a rapid assessment of pitch discrimination in CI users.

M138. A Novel Self-Unrolling Branched Cochlear Implant Electrode Design for High-Resolution Electrical Stimulation

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Category: Auditory Prostheses
Background: While cochlear implants (CI) have shown a tremendous success in hearing restoration for many patients with hearing loss, they still lack hearing resolution needed for improved hearing in noisy environments and for music appreciation. This limited hearing resolution is caused by current spread due to the conductive nature of the perilymph inside the cochlea, in which the stimulation currents spread and interfere with neighboring electrical contacts. This current spread undermines the effectiveness of deploying more contacts, posing a significant barrier in research for higher-resolution CI. Many studies have attempted to mitigate the current spread by reducing the distance between the electrical contacts and the modiolus where auditory neurons reside, but no significant result has been reported yet. Most of these studies were done using a linear electrode array, without a significant alteration to the fundamental electrode design. Our aim is to develop a novel electrode design that can not only deploy a high number of stimulation contacts but also is capable of a mechanism to mitigate the current spread, in order to achieve better stimulation resolution in CI.
Methods: Using our novel fabrication process, we successfully fabricated a CI electrode with a unique rolled-up, branched design that can support up to 200 contacts. Notably, it features an innovative self-unrolling property specifically designed to mitigate the current spread. By leveraging the elastomeric material properties, our electrode can self-unroll and fit inside the cochlea to deliver the contacts closer to the modiolus, without the use of a disruptive stylet wire as in perimodiolar electrode that can cause severe mechanical trauma to the cochlea. To test the feasibility of this self-unrolling mechanism to achieve closer contact-to-neuron distance, we implanted our electrode into a 3D-printed human cochlea phantom to observe the electrode's unrolling process and its final conformation.

Results: The electrode's unrolled conformation inside the cochlea phantom was imaged using a micro-CT scanner, and the cross-sectional images revealed that those branches that successfully unrolled had positioned themselves close to the modiolus.
Conclusions: Our novel self-unrolling, branched CI electrode design has demonstrated the feasibility of achieving a more reliable close contact-to-neuron interface through an electrode design-based approach. More design iterations are needed to further optimize the branch design and quantify the contact-to-modiolus distance, in order to ensure that a close interface can be achieved at all insertion depths. Additionally, a functional, animal version of the electrode needs to be fabricated and tested in vivo to quantify the spread of activation in auditory brain to assess the electrode design's capability to improve the stimulation resolution.

M139. In-Silico Framework for Benchmarking Optogenetic Hearing Restoration

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Category: Auditory Prostheses
Background: Optogenetic cochlear implants (oCIs) represent a promising means to better restore hearing in individuals impacted by severe sensorineural hearing loss than possible with electrical cochlear implants (eCIs). The wide spread of current and channel interactions in eCIs limit comprehension of speech in noisy environments and the enjoyment of music. By reducing the spread of neural activation, oCIs promise a greater number of independent stimulation channels.
Methods: A computational framework for the evaluation of oCIs in the human cochlea was developed using four main modules. First, a generic n-of-m sound coding strategy was implemented, which could be easily adjusted to evaluate various parameters. Second, a three-dimensional ray-tracing model of a reconstructed human cochlea was used to investigate light propagation. Third, a biophysical model of spiral ganglion neurons (SGNs) was built to simulate optogenetically evoked firing. Fourth, a similarity measure was developed to compare the input sound spectrograph to the output spikes pattern. Finally, these stages were integrated to generate a comprehensive model capable of processing an audio files dataset and computing a similarity score.
Results: The major findings indicate that the spatial spread of light using μLED- and waveguide-based oCIs is narrower than the electrical current spread. Moreover, the impact of variables such as emitter-to-SGN distance, emitter rotation, and scar tissue formation on the irradiance at SGNs was evaluated. The improved spectral resolution of oCIs compensates for the currently lower temporal fidelity of optogenetically driven firing.
Conclusions: The computational framework provides a valuable resource for researchers to explore the complex interplays of sound processing, light delivery, and optogenetic stimulation. This study supports the notion that optogenetic stimulation of the cochlea could improve the speech understanding of CI users.

M140. Feasibility of a Handheld Robotic Cochlear Implant Insertion

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Category: Auditory Prostheses

Background: Advances in cochlear implant (CI) technology and techniques have allowed for implantation of a broader cohort of patients, while preserving residual natural low frequency hearing. While initial outcomes are favorable, a subset of these patients develop further hearing loss. This is believed to be due to surgical trauma and the resulting cochlear biological response to the implant. Robotic insertion systems have thus been created to allow for atraumatic CI insertions. In this study, we assess the forces generated in a synthetic cochlea throughout implantation with both a handheld and fixated robotic system. Secondarily, we examine the importance of releasing the CI at the end of the insertion, and the forces this can generate.

Methods: Six surgeons with varying experience with the iotaSOFT insertion system (iotaMotion, Inc) were tasked with multiple CI insertions with 3 different techniques in a randomized order. These techniques include the current standard manual CI insertion and 2 robotic methods with the iotaSOFT insertion system in both a handheld and fixated configuration. Surgeons were asked to perform manual CI insertions similar to their hearing preservation cases, and all robotic insertions were set to a speed of 0.2 mm/sec. Each insertion was into a standardized 3D-printed synthetic cochlea attached to a load cell capturing insertion forces (mN). The forces collected were then analyzed to determine the maximum insertion force (mN), variation in force over each insertion (mN/sec), and the maximum force experienced on release of the implant (mN).

Results: Results showed that both robotic-assisted methods were found to have statistically significant reductions in maximum insertion force (handheld 57.38 mN, fixated 57.55 mN, manual 226.73 mN, p-value LESS THAN 0.001), and force variation (handheld 43.67 mN/s, fixated 33.81 mN/s, manual 264.58 mN/s, p-value LESS THAN 0.001). In addition, a similar statistically significant reduction was seen on the release of the device with the robotic systems in comparison to the standard manual technique (handheld 87.08 mN, fixated 66.83 mN, manual 160.12 mN, p-value 0.03).

Conclusions: The captured cochlear force loads suggest that a robotic insertion, either fixated or handheld, results in a significantly smaller force on the cochlea with less variation throughout the insertion when compared to current manual hearing preservation techniques. Implementing robotic systems such as this has the ability to allow for atraumatic CI insertions and better hearing preservation outcomes. In addition, this study highlight the importance of the CI release after insertion, and that a careless release can result in sudden traumatic forces transmitted to the cochlea.

M141. The Relationship of Neural Sensitivity and Focused Perceptual Thresholds: An Indicator for Future Cochlear Implant Programming

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Category: Auditory Prostheses

Background: The electrically-evoked compound action potential (ECAP) is an objective measure used in cochlear implant (CI) devices to measure the synchronized neural response to electrical stimuli. Recent studies have shown that ECAP responses may provide information about the efficacy of electrical stimulation. It was also demonstrated that perceptual focused thresholds can be used to select "poor" high threshold CI channels for deactivation and focused stimulation to optimize CI programming. In the current study, we investigate the relationship between ECAP peak amplitudes and perceptual focused thresholds with the ultimate goal of developing approaches to improve CI programming and speech in noise perception.
Methods: Measurements of 15 adult Advanced Bionics CI subjects were included. We used a standard forward masking artifact subtraction technique in monopolar stimulation mode to extract the ECAP responses from 50 stimulation sweeps on all clinically activated electrodes. The sweeps consisted of four frames with different masker-probe combinations with individually defined phase duration and masker-probe intervals. Stimulation levels were determined by assessing the loudness growth on every fourth CI electrode along the array and by interpolating and loudness-balancing intermediate electrodes. Measurements were performed at maximum comfortable loudness to achieve maximally large ECAP amplitudes. The perceptual focused thresholds were obtained with a sweep procedure using partial quadrupolar electrode configurations and current steering with a possible set of electrodes ranging from 2 to 15. Individual threshold profiles were extracted from an average of two apical- and two basal threshold sweeps.
Results: ECAP peak amplitudes and focused thresholds were correlated for a first intermediate analysis. Pearson correlation coefficients showed statistically significant relationships for all ears combined, with small ECAP amplitudes at high focused thresholds and vice versa. An opposite but not significant relationship between ECAP amplitudes and focused thresholds, where high ECAP amplitudes correlated with high thresholds and vice versa, was found for 4 ears.
Conclusions: The current results characterize the relationship between ECAP peak amplitudes and perceptual focused thresholds. The interplay of the two parameters revealed a significant trend of large ECAP amplitudes on channels with low focused thresholds, which might relate to the underlying neural health or density. This interpretation might support CI programming approaches, such as deactivating channels with high thresholds and small ECAP amplitudes and focusing the current for channels with low thresholds and large ECAP amplitudes, potentially improving speech perception. Further analyses to support the preliminary findings are currently ongoing.

M142. Cortical Temporal Mismatch Compensation in Bimodal Cochlear Implant Users: A Selective Attention Decoding and Pupillometry Study

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Category: Auditory Prostheses

Background: Bimodal cochlear implant (CI) users combine electrical stimulation in one ear with acoustic stimulation through either normal hearing or a hearing aid in the opposite ear. While bimodal stimulation typically improves speech perception, the degree of improvement varies significantly across subjects and can sometimes result in interference effects. This variability is associated with the integration of electric and acoustic signals, which can be influenced by several factors, including temporal mismatch between the two sides.
Methods: In previous work, we utilized cortical auditory evoked potentials (CAEPs) to estimate the temporal mismatch between the CI and the acoustic side (AS), based on differences in N1 latencies when listening with CI alone and AS alone stimulation (Dolhopiatenko et al., 2023). Building on this approach, the present study estimates the temporal mismatch and investigates the impact of compensating for this mismatch on speech perception.
In addition to traditional behavioral measures of speech understanding, this study employs selective attention decoding from electroencephalography (EEG), which has been shown feasible in bimodal CI users despite the presence of electrical artifacts from the CI (Dolhopiatenko and Nogueira, 2023). Unlike behavioral performance, selective attention decoding reflects not only speech intelligibility but also the allocation of cognitive resources to segregate speech streams such as attentional listening. Therefore, it may provide a more sensitive measure of the effects of temporal mismatch. Moreover, this study explores whether temporal mismatch compensation reduces listening effort, which is assessed via pupillometry. Changes in pupil dilation serve as an indicator of cognitive load during listening tasks.

Results: No significant effect of temporal compensation on behavioral speech understanding was observed. However, pupil dilation measurements indicated a reduced peak dilation in the compensated condition for some participants, although this finding was not consistent across the group. In contrast, the temporal response function of selective attention decoding revealed a more robust result, showing higher peak amplitudes when the temporal mismatch was compensated.
Conclusions: This study underscores the utility of CAEPs in estimating the temporal mismatch in bimodal CI users and demonstrates that compensating for this mismatch influences selective attention decoding, although no significant effect was observed in behavioral speech understanding.

M143. Cochlear Anatomy Impacts Neural Health and Current Spread at the Electrode-Nerve Interface in Children With Bilateral Cochlear Implants

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Category: Auditory Prostheses
Background: The objective of this study was to evaluate the impact of cochlear malformations on the ability of cochlear implants (CI) to deliver current to the auditory nerve. The effectiveness of this process depends on the ability of each electrode to target sites along the auditory nerve to

best portray sound cues. However, abnormal current pathways due to malformation of the cochlea may hinder the current's ability to reach neural cells or cause electrodes to stimulate overlapping populations of auditory nerve cells, obscuring sound cues. Despite frequent and successful cochlear implantation in children with abnormal cochleae, the impact of malformations on current spread remains poorly understood and programming stimulation parameters is clinically challenging. This study aimed to test the hypotheses that abnormal cochlear shape exacerbates current spread, correlates with reduced neural responsivity, and can predict the programmed electrical stimulation parameters.
Methods: CI stimulation parameters, electrophysiological recordings, transimpedance measurements and other relevant clinical information were assessed for a large cohort of children with bilateral CIs with either typically developed cochleae (n=184) and cochlear malformations (n = 27). A mixed effects modelling analysis was conducted. Child-specific models of voltage spread in the cochlea were developed by optimizing the tissue properties and dimensions of 3-D models of the implanted cochlea to accurately reproduce the spread of current in the child's cochlea as measured by the transimpedance measurements.
Results: Wider current spread was associated with increased auditory nerve electrophysiological thresholds (mean(SE) = 0.83(0.59), p LESS THAN 0.05) in the malformed cochlea, but not in the typically developed cochlea (p = 0.21). However, higher CI electrical stimulation levels were required for electrodes with wider current spread in the typical and malformed cochleae groups (mean(SE) = 5.50(0.77), p LESS THAN 0.001 for C-levels in the malformed cochlea; mean(SE) = 3.31(0.82), p LESS THAN 0.001 for C-levels in the typical cochlea; mean(SE) = 5.55(0.49), p LESS THAN 0.001 for T-levels in the malformed cochlea; mean(SE) = 4.73(0.38), p LESS THAN 0.001 for T-levels in the normal cochlea). Further, there was greater spread of CI current in the malformed cochlea group than the typical cochlea group in the mid (mean(SE) = 1.43(0.43) p LESS THAN 0.05) and apical portions of the array (mean(SE) = 1.18(0.53, p LESS THAN 0.05). Child-specific models of the voltage distribution in the cochlea indicated that the spread of current to the auditory nerve depends on child-specific anatomy.
Conclusions: The spread of current delivered by the CI in the cochlea is impacted by the of cochlear malformations, including electrode-nerve distance and extracochlear tissue properties. These differences can be captured by a child-specific model. The ability of CI electrodes to stimulate the auditory nerve is dependent on the anatomy of an individual CI user. CI programming protocols should account for these differences.

M144. Acoustic Stimulation of the Human round Window by Laser-Induced Nonlinear Optoacoustics

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Category: Auditory Prostheses
Background: For patients with profound hearing loss, the cochlear implant (CI) is a commonly used method to restore their hearing ability. However, CIs have technical limitations such as poor performance in noisy environments and a limited frequency resolution. A common approach to improve the listening experience is electro-acoustic stimulation (EAS), which is

feasible for CI-patients who still have residual hearing at low acoustic frequencies. EAS employs an acoustic stimulation in combination with the electrical stimulation provided by a CI and is beneficial for speech-perception in quiet and in noise compared to a stand-alone CI device. Here, we investigated an optical stimulation method for EAS, generating sound by the photoacoustic effect.
Methods: We used a pulsed laser to generate acoustic tones by inducing an optical breakdown in a small gel volume attached to the round window membrane. In order to generate sine wave acoustic tones at low acoustic frequencies we used an integral pulse density modulation (IPDM) technique. The IPDM converts the analog signal into identical pulses, but at varying time intervals. By controlling the laser with the resulting pulse pattern, we were able to generate acoustic tones of frequencies between 20 Hz to 1 kHz. We measured intra-cochlear pressure differences (ICPD) in three cadaveric temporal bones (TB) to determine the equivalent sound pressure level.
Results: Our setup was able to generate maximum sound pressure levels (SPL) of 90 – 140 dB SPL in all of the three tested temporal bones (TB) at acoustic frequencies of up to 1 kHz. Due to the modulation method it was possible to achieve a total harmonic distortion (THD) of LESS THAN 2%.
Conclusions: We could demonstrate that a sufficient acoustic output for EAS can be achieved using the optoacoustic effect inside the cochleae of cadaveric temporal bones. We were able to generate acoustic frequencies between 20 Hz and 1 kHz and levels up to 140 eq. dB SPL with low distortion. Further research focuses on improving the existing setup to expand the frequency range and the dynamic range.

M145. ALFIES Unwrapped: Recording Cortical Responses to Sustained High-Rate Stimulation in Cochlear-Implant Users

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Category: Auditory Prostheses
Background: Objective measures of neural responsiveness can help program the software for individual cochlear-implant (CI) users. Measuring thalamic or cortical responses typically requires short or slow-rate stimuli, reducing the correlation with behavioural responses, and requires an external EEG system. ALternating-Frequency Interleaved Electrical Stimulation (ALFIES) is a method for recording cortical responses to sustained fast-rate electrical stimuli in CI users. It uses two high-rate, interleaved, amplitude-modulated (AM) pulse trains that – when recorded with an EEG system – contain electrical artefacts at the AM frequencies but, importantly, not at their distortion product. It successfully extracts neural responses from these electrical artefacts and more closely matches the stimulation parameters of clinical coding strategies. To improve clinical applicability, we investigate recording ALFIES (i) with standard-rate EEG (vs custom-made hyper-rate EEG) (ii) the cochlear implant electrodes (iii) whilst separating distortion product components for parameter optimization.
Methods: 10 ears with cochlear implants (8 users, 2 bilateral) from Cochlear were stimulated at most comfortable level (MCL) with 2 interleaved AM high-rate pulse trains (respectively

modulated at F1=76/82/88 and F2=111/120/129Hz). We simultaneously recorded the responses from the implant itself (NIC4.3 research platform, recording in gaps between pulses) and a custom-designed 264-kHz EEG. The NIC4.3, 264kHz-EEG and down-sampled 2kHz-EEG data were investigated for significance of power at the frequencies of the quadratic (QDR: F2-F1=35/38/41Hz) and cubic (CDR: 2F1-F2=41/44/47Hz) distortion products between the two AM rates. The frequency-to-phase functions for these components were then used to calculate the group delay of the distortion products to estimate the locus of their neural generator.
Results: (i) Preliminary results suggest it is possible to measure cortical neural distortion responses to continuous high-rate electrical stimuli using 2048 kHz EEG data. (ii) When recording from the implant, 2 participants showed significant cubic distortion responses (CDRs) with group delays consistent with thalamic/cortical generators (16.9–54.1ms), and 2 different participants showed significant quadratic distortion responses (QDRs), one with a long group delay (34.1–42.1ms) and one with a short group delay (1.1-4.0ms). (iii) When recording with the hyper-rate EEG system, 6 ears showed significant QDRs (group delays: 15-78ms) and 5 showed significant CDRs (group delays: 5-70ms), all consistent with thalamic/cortical generators.
Conclusions: (i) The distortion products generated with the ALFIES method contain both quadratic and cubic components with group delays consistent with thalamic/cortical generators. (ii) It is possible to record neural responses to the same stimuli using the hardware of the cochlear implant itself, but these aren't consistent with EEG measurements, and may reflect different neural generators. (iii) It appears possible to record cortical responses to high-rate amplitude modulated pulse trains using "low-sampling-rate" 2kHz EEG systems.

M146. The Effects of Spatial and Contextual Cues on Listening Effort

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Category: Auditory Prostheses
Background: Listening outcomes are often assessed using speech intelligibility scores, which may not capture differences in listening effort (LE). LE is a key patient concern linked to fatigue, social isolation, and reduced quality of life. Critically, LE is currently lacking standardized clinical assessments. Factors contributing to increased LE also remain unclear. In real-world listening, prior studies have shown that spatial separation between target and masker improves intelligibility (spatial release from masking, SRM) and has the potential to reduce LE. However, these studies used subjective measures of LE and didn't control for contextual cues in sentence testing. Studies suggest that pupillometry, which uses pupil dilation as an index of LE, is more reliable than subjective LE evaluations, and sentence context (low vs. high) influences both percent correct scores and SRM. We aim to objectively assess the relationship between SRM and LE using pupillometry, while controlling for contextual cues during testing. We hypothesize that both spatial and contextual cues reduce LE: smaller pupil dilation when targets are spatially separated and larger dilation for low-predictable sentences due to fewer semantic cues.
Methods: Eight participants with typical hearing were recruited to listen to and repeat male-voiced target sentences in the presence of competing male-voiced 2-talker mixtures. The target sentences varied in context (coherent vs. anomalous) and were presented at 0° azimuth, while maskers were either co-located with the target at 0° or were at 90° (asymmetric-right). The target

and masker were presented at a 1 dB signal-to-noise ratio (SNR). 1 dB SNR was chosen to help listeners pick out the target. Speech intelligibility was assessed by the number of correctly repeated words, and pupil size was concurrently measured using an eye tracker.
Results: Preliminary data show SRM of around 31% increase in percent correct scores for both coherent and anomalous sentences. However, coherent sentences led to better performance than anomalous sentences within each spatial configuration. Greater pupil dilation (indicating higher LE) was observed in the co-located than the asymmetric-right condition, reflecting a reduction in LE along with SRM. Notably, we observed greater reduction in LE with anomalous than coherent sentences, even though SRM is comparable between two sentence types.
Conclusions: These results underscore the importance of assessing LE to capture insights beyond standard percent correct measures. The finding that SRM reduces LE has significant implications for hearing technologies such as cochlear implants, which currently do not adequately encode spatial cues. The encoding of spatial cues in cochlear implants might help manage exacerbated LE. Additionally, the study highlights the need to control for contextual cues when using sentence materials in testing.

M147. Remote Auditory Training to Improve Listening Comprehension of Adult Cochlear Implant Users

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Category: Auditory Prostheses
Background: Adults with cochlear implants (CI) often report various communication difficulties, even many years after the implantation. Auditory training for this group is rarely provided, and when it is, it mainly focuses on basic aural skills in the early period following cochlear implantation. There is a lack of data-based auditory training programs for adults. We aim to develop a data-based auditory training program to improve listening comprehension of progressively longer speech passages.
Methods: 15 CI users (postlingual and prelingual) who are considered good CI users with above 70% score of open set sentences understanding in quiet, (ages 18-60) are participating in an individualized intervention program with hierarchical and structured auditory training. The program consists of 16 individual sessions, including two assessment sessions conducted at a CI center and 14 online training sessions. Training materials include progressively longer listening passages (from 100 to 500 words each) with decreasing use of supportive speech-understanding strategies (e.g. two readings, prior context clues). Each session also provides guidance on using hearing in daily life. CI center assessment includes word recognition (HAB) and sentence comprehension (HeBio) tests presented in quiet and competing noise listening condition, and auditory working memory test (Digit Span), and two quality-of-life questionnaires. To assess the impact of the intervention program, listening comprehension was assessed two months before the intervention, at the first and final intervention period, and two months after its completion.
Results: Preliminary results from ten participants indicate increases in their listening comprehension span of speech materials (from 100-200 to 200-400 words) which were maintained at least two months after the intervention. Participants also reported increased

involvement in hearing-based activities- especially when it involved listening to recorded materials (e.g. Podcasts) after the intervention.
Conclusions: The remote auditory training provided is feasible and can contribute to adult CI users' auditory functioning.

M148. Auditory Outcomes in Cochlear Implantation for Children With Usher Syndrome

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Category: Auditory Prostheses
Background: Usher Syndrome type 1 (US1) is an inherited disorder, most commonly due to mutations in MYO7A, that leads to mild to profound sensorineural hearing loss within the first year of life, vestibular impairment, and early vision loss called retinitis pigmentosa (RP). The sensorineural deafness in US1 is due to abnormal development of the inner and outer hair cells of the cochlea.
Methods: A retrospective chart review was performed at a tertiary care hospital to identify children with US1 who had been implanted between 2000-2021. The impact of multiple disabilities, especially visual impairment, was studied and analyzed. Inclusion criteria included a diagnosis of US1, either clinically or genetically confirmed, and available audiogram data. Information including age of cochlear implantation (CI), complications of implantation, primary language (English, Spanish, sign language), vestibular status, and the presence or absence of visual impairment at the time of implantation were gathered. Cochlear implant outcomes were measured by pre- and postoperative hearing thresholds, AzBio sentence scores, vowel and consonant identification, pure tone averages (PTA), ling sounds, phonetically balanced kindergarten (PBK) test scores, and multisyllabic lexical neighborhood Test (MLNT). Exclusion criteria included any other co-morbidity that could lead to sensorineural hearing loss.
Results: 14 implanted patients with a history of US1 were identified. The average age at implantation was 3.3 years old (SD = 3.20), 60% of patients were implanted bilaterally sequentially, and 50% were female. Patients reported English (40%), Spanish (40%), and sign language (20%) as their primary language. The most common causative mutation of US1 was homozygous MYO7A mutation (n=3). Across all implanted patients with US1, there was a high gain of PTA scores and high AzBio and CNC percentages. Patients with previous diagnosis of RP (n=7) have greater improvement post-implantation compared to those without previous eye pathology. Post-operative CNC scores for those with eye pathology demonstrated high scoring on both words (average = 85%) and phonemes (average = 89%). AzBio percentages and PTA scores were significantly higher post-operatively for those presenting with eye pathology compared to those without history of eye pathology (p=.0179 and p = .0261, respectively).
Conclusions: Individuals with US1 greatly benefit from CI. Visual impairment appears not to have a negative impact on CI outcomes and may be a predictive factor for better outcomes. The presence of RP leads to early identification for US1 and therefore, allows an anticipated management of multiple sensory impairments. In addition, early visual impairment has been

shown to reshape the cortical representation of alternate sensory functions, including auditory cortex. The increased reliance on alternative sensory function might lead to an improved rehabilitation after CI.

M149. Decoding Auditory Selective Attention in Normal Hearing and Cochlear Implant Listeners

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Category: Auditory Prostheses

Background: We aimed to decode auditory selective attention from the single-trial EEG signals of individuals with cochlear implants (CI) to provide neurofeedback during their perceptual training of auditory selective attention. Communication difficulties in noisy situations are a common issue among CI users. Recent studies suggest that the inability to inhibit neural responses to background noise significantly contributes to this challenge, alongside degraded sensory input. Auditory selective attention may function as a central mechanism that inhibits neural responses to background noise.

Our previous research has demonstrated that neurofeedback training, tailored to target the attentional modulation of cortical auditory-evoked responses, can enhance auditory selective attention in the normal hearing population. Furthermore, this training effect transferred to better speech perception in noise. Building upon these findings, we sought to assess the effectiveness of neurofeedback training for CI users.

Although decoding auditory selective attention from an individual's EEG signal is a crucial initial step for valid neurofeedback and measuring attentional modulation, it has been unclear whether attention can be decoded from CI users' EEG due to possible degradation of their attention mechanisms as well as significant device-related artifacts. Therefore, our primary objective was to develop an attention-decoding algorithm that provides both feedback validity and interpretability to understand attentional modulation in a relatively large cohort of CI users.

Methods: For this purpose, we estimated a temporal response function that predicts the envelope-following EEG responses from the incoming speech envelope and decoded selective attention based on the model output. While recording their neural responses with 64-channel scalp EEG, participants were asked to attend one of two competing speech streams—a female saying "Up" five times and a male saying "Down" four times. We calculated the similarity between the model-predicted neural responses and single-trial EEG across all channels using correlation coefficients. Based on those correlations, the machine learning classifier predicted which speech stream a participant was attending to. To assess the accuracy of our predictions, we used leave-one-participant-out cross-validation, where we tested the model on one participant's data while training it on the others and repeated this process for all participants.

Results: The temporal response function successfully predicted time-locked envelope-following EEG responses that model attentional modulation in both populations. The time course of model

coefficients and topography of correlation coefficients showed a similar morphology to that of auditory evoked response. Our linear (logistic regression) classifier decoded auditory selective attention with 63% accuracy in normal hearing and 59% in CI listeners.
Conclusions: Our study shows we can decode auditory selective attention from CI listeners' single-trial EEG. Moreover, interpretable model coefficients verify the encoding of attentional modulation of auditory response. We are using this method for neurofeedback training of auditory selective attention in CI listeners and will present the training effects in the future.

M150. Differential Use of Auditory Feedback in the Real-Time Control of Speech Movements by Deaf Talkers With Cochlear Implants and Peers With Normal-Hearing

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Category: Auditory Prostheses
Background: Auditory input is essential to the acquisition and maintenance of speech production skills. The partial neurosensory restoration of hearing through cochlear implants (CIs) — regardless of whether the hearing loss (HL) is congenital or acquired — improves speech production, but some aspects of speech remain impaired in CI recipients relative to normal hearing (NH) talkers, even years after implantation. However, given that speech production by deaf talkers with CIs has for the most part only been studied acoustically, it remains unclear how, exactly, degraded auditory input impacts the online coordination and control of multi-articulator speech movements. In this pilot study, we used electromagnetic articulography to capture direct kinematic measures of jaw and tongue-tip movements during the production of intervocalic alveolar segments, with and without auditory feedback, by adults with CIs and congenital and acquired HL, and peers with NH.
Methods: Four adults with bilateral CIs (2 prelingually deaf; 2 postlingually deaf) and ten adults with NH produced 480 vowel-consonant-vowel (VCV) sequences, recorded using articulography, with variation in production rate (fast-normal) and syllable stress (first syllable stressed-unstressed). V was /ɑ/−/ɛ/ and C was /t/−/d/. Utterances were split between two listening conditions for the CI groups: speech processor turned on and turned off. NH controls performed the same speaking task with and without auditory masking. To quantify the effect of immediate auditory feedback on the coordination between the jaw and tongue-tip, the timing of the tongue-tip raising onset for C, relative to the jaw opening-closing cycle for the flanking vowels, was obtained in each listening condition for all experimental groups.
Results: Across experimental groups and listening conditions, any manipulation that shortened the jaw opening-closing cycle reduced the latency of tongue-tip movement onset, relative to the onset of jaw opening. Moreover, tongue-tip latencies were differentiated reliably across the manipulations in rate and stress, as well as phonetic structure. The absence of immediate auditory feedback, however, differentially impacted articulatory coordination in the two experimental groups: tongue-tip latencies were more variable in the NH group during masking, whereas tongue-tip latencies were less variable in both CI groups when speech processors were turned off.

Conclusions: Preliminary results suggest that the normal process of speech motor control makes use of immediate auditory feedback to regulate speech movement timing, and that this feedback-based regulation breaks down in deaf talkers with CIs, undoubtedly because of the degraded nature of auditory signals available through CIs. Two alternative, but not mutually exclusive, explanations are considered: (1) Processing degraded auditory feedback about one's own speech increases cognitive effort and that increased cognitive effort indirectly affects the precision of speech movement timing control. (2) Degraded auditory input leads to increased reliance on somatosensory inputs from the vocal tract to control speech movements.

M151. Electric Auditory Brainstem Response (EABR) Properties and Histology of a New 32-Channel Cochlear Implant System

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Category: Auditory Prostheses

Background: Research shows that increasing cochlear implant channels improves auditory performance and sound quality while reducing the effort needed for recognition. To evaluate a new 32-channel device, we implanted this device in minipigs and measured Electric Auditory Brainstem Response (EABR) to verify functionality. We also conduct histological analysis to assess tissue response and biocompatibility. This study aims to determine the properties of the 32-channel system.

Methods: The cochlear implant, ranging from 16 to 26 channels, was inserted using the round window approach, with the internal device coil and body placed at the minipig's forehead. EABR measurements were taken over three months, at intervals of two weeks to one month. Electric ABR responses were recorded by stimulating channels 1, 2, 8, 9, 14, and 15. Parameters included a pulse rate of 40 Hz, pulse width from 13 to 50 μs/ph, and pulse amplitude levels ranging from 0 to 255 current level. After cochlear explantation, H and E staining was performed for histological analysis.

Results: EABR amplitude was 1.56 times larger when stimulus pulse width increased from 25 μs/pulse to 50 μs/pulse ($p = 0.0313$). The amplitudes were 0.764 ± 0.558 μV for 25 μs/pulse and 1.193 ± 0.823 μV for 50 μs/pulse. Conversely, EABR threshold was significantly higher in the 25 μs/pulse group (p LESS THAN 0.001): 740 ± 242 μA and 513 ± 136 μA, respectively. The input-output growth was fitted to a sigmoidal exponential function (amplitude = max / (1 + exp(-k * (CL - CL0)))). The inclination (k) was significantly greater in the apical turn channels (112.80 ± 0.05 μA) than in the basal turn channels (57.14 ± 0.06 μA, p LESS THAN 0.001). The maximum EABR amplitude increased over two months after implantation (p LESS THAN 0.001). It was 0.552 ± 0.377 μV in the 1st month and 1.080 ± 0.647 μV in the 2nd month for the 25 μs/pulse group ($p = 0.0095$), and 0.988 ± 0.58 μV in the 1st month and 1.588 ± 1.121 μV in the 2nd month for the 50 μs/pulse group.

Conclusions: The 32-channel cochlear implant system using minipigs is an ongoing experiment. The EABR amplitude was significantly larger when stimulus pulse width increased, and the duration of implantation was longer over two months. The sigmoidal input-output growth was

1.97 times steeper in apical turn electrodes than in basal turn electrodes. These outcomes provide insight into understanding neural response with a 32-channel CI system. Unlike rodent models, minipigs have anatomical similarities to the human cochlea, allowing more accurate extrapolation to clinical applications.

M152. Computational Loudness Model of an Electrically Stimulated Cochlea

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Category: Auditory Prostheses

Background: Cochlear implants (CIs) are devices that restore the sense of hearing in people with severe sensorineural hearing loss. An electrode array inserted in the cochlea bypasses the natural transducer mechanism that transforms mechanical sound waves into neural activity, by artificially stimulating the auditory nerve fibers (ANFs) with electrical pulses. The perception of sounds is possible because the brain extracts features from this neural activity, and loudness is arguably the perceptual feature closest to the neural activity at the periphery of the auditory pathway.

Methods: A computational framework that uses a three-dimensional model and a simplified model of the electrically stimulated cochlea are used to reproduce loudness summation experiments performed by real CI users. These experiments studied the effect of rate of stimulation, electrode separation and amplitude modulation when using sequential stimulation (only one electrode active at a time). To obtain a loudness index, a spatio-temporal integration of the loudness contribution was performed. An exponential transform function was used to convert the instantaneous neural excitation density into loudness contribution in time steps of 200 μs. The simulated threshold of hearing (Th) and most comfortable loudness (MCL) levels for each electrode were determined using a proposed method where the loudness growth function (LGF) obtained at different stimulation rates were compared. The criteria was based on various features observed in published data of real CI users' LGFs.

Results: The dynamic range of all electrodes, which is defined as the difference in dB between Th and MCL level, monotonically increased with the rate of stimulation when using pulse trains stimuli. However, this increment was highly dependent on the shape and selectivity of the excitation profile. Using two-electrode interleaved stimuli, the computational model predicted almost no difference (bellow 1 dB) in loudness summation when separating the stimulating electrodes. Finally, the loudness index of amplitude modulated stimuli was closer to the loudness index obtained by non-modulated stimuli at peak level when the carrier stimulation rate was low and the stimulation current was high. Using the simplified model ended in similar results as with the three-dimensional model, however, the dynamic range and loudness summation was generally higher.

Conclusions: Results showed that the proposed computational model, using both the three-dimensional and simplified peripheral model, is able to reproduce a wide range of experiments with real CI users predicting loudness summation in sequential stimulation. The LGF at low stimulation currents was highly related to the increase of neural activity in the peripheral ANFs,

however, without the exponential transform function, it was not possible to obtain the rapid increase of the LGF slope at higher stimulation levels. This observation suggests that a more central mechanism in the auditory pathway influences the loudness perception.

M153. Electrical Modelling of Cochlear Implant Electrodes for Detection of Cell Occupation and Monitoring Stimulation Efficiency

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Category: Auditory Prostheses

Background: Cochlear implants (CIs) have achieved remarkable success as neural prostheses, globally utilized to restore sensorineural hearing loss through direct electrical stimulation of the spiral ganglion cells. However, they are known to elicit an immune response resulting possibly in fibrotic tissue formation in the cochlea that is linked to increased impedance of the electrodes, low stimulation efficiency and suboptimal outcomes. Electric Impedance Spectroscopy (EIS) is a versatile tool for intra- and post-operative diagnosis of cochlear implant functionality. Despite this, EIS is not used with its full potential especially for analysing the electrical properties of CIs and differentiating cell types adhering to the CI electrodes after implantation.

Methods: The study involves four CI electrode arrays from different manufacturers (MED-EL, Advanced Bionics, Oticon, and Cochlear). Impedance measurements were conducted using an HP4192A impedance analyzer across neighboring stimulation electrodes (SEs) in a frequency range from 5 Hz to 13 MHz. The electrical equivalent circuit (EEC) of the CI electrodes was modeled involving linear elements, focusing on wire resistances and capacitances, as well as the electrode-electrolyte interface, using two non-linear bilayer models (Cole-Cole and Schwan-Faraday). Finite element method (FEM) simulations were performed to further characterize the capacitive interaction between the electrodes and the surrounding medium.

Results: The results of the study show that the wire resistances and capacitances of cochlear implant (CI) electrodes vary across different manufacturers and designs. Finite element method (FEM) simulations demonstrated that capacitance contributions from the medium (electrolyte) become significant at higher frequencies, affecting the overall impedance. The study also found that the Cole-Cole and Schwan-Faraday bilayer models could adequately describe the impedance behavior across the frequency spectrum, with the Schwan-Faraday model providing slightly better accuracy. From these results a general, applying to all types of CIs, a nonlinear EEC model was derived that allows the determination of local impedances between neighboring CI array electrodes with an accuracy of LESS THAN 10% between 5Hz – 13Mhz.

Conclusions: In conclusion, our study developed and validated an electric equivalent circuit (EEC) model to describe the electrical properties of cochlear implant (CI) electrode arrays in a 2-pole configuration using precision impedance spectroscopy. The model accurately captured the impedance characteristics across a frequencies range between 5 Hz – 13 MHz, revealing distinct frequency domains influenced by the bilayer and resistive and capacitive properties of the arrays.

Our findings underscore the potential of integrating impedance spectroscopy into CIs for advanced diagnostics, though technical, manufacturing, and regulatory challenges remain.

M154. Contralateral Botulinum Toxin Injection Accelerates Recovery in a Animal Model of Facial Nerve Palsy

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Category: Clinical Otolaryngology & Pathology

Background: Botulinum toxin (BTX) is commonly used to treat facial palsy (FP) in conjunction with oral steroids, yet its exact role in accelerating recovery via contralateral BTX injection is not fully understood. This study evaluates the efficacy of contralateral BTX injections in a rat model of peripheral FP and examines its impact on neuromuscular junction regeneration and cortical involvement.

Methods: Adult Sprague-Dawley rats were divided into three groups: (1) Control group (CG), (2) Facial palsy group (FPG), and (3) Facial palsy with contralateral BTX injection group (FPBG). Peripheral FP was induced by crushing the main trunk of the facial nerve for 1 minute. One day post-injury, 20 mouse units of BTX were injected into the contralateral side of the face in the FPBG group. Functional assessments were conducted at 3 days, 1 week, 2 weeks, and 4 weeks post-injury, including vibrissae movement observation and facial symmetry measurements. Immunofluorescence (IF) study was used to assess neuromuscular junction regeneration, focusing on SY38/a-bungarotoxin ratios. H and E staining was performed on facial nerve samples across all time points to evaluate histological changes, and Western blot analysis examined protein levels of Pituitary adenylate cyclase-activating peptide (PACAP) Galanin, and Calcitonin gene-related peptide (CGRP). IF was used to evaluate c-fos and NeuN expression in the brain's motor cortex.

Results: FP was induced in all rats. At 3 days and 1 week, vibrissae movement scores were significantly reduced in FPG and FPBG compared to controls. By 2 and 4 weeks, FPBG showed significant improvement with facial symmetry resembling controls. Neuromuscular junction regeneration in FPBG improved at 2 weeks, with SY38/a-bungarotoxin ratios nearing control levels by 4 weeks. Western blot revealed increased PACAP, Galanin, and CGRP in FPG at 3 days, 1 week, and 2 weeks, while FPBG showed lower levels, indicating that BTX modulates the neurochemical response to FP. H and E staining showed severe inflammation and nerve degeneration in FPG at 3 days and 1 week, with gradual recovery by 4 weeks. In FPBG, nerve damage was reduced, and regeneration was observed by 2 weeks, matching controls by 4 weeks. IF analysis of the motor cortex revealed increased c-fos and NeuN expression in FPG, indicating neuronal stress. FPBG showed reduced c-fos expression at all time points.

Conclusions: This study confirms that a 1-minute nerve crush induces FP in rats, and contralateral BTX injection significantly accelerates recovery, as evidenced by functional, histological, and molecular findings. BTX promotes faster neuromuscular junction regeneration,

reduces nerve inflammation and degeneration, and modulates the central nervous system's response to injury. Further research will explore the underlying mechanisms.

M155. Seasonal Variation in Peripheral Vestibular Disorders Based on Korean Population Data

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Category: Clinical Otolaryngology & Pathology
Background: Comprehensive studies in which the seasonal variation in peripheral vestibular disorders was evaluated using data from an entire population are insufficient. The seasonal variation in peripheral vestibular disorders based on data from the entire Korean population was investigated in the present study.
Methods: Retrospective data from the National Health Insurance Service of Korea from 2008 to 2020 was analyzed. Benign paroxysmal positional vertigo (BPPV), vestibular neuritis (VN), and Meniere's disease (MD) were defined based on diagnostic, treatment, or audiovestibular test codes. The seasonal incidence for each peripheral vestibular disorder was calculated among all study subjects.
Results: For the entire study cohort, the incidence of BPPV was significantly higher in spring (odds ratio [OR] = 1.031, 95% confidence interval [CI] = 1.026–1.037), autumn (OR = 1.024, 95% CI = 1.019–1.029), and winter (OR = 1.051, 95% CI = 1.046–1.056) than in summer. The incidence of VN was significantly lower in winter (OR = 0.917, 95% CI = 0.907–0.927) than in summer. The incidence of MD was significantly higher in spring (OR = 1.027, 95% CI = 1.015–1.039) and autumn (OR = 1.029, 95% CI = 1.017–1.041) and significantly lower in winter (OR = 0.919, 95% CI = 0.908–0.931) than in summer. Differences were also observed in seasonal variation based on sex and age.
Conclusions: Significant seasonal variation occurred in peripheral vestibular disorders including BPPV, VN, and MD based on the entire Korean population data. Furthermore, seasonal variation showed differences based on sex and age.

M156. Identifying Barriers to Vestibular Rehabilitation Therapy in South Florida

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Category: Clinical Otolaryngology & Pathology
Background: Vestibular rehabilitation therapy (VRT) is an exercise-based treatment aimed to alleviate vestibular symptoms such as dizziness and imbalance. Otolaryngologists prescribe VRT to patients with diagnoses such as vertigo, Meniere’s Disease (MD), vestibular neuritis (VN), vestibular schwannoma (VS), mild traumatic brain injury (mTBI), benign paroxysmal positional

vertigo (BPPV), and vestibular migraine (VM). VRT is typically performed by physical therapists and incorporates head-eye movements to enhance gaze and postural stability as well as improve symptoms of dizziness and activities of daily living. It is estimated that 35.4% of the United States (US) adult population suffers from vestibular dysfunction that could benefit from VRT. Unfortunately, adherence to VRT is reported to be less than 50%. This study aims to identify and assess potential social, epidemiological, and economic barriers to VRT in the South Florida population to increase therapy attendance and compliance.
Methods: Through a retrospective chart review (IRB #20230698), we analyzed 243 patients at the University of Miami Ear Institute who were prescribed VRT in a ten-year period between 2012-2022. Demographics and pertinent healthcare data were collected. Chi-square tests and logistic regression were conducted to ascertain relationships between sociodemographic or health factors against VRT attendance.
Results: Overall, 1.6% (n=4) of patients had been diagnosed with BPPV, 2.5% (n=6) with mTBI, 3.3% (n=8) with vestibular migraine, 16.9% (n=41) with vestibular schwannoma, 32.3% (n=79) with vestibular neuritis, 33.7% (n=82) with Meniere's disease, and 9.5% (n=23) were classified as other. Patients mostly identified as White (81%) and Hispanic or Latino (47%). Forty percent (n=98) of all patients followed through with VRT, with an average of 8 (±15) visits. Chi-square tests revealed no significant differences between VRT attendance and sex, race, or ethnicity. Differences in VRT attendance was noted, however, across different insurance types ($X^2(27) = 59.41$, p = LESS THAN 0.001) as well as different diagnoses ($X^2(17) = 30.96$, p = 0.020). Simple logistic regression was subsequently performed to examine insurance types and vestibular diagnoses against VRT attendance. Blue Cross Blue Shield (β = -2.3914, p LESS THAN 0.001), CIGNA (β = -2.1972, p = 0.004), Medicare (β = -1.1474, p = 0.035), Oscar Health Exchange (β = -2.4204, p = 0.039), and United Healthcare (β = -2.5455, p = 0.001) were significantly associated with lower VRT attendance compared to the reference group (Aetna). Lastly, although not statistically meaningful, individuals with MD and VM, VN, and VS were less likely to attend VRT as compared to individuals with BPPV and TBI.
Conclusions: Identifying and understanding sociodemographic and health factors influencing low patient attendance/adherence to VRT is essential. Associations between insurance type and VRT attendance suggest that higher costs or administrative hurdles may be affecting patients' decisions to attend VRT. Underlying individual-level predictors remain unclear, highlighting the need for further prospective investigation.

M157. Predicting Variability in Pediatric Cochlear Implant Outcomes Through Synchronous Brain Activation Patterns: Insights From fNIRS

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Category: Clinical Otolaryngology & Pathology
Background: Pediatric cochlear implant (CI) outcomes vary widely, and the neural underpinnings of this variability remain poorly understood. This study examines brain activation patterns in children with CIs and normal hearing (NH) controls during auditory tasks to identify neural predictors of CI outcomes.

Methods: Eighteen pediatric CI users and 17 NH controls underwent auditory testing (non-speech sound discrimination and sentence recognition) while functional near-infrared spectroscopy (fNIRS) data were collected. Estimated response amplitudes (ERAs) were analyzed to identify brain activation patterns associated with task performance and group differences.
Results: CI users showed poorer sentence recognition but comparable non-speech sound discrimination to NH controls. Brain activation patterns differed significantly between groups. CI users showed more focal activation in the right temporal lobe during non-speech sound processing and greater reliance on left parietal and frontal regions for sentence recognition, suggesting compensatory mechanisms. In contrast, NH individuals showed more distributed activation patterns across multiple brain regions for both tasks. Importantly, activation in specific brain regions significantly predicted behavioral accuracy in CI users but not in NH controls.
Conclusions: This study provides novel insights into the neural mechanisms underlying auditory processing in CI users and highlights the role of specific neural circuits in explaining variability in CI performance. The distinct activation patterns observed in CI users suggest a reliance on compensatory mechanisms for speech processing. These findings have important clinical implications for developing targeted rehabilitation strategies and improving CI outcomes in children.

M158. DMSO Does Not Aid in Reducing Decalcification Time of Human Temporal Bones

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Category: Clinical Otolaryngology & Pathology
Background: The standard method for decalcifying human temporal bones for histology typically employs ethylenediaminetetraacetate (EDTA) as a chelating agent due to its superior ability to preserve protein and DNA integrity compared to other acids. However, EDTA requires, on average, about one year for complete decalcification, which can lead to protein degradation and increased signal noise in immunohistochemistry. To address this issue, we hypothesized that a penetration enhancement vehicle could accelerate decalcification while preserving proteins and DNA. Dimethylsulfoxide (DMSO) was identified as a potential candidate, given its effectiveness in enhancing fixation with formalin and paraformaldehyde.
Methods: Three pairs of human temporal bones from the Anatomy Bequest Program at the University of Minnesota were utilized. For each pair, one bone was assigned to the experimental group and the other to the control group. Control group bones were decalcified in 500 mL of EDTA, while experimental group bones were decalcified using a solution of 10% DMSO (50 mL DMSO and 450 mL EDTA). Both groups were gently oscillated throughout the decalcification process to maximize EDTA infiltration. A standard calcium test was performed to assess decalcification: 500 µL of the sample EDTA was combined with 1 mL of citric phosphate and 2.5 mL of ammonium oxalate and then observed for precipitation after 2 hours or overnight.
Results: The control group bones required between 9 to 12 months (mean: 10.3 months) for full decalcification. In the experimental group, two specimens remained solid after 14 months, while one decalcified in 10 months. Given these prolonged decalcification times, we decided to

discontinue further calcium tests for the experimental group and these bones were further decalcified using the standard method (EDTA only).
Conclusions: A 10% concentration of DMSO mixed with EDTA does not expedite decalcification time and may even prolong it. Future studies should explore different DMSO concentrations to better understand their impact on decalcification speed.

M159. Primary Culture of Inner Ear Schwannoma

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Category: Clinical Otolaryngology & Pathology
Background: Vestibulocochlear schwannomas (VS) are benign tumors that develop from Schwann cells of the eighth cranial nerve. While VS typically occur in the internal auditory canal (IAC) or cerebellopontine angle (CPA), a subset forms within the inner ear, known as inner ear schwannomas (IES). These tumors usually grow slowly, and their location significantly impacts treatment strategies, including tumor control, hearing preservation, and maintaining vestibular function. Despite the clinical importance of IES and the anatomical and epidemiological differences from "classical" VS, little is understood about their biological characteristics.
Methods: Primary cells from tumor samples of four patients with IES were cultured for 28 days. Schwannoma cell proliferation and stability, along with monocytic cell involvement, were assessed through immunofluorescence staining and semiquantitative image analysis.
Results: In all samples, viable cells could be cultured for at least 28 d. Primary cells showed similar morphological features and proliferation. Sequential staining after 7 d, 14 d and 28 d showed stability of cultured schwannoma cells.
Conclusions: This study presents the first successful establishment of reproducible and stable primary cultures of inner ear schwannomas from different locations within the inner ear. This method offers a valuable in vitro model system for investigating biological properties of IES.

M160. A First Look at Human Inner Ear Pathology in POU4F3 Variants: Findings From Three Human Temporal Bone Donors

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Category: Clinical Otolaryngology & Pathology
Background: Genetic variants account for nearly half of sensorineural hearing loss (SNHL) cases worldwide. The POU4F3 gene, a class IV POU domain transcription factor, is essential for inner ear hair cell survival. Mutations in POU4F3 underlie DFNA15, an autosomal dominant non-syndromic hearing loss with variable clinical presentations. While animal models offer insights, human temporal bone histopathology for POU4F3 mutations has not been reported.

This study describes the histopathological findings in three donors with POU4F3 variants, complementing existing clinical and animal data.
Methods: Sixty-nine temporal bone donors with histories suggestive of hereditary hearing loss were identified. Samples were obtained via buccal swabs (n=29) or frozen muscle specimens (n=40) and Whole Exome Sequencing was performed using the Illumina NextSeq 500. Temporal bones from candidate donors were embedded in celloidin, sectioned, and stained with hematoxylin and eosin (H and E). Clinical records and family histories were reviewed using data from the NIDCD National Temporal Bone Registry. Histopathological analysis was conducted to identify inner ear abnormalities, and differential interference contrast (DIC) microscopy was used to assess hair cell and supporting cell survival. A machine learning algorithm in Dragonfly 3D was used to evaluate spiral ganglion neuron (SGN) counts, which were then compared to age-matched controls.
Results: Three donors with POU4F3 variants were identified: two with the c.602T GREATER THAN C p.Leu201Pro missense variant (likely pathogenic) and one with the c.709T GREATER THAN G p.Ser237Ala (variant of unknown significance). The cases included two females and one male, all in their 10th decade of life. Hearing loss onset ranged from childhood to middle adulthood, with a strong family history suggesting autosomal dominant inheritance. Audiological history showed progressive bilateral SNHL, varying from moderate to profound, with better preservation of lower frequencies.
Histopathology revealed consistent findings: a flat organ of Corti in the basal cochlea with no surviving hair or supporting cells. In the apical regions, inner hair cell survival increased to 65-80%, while outer hair cell survival remained below 30%. Supporting cells were absent in the basal turn; toward the apex, some regions showed a collapsed tunnel of Corti with partial differentiation into supporting cells but without normal architecture. Other areas had more preserved cytoarchitecture but with evident loss of outer pillar cells.

SGN counts ranged from 10,550 to 15,820 (raw counts, multiplied by ten, as only every 10th section was counted, no correction factor used). These values corresponded to 61.98% to 96.5% of the SGN counts found in age-matched controls.

Conclusions: Our analysis presents the first human histopathological insights into two POU4F3 variants, revealing severe hair cell loss and supporting cell disruption, especially in the basal regions. These findings enhance our understanding of the underlying pathology and raise important questions about potential therapeutic approaches for this patient group.

M161. Evaluating Underexplored Factors Contributing to Sudden Sensorineural Hearing Loss Recovery

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Category: Clinical Otolaryngology & Pathology
Background: Sudden sensorineural hearing loss (SSNHL) affects 5-20 in 100,000 people annually with a standard treatment comprising oral steroids, intratympanic (IT) injections, or a

combination of both. Despite established treatment modalities, patient outcomes and hearing recovery vary dramatically. This study aimed to assess how hearing recovery outcomes are affected or influenced by clinical presentation and treatment course, including initial severity of hearing loss, time from symptom onset to treatment, and number of IT injections.
Methods: Through a retrospective chart review (#20230698), 255 patients at the University of Miami Ear Institute with SSNHL who received IT injections were identified. Demographic information, risk factors/medical comorbidities, audiometric measurements (i.e., pure-tone averages and word recognition scores), and detailed SSNHL history were collected. Pearson correlation and logistic regression models were generated to examine how hearing loss severity, time from symptom onset to first IT injection, and total number of IT injections received were predictive of SSNHL recovery. Patient recovery was labeled as none (≤10 dB PTA recovery), partial (return to serviceable hearing, ≥10 dB PTA recovery, or ≥10% WRS recovery), or complete (≤10 dB PTA from unaffected ear and ≤10% WRS from unaffected ear). Serviceable hearing was established as ≤50 dB PTA and ≥50% WRS. Recovery categories were selected based on American Academy of Otolaryngology-Head and Neck Surgery (AAOHNS) hearing classification guidelines.
Results: Collectively, the cohort was comprised of 117 males (45.9%) and 138 females (54.1%), at an average age of 52.1 years old (± 12.3). Mean PTA of the affected ear at initial presentation was 61.8 (± 41.3) dB, with a final PTA of 52.6 (± 42.3) dB following treatment. The average time from symptom onset to first injection was 15 weeks (± 47.5) and mean number of IT injections received was 2.8 (± 1.4). Following logistic regression to investigate the relationship between initial SSNHL severity and recovery, we observed no significant relationship between serviceable hearing at the time of presentation and achieving complete or partial recovery (Estimate = -0.402, SE = 0.416, p = 0.334). There was a slight but statistically significant positive correlation between final PTA and delayed time to treatment (Pearson's r = 0.145, p = 0.021). Interestingly, multinomial logistic regression showed total injections was a significant predictor of no recovery, where each additional injection increases the odds of no recovery by 57%. There was no significant correlation between final PTA and the total number of injections received (Pearson's r = -0.049, p = 0.434).
Conclusions: SSNHL outcomes are highly variable despite an established treatment paradigm. Patients experiencing symptoms of SSNHL and clinicians should be wary of potential risks associated with delaying treatment. Further investigation of SSNHL recovery and contributory factors is warranted.

M162. Improvement of Patient Reported Outcomes of Bimodal Ci-Users Compared to Binaural Hearing Aids: A Randomized Controlled Trial

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Category: Clinical Otolaryngology & Pathology
Background: Replacement of bilateral hearing aids (HAs) with a cochlear implant (CI) and a hearing aid in the bimodal solution is a challenging decision especially in the case when some benefit of the bilateral hearing aid treatment exists. It is not given that patients report benefits of

the bimodal solution in all situations. Patients can have improvement in speech intelligibility in challenging conditions, but may report poorer outcomes on localisation and quality of sound in special listening situations. This study investigates if patients with new binaural replacement hearing aids gain a significant subjective advantage using the SSQ-12 questionnaire when receiving a cochlear implant to the worst hearing ear and a new and recently fitted hearing aid to the better hearing ear.
Methods: The population consist of 56 bilateral HA users, mean age of 63.4 years (SD 17.1), referred for CI and randomized to a short period of one month vs. a longer period of four months of HA use of new replacement hearing aids before cochlear implantation on the poorer hearing ear. The existing hearing aids were replaced with new HAs (Phonak Link M or GN (ReSound LiNXQuattro, ENZOQ) fitted to target using NAL-NL2 and verified using Real Ear Measurements (REM).
The patients answered the SSQ-12 and speech intelligibility was tested using HINT determining the 70% correct word recognition score before and after HA replacement with follow-up at one- or three-months post HA fitting. The patients were tested again 3,6 and 12 months following cochlear implant switch-on.

Results: Overall patients reported a small but significant improvement in SSQ-12 total scores of 0.55 (n=54) (95% CI 0.2 to 0.9) at 1 month post fitting of new hearing aids. This was not improved for the group (n=27) using the hearing aids for additional 3 month. However, at 3 months after switch on of the CI on the worst hearing ear, interim analysis of data (n=28) shows a robust and significant improvement in SSQ-12 total scores of 2.2 (95% CI 1.2 to 3.2) compared to the SSQ-12 scores with new replacement HAs. Speech, spatial and quality domaines of the SSQ-12 all contributed significantly to the improvement in SSQ-12 total scores. There was no significant effect of the period of use with new hearing aids on the outcomes following CI. SNR70% using HINT improved -1.8 dB (95% CI -3.0 to 0.0 dB) with the new replacement HAs, and SNR70% improved additional -10.9 dB (-16.7 to -5.1) compared to the situation with new HAs.
Conclusions: Replacement of HAs only give minor improvement of SSQ-12 scores. However, CI+HA in the bimodal solution gives a clinical important increase of SSQ-12 scores including improvement in speech recognition and spatial hearing as well as improvement of SNR70% values.

M163. Serum Level of Mmp-9 and its Genetic Polymorphism as a Biomarkers of Neuroplasticity in Prelingual Deafness Treatment by Cochlear Implantation

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Category: Clinical Otolaryngology & Pathology
Background: Molecular and genetic biomarkers of neuroplasticity in congenitally deaf children treated with cochlear implantation (CI) would allow to implement better clinical management, taking into account individual, personalized needs, especially giving them better chances of

spoken language rehabilitation. The objective of the study was to verify the prognostic value of carrying a certain variant of MMP-9 gene and plasma level of matrix metalloproteinase 9 (MMP-9), measured at cochlear implantation, to the outcome of speech and language rehabilitation after 18 months of CI use in long term follow-up.
Methods: We performed a prospective observational study analysis of serum activities of MMP-9 at CI activation, 8, and 18 months after CI activation in the cohort of 61 children, diagnosed with bilateral profound sensory- neural non-syndromic hearing loss, aged below 2, treated with unilateral cochlear implantation. Language acquisition was assessed with Little Ears Questionnaire (LEAQ). We studied associations between serum activities of MMP-9 n the aforementioned intervals and LEAQ scores over follow-up intervals of the implanted children. In the other group of 100 deaf born children enrolled according to the same inclusion criteria association analysis of functional MMP9 rs3918242 variant and the child's auditory development measured at CI activation and 1, 5, 9, 14 and 24 months post CI activation with LittlEARS Questionnaire (LEAQ) was conducted
Results: Correlation analysis shows that there is a significant relation between plasma level of MMP-9 measured at cochlear implantation and LEAQ score in 18 month follow up (rho= - 0.25, p LESS THAN 0.05). Statistical analysis in the subgroup implanted after 1 year of life (n=53) showed significant association between MMP9 rs3918242 and LEAQ scores at 1 month (p=0.01), at 5 months (p=0.01), at 9 months (p=0.01) and at 24 months (p=0.01) after CI activation. No significant associations in the subgroup implanted before 1 year of life were observed. Multiple regression analysis (R2 = 0.73) in the subgroup implanted after 1 year of life revealed that MMP9 rs3918242 was a significant predictor of treatment outcome.
Conclusions: MMP-9 plasma level measured at cochlear implantation below 150 ng/ml predisposes deaf children to good response to cochlear implantation after 18 months follow-up. C/C rs3918242 MMP9 predisposes their deaf carriers to better CI outcomes, especially when implanted after the 1st birthday, than carriers of C/T rs3918242MMP9

M164. Mechanisms of Medial Olivocochlear Reflex Enhancement Based on Temporal Prediction - An Investigation by Simultaneous Measurements of Delta-Band Brain Rhythm and Brainstem

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Category: Otoacoustic Emissions
Background: Medial olivocochlear (MOC) fibers are efferent projections that emerge from the brainstem and extend to the outer hair cells (OHCs). The MOC fibers are activated by acoustic stimulation and exert an inhibitory effect on OHC motility. This effect is called the medial olivocochlear reflex (MOCR). In our previous study, the MOCR and phase locking of delta activity in cortical regions showed a similar decreasing tendency with increasing jitter added to the preceding sound sequence. Efferent nerves connect from the cortical region to the MOC bundle via several brainstem nuclei. Given this, it is assumed that a comprehensive measurement of the activity from the brainstem to the cortex is necessary to identify the neural basis for the MOCR regulation. Therefore, in this study, the cortical delta-wave, auditory brainstem response (ABR) and MOCR were measured simultaneously and analyzed their temporal correlation.

Methods: As stimuli for the ABR elicitation, clicks with different interstimulus intervals (ISIs) based on maximum length sequences (MLS) were used. The duration and the sound pressure level (SPL) of the MLS-clicks were set to 508 ms and 60 dB, respectively. The MLS-clicks were presented in two conditions alternately. In the Regular condition, ISIs among the MLS-clicks was fixed at 500 ms. In the Irregular condition, the ISI was randomly selected from 200, 500, and 900 ms at each trial.
MOCR was assessed noninvasively using otoacoustic emissions (OAEs), which are sounds that originate in the cochlea and reflect OHC motility. OAEs evoked by clicks presented right after the MLS-clicks offset were measured. The presentation rate and SPL of the clicks was 40 Hz and 60 peak equivalent dB, respectively. OAE level differences between the irregular and regular condition can be considered as the MOCR changes (ΔMOCR). Delta-waves synchronized with the MLS-clicks onset were measured. The band-pass filter between 0.5-30 Hz was applied to the recorded signals and calculated the phase locking value (PLV). One measurement time was eight minutes-long, and the time variation of the PLV of delta-wave, the wave-V amplitude of the ABR and the ΔMOCR were estimated.

Results: The time variation of ΔMOCR was positively correlated with that of the PLV of delta-wave. On the other hand, no correlation was observed between the time variation of the ΔMOCR and the wave-V of ABR.
Conclusions: MOCR strength in the Regular condition increases compared to the Irregular condition, when the PLV of delta-wave increases. Given the wave-V of the ABR is said to originate from the inferior colliculus, its no correlation with the temporal variation of MOCR suggests that the inferior colliculus dose not contribute to the predictive control of MOCR. Delta-band brain rhythms may predictably regulate MOCR through the direct efferent pathway from the cortex to MOC neurons.

M165. Preliminary Evaluations of Speech, Language, and Hearing Functions of Students in Deaf Schools

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Category: Development: Human Subjects
Background: Longitudinal evidence supports that communication domains are connected to each other in the development of children with hearing impairment (HI). For children with HI, speech production and perception are largely associated with cognitive and language abilities. One major research and clinical gap is how speech production, auditory perception, and language processing are developed for children with HI, particularly those in the Deaf School, a unique education and communication environment. The primary goal of this study was to evaluate the functions in speech, language, and hearing for hearing-impaired children in the Deaf School at Nanjing, China, while our long-term goal is to investigate how these functions are developed with practical and clinical training.

Methods: We recruited 27 children and adolescents (1st grade to 5th grade; age range: 6-14 years) with HI from Nanjing School for the Deaf. They are all Mandarin Chinese native listeners who have mild-to-profound hearing loss, and there are no diagnosed cognitive or behavioral disabilities. In this study, a comprehensive evaluation was conducted to identify weaknesses of these HI students in speech, language, and hearing functions. We conducted evaluations on four domains besides pure tone audiogram: speech perception, language, literacy, and cognitive abilities.
Results: For cognitive domain, three of the 26 students' scores are lower than or equal to 90 percent of the norm group as indicated in Raven's Progressive Matrices. In 22 students who attended speech perception evaluation, only a small portion could complete the speech recognition tasks. For example, two students completed Mandarin Hearing in Noise test (MHINT-C) tasks, five did for Mandarin Tone Identification Test (MTIT), and eight completed Mandarin Early Speech Perception (MESP) test with their percentage correction ranging broadly from 0-100%. In vocabulary evaluation, only 16 students in 22 completed Mandarin PPVT-4 tasks with standard oral administration, and for 15 students' raw score, age equivalents are remarkably lower than their chronological ages. Narrative tasks yielded floor effects in intelligibility and story components.
Conclusions: Based on the preliminary pre-treatment evaluation, researchers found relative strength in cognitive domain compared to all other domains. Lexical tone discrimination and overall speech perception are weaknesses of the recruited student participants, and thus better lexical tone discrimination in word-level minimal pairs is considered for the school-based intervention program design in our next step. The profiles for student participants' communication abilities call for further attention on the student populations in Deaf schools in China and exploration on school-based interventions.

M166. Preferences for Loudness and Pitch Vary Across Cultures

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Category: Psychoacoustics
Background: Human preferences for sounds may shape musical systems and influence the designs of sound-producing objects in our world, such as machinery and household appliances. Yet it remains unclear why humans have the auditory preferences they do, and whether any such preferences are universal. To address these issues, we tested preferences for natural sounds and various basic sound features, including pitch height, loudness, and roughness (amplitude modulation), across cultures.
Methods: Participants completed experiments in which they rated how much they liked or disliked sounds. Participants were drawn from five groups: 1) the USA, 2) a small rural town in Bolivia, 3) an Indigenous community in the Bolivian Amazon (the Tsimane'), 4) an Indigenous community in the Peruvian Amazon (the Shipibo), and 5) Shipibo individuals who had lived in larger Peruvian cities like Pucallpa and Lima. Some experiments presented synthetic sounds: pure and complex tones and bandpass noise that varied in frequency, loudness, and amplitude

modulation. Participants also rated the pleasantness of a large set of environmental sounds (e.g., animal vocalizations, machines, running water).
Results: Preferences for sounds varied across cultures. In particular, preferences for loudness and pitch height were opposite between Amazonian and USA participants: Tsimane' and Shipibo participants preferred high-pitched and loud sounds, whereas participants in the USA preferred low-pitched and quiet sounds. Participants from the small Bolivian town, as well as Shipibo who had lived in large cities, demonstrated preferences that were intermediate between USA participants and the Amazonian populations who had never lived away from their villages. Control tasks and audiometric tests indicated that differences between groups cannot be explained by differences in task comprehension, stimulus interpretation, or hearing thresholds. Cross-cultural variation extended to recorded environmental sounds, and was partly explained by variation in preferences for frequency (sounds with more low-frequency energy tended to be preferred by USA participants but not by Amazonian participants).
Conclusions: The gradation in responses observed in Bolivia and in Peru is consistent with the hypothesis that aversion to high frequencies and loud noises is caused by exposure to noise sources that are prevalent in larger towns and cities. More broadly, the results suggest that cultural and environmental forces may be strong determinants of acoustic preferences

M167. Tests of Human Auditory Temporal Resolution: Psychophysical Measurements of Normal Hearing Listeners by Bayesian Estimation

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Category: Psychoacoustics
Background: Auditory temporal resolution plays an important role in speech perception. The gap detection threshold (GDT) and temporal modulation transfer function (TMTF) have been proposed as indices of auditory temporal resolution. The GDT is the minimum length of a perceptible silent interval inserted into a carrier, and the TMTF is a function of the modulation frequency against the modulation detection threshold (MDT). Although these indices are considered useful, they are not used in clinical settings as their measurement is time-consuming. Therefore, we used Zippy Estimation by Sequential Testing (ZEST; King-Smith et al., 1994), an adaptive Bayesian threshold estimation procedure, to measure the GDT and TMTF and thereby shorten the measurement time. ZEST requires the psychometric function and initial probability density function for each threshold. We previously simulated the GDT and MDT measurements using ZEST to evaluate these parameters [Mori et al., 2023; Mori et al., 2024]. In this study, we conducted psychophysical measurements using ZEST to evaluate its accuracy and efficiency.
Methods: GDT and MDT measurements with ZEST (the proposed method) and the transformed up-down method (the conventional method) were conducted on 112 young adults with normal hearing and 34 children with normal hearing. For young adults, each index was measured once using the proposed method and twice using the conventional method. However, only the proposed method was used with children. For ZEST, the parameters determined by computer simulations were used.

Results: The results obtained using the proposed method for young adults and children indicated that the measured values were almost constant after approximately 15 trials. This implies that the results can be obtained within approximately 1.5 min. A Bland-Altman analysis of the results for young adults showed that for most of the indices, the limit of agreement (LOA) between the results obtained using the proposed method and the conventional method was lower than the LOA between the two results obtained using the conventional method. Finally, there were no significant differences between the results obtained using the proposed method in young adults and those obtained in children for the GDT, whereas the MDT measurement was more sensitive in young adults, as previously reported [Trehub et al., 1995; Hall, 1994].
Conclusions: The use of ZEST to measure the GDT and MDT could reduce the measurement time while maintaining accuracy. In particular, it was possible to obtain a GDT of approximately 1.5 min. For the TMTF, it is necessary to measure the MDT at several modulation frequencies; nevertheless, it is still possible to further reduce the measurement time by using a method that estimates the TMTF from only two measurement points [Morimoto et al., 2018].

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M168. Within-Subject Standard Deviations in Auditory Masking Tasks Are Higher for Children with Language-Based Learning Impairments Than Controls

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Category: Psychoacoustics
Background: Language-Based Learning Impairments (LBLIs) encompass a group of diagnoses related to difficulties producing and understanding spoken and/or written language. LBLIs have been proposed to arise, at least in part, from "fuzzy" internal representations of sound. Here we test a prediction of that proposal: namely, that the slope of the psychometric function for the detection of tones in noise should be shallower for children with LBLIs than controls. To do so, for convenience, we examined a proxy for the psychometric-function slope--within-subject standard deviation--for signal-in-noise detection in children with LBLIs and controls. Lower within-subject standard deviations are indicative of steeper psychometric-function slopes and sharper internal representations, because a small change in stimulus level can lead to a large change in performance (steep function), making consistent performance more likely (low within-subject standard deviation), across the stark perceptual boundary (sharp internal representation). We also examined the relationship between within-subject standard deviations and mean detection thresholds.
Methods: We assessed within-subject standard deviations and mean thresholds for signal-in-noise detection in normal-hearing ~8-year-old children with two types of LBLIs--developmental language disorder (DLD) and dyslexia (DYS)--and age-matched controls (n=8/group). The signal was a 20-ms, 1-kHz tone. The noise was a 300-ms bandpass noise (0.6-1.4 kHz) or notched noise (0.4-0.8 kHz and 1.2-1.6 kHz). The signal was presented either 200 ms after noise onset (simultaneous masking) or immediately after noise offset (forward masking).

Results: Within-subject standard deviations computed across three threshold estimates were larger in the DLD group than the control group in three of the four conditions (all but simultaneous-bandpass), intermediate in the DYS group between the DLD and control groups in the two notched-noise conditions (simultaneous and forward), and similar in the DYS and control groups in the two bandpass conditions. Within-subject standard deviations were not correlated with mean thresholds, overall, in three of the four conditions (all but forward-notched). In forward masking, there were cases in which the overall mean within-subject standard deviations differed between groups when the overall mean thresholds were similar, and vice versa.
Conclusions: (1) Within-subject standard deviations were larger in children with LBLIs than controls in two (DYS) or three (DLD) of the four conditions. Thus, the auditory perceptual deficits in children with LBLIs appear to include shallower-than-normal psychometric-function slopes for signal-in-noise detection, consistent with the proposal that children with LBLIs have fuzzy internal representations of sound. (2) Within-subject standard deviations were separable from mean thresholds, aligning with the independence of the slope and position of the psychometric function in mathematical formulations. This outcome serves as a reminder that the evaluation of within-subject standard deviation (psychometric-function slope) can provide information of theoretical and practical value beyond the evaluation of mean performance alone.

M169. Frequency Resolution and Processing Efficiency in Children With Language-Based Learning Impairments

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Category: Psychoacoustics
Background: Language-Based Learning Impairments (LBLIs) encompass a group of diagnoses related to difficulties producing and understanding spoken and/or written language. People with LBLIs have normal hearing thresholds, by definition, but many have auditory-perceptual deficits. Here we focus on two aspects of auditory perception that have received relatively little attention in this population: frequency resolution and processing efficiency. Frequency resolution refers to the capacity to separate complex sounds into their component frequencies. We quantify it by estimating auditory-filter width. Processing efficiency refers to how effectively the information available from the filter is used. We quantify it as the signal-to-noise ratio at the output of the filter that was necessary for signal detection.
Methods: We tested normal-hearing children (~8 years old) with two types of LBLIs--developmental language disorder (DLD) and dyslexia (DYS)--and age-matched controls (n=8 per group). To assess auditory filter width and processing efficiency, we measured the detection threshold for a 20-ms, 1-kHz tonal signal in 300-ms bandpass and notched (notch width: 0.4 kHz) noises, where the noises and the notch were spectrally centered at 1 kHz. Signal onset was either 200 ms after noise onset (simultaneous masking) or 0 ms after noise offset (forward masking). We then fit the threshold estimates separately for each individual and signal-onset time using the roex(p) model to obtain estimates of auditory filter width (p) and processing efficiency (K).

Results: Auditory filter widths were wider and/or more variable in children with LBLIs than in controls in both simultaneous and forward masking. Filter widths were also wider in simultaneous than in forward masking, on average, but this difference was larger and more variable in children with LBLIs than in controls. Processing efficiency was poorer in children with LBLIs than in controls, particularly in forward masking. Efficiency was also poorer in simultaneous than in forward masking, on average, but this difference was smaller and more variable for children with LBLIs than controls. In general, children with DLD and children with DYS performed similarly, and more poorly than controls, though children with DLD tended to have the widest filters in simultaneous masking of the three groups.
Conclusions: The present results suggest that the auditory perceptual deficits in children with LBLIs (1) extend to resolution in the frequency domain, manifesting as wider-than-typical filter widths overall and greater-than-typical narrowing of filter widths from simultaneous to forward masking, (2) are frequency specific in the time domain (forward masking), illustrating a constraint on a deficit in temporal processing previously observed in this population, and (3) involve poor processing efficiency primarily in forward masking. In sum, children with LBLIs appear to have deficits in the resolution and effective use of auditory information in the frequency domain.

M170. Investigating the Effect of Head Movements on Front-Back Discrimination and Sound Externalization with Hearing Aids

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Category: Psychoacoustics
Background: Head movements are an intuitive, often spontaneous, behaviour that can, among other things, help us localize the origin of a sound source. In normal-hearing listeners, head movements during sound presentation are known to improve localization performance in both horizontal and vertical dimensions, as well as front-back discrimination. The perceived externalization of sound sources can also be improved by introducing head movements. Hearing-aid users, especially users with behind-the-ear microphone positioning, exhibit diminished front-back localization abilities as well as a breakdown of externalization. One speculation is that this externalization breakdown is caused by the increase in front-back ambiguity. If this is the case, manipulations that resolve front-back confusion (such as head movements) may also restore externalization with hearing aids. The current study aimed to provide quantitative data on this issue.
Methods: Participants with normal hearing were fitted with low-gain hearing aids and presented with speech stimuli from a loudspeaker directly in front or behind. In separate blocks of trials, they performed front-back discrimination or rated their perceived externalization, and did so with or without head movements. We hypothesized that head movements would fully resolve front-back confusions and restore externalization ratings to unaided levels.
Results: Preliminary data does not support this hypothesis,
Conclusions: which indicates that the externalization breakdowns associated with hearing aids are not entirely driven by front-back ambiguity, and that there are other contributing factors that are likely to persist even when a listener is actively moving within their environment.

M171. Blindness, Cortical Reorganization, and the Neuroscience of Creativity: A Case Study Investigation of Blind Piano Prodigy Matthew Whitaker

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Category: Multisensory Processing/Interactions

Background: Musical improvisation is a highly creative process, based on neural interplays between idea generation, retrieval, and evaluation. Many researchers have explored the neural correlates of improvisation through fMRI studies requiring participants to play improvised music vs pre-memorized, pre-learned music.1-4 However, improvisation in highly creative individuals and the impact of sensory deprivation, still needs to be explored. Matthew Whitaker (MW) is a congenitally blind jazz pianist who has trained at The Julliard School and is a jazz prodigy.5 Due to retinopathy of prematurity, his congenital blindness allowed us to study the interaction of audition, neural plasticity, and creativity in a single individual using fMRI.

Methods: MW was scanned in two fMRI sessions, playing a custom-made non-ferromagnetic piano keyboard held on his lap as he lay supine in the scanner. He was scanned while performing two different tasks that allowed for subtractive analysis to reveal areas of neural activity. He listened to multiple auditory stimuli of Boring and Interesting speech and music to assess the perception of different auditory stimuli. He was also asked to play an alternating scale pattern at two difficulty levels to test his proprioceptive ability when moving over the keyboard. Finally, his creative ability was tested by playing combinations of improvised and memorized music in Generative paradigms: 1) playing a melody of a pre-written piece and improvising over the same chord structure, 2) trading playing four measures with another musician over pre-written music, and 3) freely playing and improvising over a chosen piece of music with no restrictions.

Results: Results showed that music appears to be a privileged stimulus for MW, as he displayed global neural activity for musical stimuli as compared to minimal activity for speech. In particular, MW showed areas of activity in his occipital regions. Connectivity between the fusiform gyrus and motor cortices was found to be related to MW's movement around the keyboard. Additionally, his creative processes were markedly associated with occipital deactivation, mainly of the middle occipital gyrus, fusiform gyrus, and cuneus. It appears that MW recruits these occipital areas for planning or memorization, and during improvisation, he does not require these areas to the same extent. This corroborates previous research of improvisation being a dissociative process.

Conclusions: Thus, MW utilizes occipital areas for audition, spatial processing related to sound, and generative musical creativity. He is a unique model of plastic neural reorganization, recruiting unused visual areas of the brain to support auditory perception and production. To our knowledge, this is the first case study to identify neural reorganization and creativity in a blind musical prodigy, deepening our current understanding of sensory reorganization in the brain and the neural correlates that support prodigious musical creativity.

M172. Establishing a Rat Chronic Suppurative Otitis Media Model With Eustachian Tube Blockage Using Gelatin Sponge

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Category: Middle & External Ear

Background: Chronic suppurative otitis media(CSOM) is a persistent inflammation of the middle ear or mastoid cavity characterized by ongoing or recurrent discharge from the middle ear through a perforation of the tympanic membrane(TM) lasting over 2 to 6 weeks. Understanding the mechanisms behind chronic inflammation in CSOM is critical for developing effective therapies. However, replicating chronic infections in animal models is challenging due to the rapid natural clearance of pathogens. Existing methods, such as surgically blocking the Eustachian tube or using mutant mice, do not accurately replicate the CSOM development process observed in humans. Therefore, we developed a relatively simple rat model of CSOM by inserting an absorbable gelatin sponge into the bony part of the Eustachian tube, followed by inoculation with Pseudomonas aeruginosa.

Methods: A total of 72 male Sprague-Dawley rats were used and divided into two groups: Control (n=36, distilled water injection through the TM perforation) and CSOM(n=36, gelatins sponge insertion to the Eustachian tube and bacterial inoculation through the TM perforation). Each group was further divided into subgroups of 6 rats, sacrificed at 0(3 hours post-inoculation), 3, 7, 14, 28, and 56 days after bacterial inoculation or distilled water injection. Otoscopic evaluations were conducted to monitor tympanic membrane perforations and suppuration. Histological analysis included H and E staining to assess mucosal and submucosal thickness. ELISA was performed to measure cytokine levels (IL-1β, IL-6, TNF-α, VEGF, and HIF-1α) at various time points (0, 3, 7, 14, 28, and 56 days), while immunohistochemistry/immunofluorescence (IHC/IF) was used to detect Pseudomonas aeruginosa in the day 56 group.

Results: Otoscopic findings showed that in the CSOM group, tympanic membrane perforations persisted, and suppuration was observed in 58% (7/12) of the ears by day 56. Histological examination revealed significant mucosal and submucosal thickening, with mucosal thickness peaking at day 28(216.79 μm) and partially resolving by day 56(83.27 μm). Submucosal thickness peaked on day 14(230.25 μm) and decreased by day 56(89.3 μm). IHC/IF analysis detected Pseudomonas aeruginosa in 3 out of 6 ears (50%), localized near the mucosal layer. ELISA results showed elevated levels of IL-1β, IL-6, TNF-α, VEGF, and HIF-1α, indicating sustained inflammatory and hypoxic responses throughout the study period.

Conclusions: This study successfully developed a rat model of CSOM that mirrors key pathological features seen in humans, including persistent bacterial presence and chronic inflammation. By utilizing an absorbable gelatin sponge to induce temporary Eustachian tube blockage, our model closely replicates the pathophysiology of human CSOM without the need for surgical manipulation. These findings underscore the importance of targeting both bacterial infection and the inflammatory response in CSOM management. This model provides a valuable platform for testing new therapeutic strategies aimed at reducing chronic inflammation and improving outcomes for patients with CSOM.

M173. Automated Classification of Middle- And Inner-Ear Mechanical Pathologies Based on Impedance and Air-Bone Gap

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Category: Middle & External Ear

Background: Automated classification methods using machine learning can enhance the accuracy and efficiency of diagnosing mechanical pathologies of the ear, especially when leveraging data from wideband tympanometry (WBT) and the audiogram. Prior studies have demonstrated that using both air-bone gap (ABG) and absorbance, the most commonly used WBT metric, improves differentiation between stapes fixation (SF) and superior canal dehiscence (SCD) with a smaller classification error than either feature alone. Another WBT metric, impedance, provides additional benefits over absorbance by preserving phase information, which can provide deeper insights into ear mechanics. By integrating impedance measurements with a physical model of the ear, we can negate the acoustic effects of ear canal variability, a known challenge in utilizing impedance as a diagnostic tool. Therefore, this study aims to determine whether an estimate of acoustic input impedance (Z) – combined impedance of both middle and inner ear – can further reduce classification error.
Methods: Data from 70 pathological ears diagnosed with either SCD or SF were analyzed. For each ear, Z was estimated by fitting parameters of an analog circuit model to individual WBT measurements. The model includes mechanical properties of the ear canal, middle-, and inner-ear. The ABG feature was simplified to an optimized weighted average of the ABG measured from 250 Hz to 4 kHz. Leave-one-out cross-validation (LOOCV) was used to train and validate the logistic regressions used to classify ears, and regularization was applied to avoid overfitting.
Results: Two-way classification of SCD and SF based on ABG+|Z| achieved an error of 0%, outperforming ABG+Absorbance, which had a 2.9% error. When using ABG alone, the error was 4.3%, while absorbance and |Z| alone produced errors of 21.4% and 20%, respectively.
Conclusions: While these results are based on a limited dataset, the reduction in classification error suggests that using impedance in combination with ABG can improve diagnostic accuracy for mechanical pathologies of the ear. Future work will involve increasing the size of the dataset and including a broader array of pathologies in our analysis. We aim to extend the classification to include ears with ossicular discontinuity and malleus fixation. Additionally, we plan to explore alternative ear models that better represent anatomical structures, to enhance our understanding of pathology-specific mechanical impacts.

M174. Developing a Time- And Frequency-Domain Nonlinear Finite Element Model for the Human Middle Ear

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Category: Middle & External Ear

Background: In response to low-to-moderate-level sounds, the middle ear works as a broadband linear transformer, faithfully transmitting sound to the cochlea. However, for high-level sounds (GREATER THAN 130 dB SPL), the middle ear behaves nonlinearly, which can reduce or enhance sound transmission. Recent studies (Gottlieb et al., 2018; Jiang et al., 2021; Cheng et al., 2021; Tang et al., 2021) reported measurements of frequency- and time-domain middle ear nonlinear behavior arising from moderate-to-high-level sounds. Nevertheless, there is no known middle ear model that adequately simulates these nonlinear behaviors in either the time or frequency domain. This study develops a finite element middle ear model that incorporates multiple nonlinear mechanisms to predict the transmission of high-level impulsive sound and continuous tones.
Methods: An anatomically accurate human middle ear model, previously developed with COMSOL Multiphysics (O'Connor et al., 2017), was employed for this study. It includes the ear canal, tympanic membrane (TM), ossicles, soft tissues, and middle ear cavity. Unlike the previous linear, frequency domain computations, this study incorporates nonlinear mechanics and expands analysis to the time domain. We explored both geometric nonlinearity and material nonlinearity by representing the TM with a hyperelastic one-term Ogden material model (e.g., Cheng et al., 2007). The stimulus is applied as a point pressure source (pure tones in frequency, or 0.2 ms duration impulse in time) in the ear canal, 4 mm from the TM, at levels ranging from 60 to 200 dB SPL. Resulting umbo and stapes motions were analyzed in both domains to characterize nonlinear responses.
Results: In the frequency domain analysis, the umbo displacement shows nonlinearity for levels higher than 180 dB SPL. The umbo shows compressive nonlinearity below 1 kHz and expansive nonlinearity at higher frequencies. Although Cheng et al. (2021) observed that expansive nonlinearity of the umbo tended to occurs at low frequencies than high frequencies, and the onset of nonlinearity was at a lower level (i.e., LESS THAN 120 dB SPL), the ability of the model to produce both expansive and compressive nonlinearity is encouraging. Nevertheless, the results suggest that incorporating nonlinear TM material properties alone is insufficient to simulate measured middle ear nonlinearity. We are investigating including other middle ear nonlinear structures such as the stapedial annular ligament in our model, as well as tuning material properties to fit the model with experimental nonlinear behaviors.
Conclusions: In the COMSOL finite element modeling software, there are many different types of non-linear material models for tissue mechanics to choose from. That the one-term Ogden model for the TM can simulate expansive non-linearity for some frequencies and compressive non-linearity for other frequencies suggests that this approach will be fruitful for incorporating into the other middle ear structures.

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M175. Implantation of a Eustachian Tube Stent in a Model of Eustachian Tube Dysfunction

Katharina Schmitt[1], Malena Timm[1], Philipp Krüger[2], Niels Oppel[1], Alexandra Napp[1], Friederike Pohl[1], Robert Schuon[1], Marion Bankstahl[1], Thomas Lenarz[1], Tobias Stein[2], Gerrit Paasche*[1]

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Category: Middle & External Ear
Background: Stenting the Eustachian tube (ET) could be an option to treat ET dysfunction. The aim would be to restore natural function without generating a patulous ET. Whereas some patients might need permanent support, temporary support might be sufficient in other patients. In the current study, a shape-adapted permanent stent developed for the human ET was investigated regarding implantation in a model of artificially induced ET dysfunction.
Methods: After confirmation of regularly ventilated middle ears, both ETs of eight sheep were augmented with up to 4.25 ml stabilized hyaluronic acid. A permanent stent made from Nitinol with a length of 14 mm (including X-ray markers) and a diameter of 3 to 5 mm was inserted in one ET after proven ET dysfunction in that ear. Middle ear ventilation was investigated by tympanometry once per week. Before and after augmentation and stent insertion, and during half-time and final follow-ups, tympanic membranes and pharyngeal orifices of both ET were inspected. Finally, after an observation period of three months, CBCT images were taken, and the ETs were processed for histology.
Results: ET dysfunction was successfully induced bilaterally in four animals and unilaterally in the remaining animals. Vision on the ET orifices was hampered due to the hyaluronic acid depot protruding into the pharynx lumen and a stronger than normal accumulation of secretion in the pharynx. Nevertheless, seven stents could be positioned as intended in the cartilaginous part of the ET, one stent was found to be positioned in the surrounding soft tissue. ET orifices were closed, except for one slightly open orifice right after stent insertion and another one at final control. Animals did not show any sign of discomfort related to stent placement. In three (including the misplaced stent) of the four cases with bilateral ET dysfunction, ventilation of the middle ear was restored earlier on the stented side. Histology revealed that the hyaluronic acid mainly accumulated directly at the pharyngeal orifice of the ET. Tissue growth on top of the stent struts was observed in all animals. However, the measured open lumen was always larger in stented ETs than in control ETs.
Conclusions: The permanent stent can be inserted in the model of ET dysfunction without causing a patulous ET. However, due to the accumulation of the hyaluronic acid at the pharyngeal orifice of the ET and the position of the stent deep in its cartilaginous part, a true evaluation of the functionality of the stent can not be made.

M176. In Vivo Investigation of a Degradable Polymeric Stent for the Eustachian Tube

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Category: Middle & External Ear
Background: Stenting the Eustachian tube (ET) could be an option to treat ET dysfunction. The aim would be to restore natural function without generating a patulous ET. Whereas some patients might need permanent support, temporary support might be sufficient in other patients. In the current study, a degradable polymeric stent developed for the human ET was investigated regarding its implantability and stability after implantation.

Methods: Polymeric stents made from PLLA were inserted single sided in the ET of nine healthy sheep for observation times of 3, 6, and 12 months (n=3 each). Middle ear ventilation was tested by tympanometry once per week. Before and after implantation, and during half-time and final follow-ups, tympanic membranes and pharyngeal orifices of both ET were inspected. Finally, CBCT images were taken, and the ETs were processed for histology.
Results: All stents were positioned as intended in the cartilaginous part of the ET. Right after insertion, ET orifices appeared slightly open in some cases; at half-time and final follow-ups, all ETs were closed. Animals did not show any sign of discomfort. Stents remained in position and could be confirmed during imaging at the end of the individual observation period. In two cases, a period of disturbed middle ear ventilation of a few weeks was detected. After 3 months, the shape of the stent was round, but first fragmentation of the stent could be observed. After 6 months, the stents were partly collapsed but still held a residual lumen open. Twelve months after insertion, the ETs were completely collapsed close to their natural shape even though the ET lumen appeared still larger than on the control side. Fragments of various sizes were found distributed in the tissue beneath an epithelialized ET. Tissue growth on top of the stent struts was observed in all animals.
Conclusions: The degradable polymeric stent was reliably positioned in the cartilaginous part of the ET without causing patulous ET. The stents seem to fully support the ET during the first 3 months before fragmentation leads to a loss in functionality. These stents provide the basis for the further development to a degradable ET stent intended for human use.

M177. A Deep Neural Network Trained on Finite-Element Simulation Data as a Surrogate Model of the Middle Ear

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Category: Middle & External Ear
Background: Despite its effectiveness, the significant computational resources required for finite-element (FE) simulation pose a challenge when dealing with the intricate geometry of the middle ear and with large parameter spaces. To mitigate this issue, neural networks (NN's) have been explored to reduce computational load while maintaining accuracy in middle-ear modelling.
Methods: We used data from 10,000 simulations with random variations of seven of the parameters of a previously developed linear FE model of an adult human middle ear. Five output quantities (absorbance, umbo velocity Vmag and Vph, and impedance Zmag and Zph) were computed at 50 frequencies from 200 to 8000 Hz. A deep NN was implemented using Keras (https://keras.io) and trained on the FE simulation data and then used to predict the five output quantities. The inputs of the NN consisted of the seven FE model parameters and a frequency. After the input layer there were three fully connected hidden layers with rectified linear unit (ReLU) activation functions to capture complex, non-linear relationships in the data. A dropout layer was included to prevent overfitting. The NN output layer included the five model output quantities. We used the adaptive moment estimation (Adam) optimizer and used mean squared error as the loss function. Data from 6,000 and 4,000 simulations were allocated to the training and test sets, respectively, with 20% of the training data reserved for verification during the training. To assess the prediction accuracy of the NN, the predicted outputs across all frequencies

were compared with the FE outputs using the Spearman rank correlation coefficient and the cosine vector similarity coefficient. The means and standard deviations of these metrics were computed across all 4000 test samples. Other metrics have also been investigated.
Results: Strong correlations were observed between the values generated by the NN and the actual FE outputs across the frequency range. The means (and standard deviations) for the Spearman correlation coefficients were 0.99 (0.100) for absorbance, 0.92 (0.079) for Vmag, 0.98 (0.045) for Vph, 0.98 (0.010) for Zmag, and 0.96 (0.034) for Zph. The cosine similarity coefficients were 0.99 (0.004) for absorbance, 0.97 (0.045) for Vmag, 0.99 (0.009) for Vph, 0.99 (0.006) for Zmag, and 0.998 (0.002) for Zph.
Conclusions: Both of our validation metrics indicated a good agreement between the FE model output and the corresponding values obtained by the NN. This suggests that use of a NN could make advanced FE simulation results more accessible by greatly reducing the need for computational resources. Such NN-based surrogate models offer a possible avenue for enhancing the application of middle-ear modelling, with potential applications in understanding and diagnosing various auditory conditions.

M178. Modeling Vibrations of the Human Middle Ear in Bone Conduction

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Category: Middle & External Ear
Background: The middle ear plays a nontrivial role in bone-conduction (BC) hearing. When the skull vibrates, the ossicles, which are loosely coupled to the skull, can vibrate relative to the skull. The stapes footplate hence can move with respect to the rim of the oval window, contributing to hearing in a manner like air conduction. Previous experiments have shown that the degree of this differential motion was affected by the mass and stiffness of the ossicular chain. While the middle ear's contribution to BC hearing has been recognized, the underlying mechanism is not fully understood in part due to the lack of analytical modeling and solution of the middle-ear vibrations in BC.
Methods: In the present study, a computational BC human middle ear model is developed. The model comprises lumped mechanical components – masses, springs and dampers – to represent various middle-ear structures such as the eardrum, ossicles, ligaments, and joints. The parameters of those components are adopted from existing air-conduction human ear models. The BC-elicited vibrations of the umbo and stapes are simulated in the normal condition and after manipulations such as adding mass at the umbo and stiffening the ossicular joints or ligaments.
Results: The model-predicted results generally agree with the experimental data measured with similar manipulations in human cadaveric temporal bones.
Conclusions: I believe this is the first lumped-element model that can simulate the vibrations of the human middle ear in BC. The model will help elucidate how the middle ear contributes to BC and how implantable middle-ear devices affect BC hearing. This work is supported by the NIH/NIDCD R21DC017251.

M179. Development of Ossicular Palpation Training Simulator Using Haptic Device

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Category: Middle & External Ear

Background: Ossicular mobility is usually measured by intraoperative palpation in order to diagnose the ossicular fixation and determine surgical procedures, e.g., Ossiculoplasty, Stapedectomy, Malleo-stapedotomy. Although the assessment of ossicular mobility is important to improve postoperative hearing level, the assessment through a palpation may rely on surgeons' individual experiences and skills. A training system is necessary to perform effective palpation for a precise diagnosis, especially in surgeons who have little experience. Our research group has been developing an intraoperative measurement system (Koike et al., Hear. Res., 2019) to measure the ossicular mobility quantitatively and suggesting effective palpating methods using a computational model of the human middle ear (Lee et al., Sci. Rep., 2024). In this study, we aim to develop a training simulator using virtual reality technologies and taking advantage of our research resources.

Methods: A virtual middle ear model was built by setting each parameter based on ossicular compliances obtained from measurements using our system (Koike et al., 2019) and simulation by our finite-element model of the human middle ear (Lee et al., 2024). The ossicular mobility were calculated by the ratio of the displacement to the reaction force. Normal ossicular mobility was set based on the measurement result using the intraoperative measurement system in temporal bones (Lee et al., 2024). Several pathological conditions, such as otosclerosis or combined fixation around the malleus or incus with otosclerosis, were represented by increasing the stiffness of ligaments which is in the same way as that used in Lee et al. (2024). A haptic device (Touch TM, 3D SYSTEMS) was used to provide feedback force to palpation as a training simulator.

Results: As a result, ossicular mobility in the normal model and fixation models were distinguishable through palpation using the developed training simulator. The sense of feedbacked force by the simulator was assessed based on comparisons with that obtained from palpating artificial ossicles (Koike et al., 2019). The artificial ossicles were built by various coil springs (0.3 N/mm ~ 4.9 N/mm) which represent several pathological conditions including intact case.

Conclusions: The training simulator using virtual reality technologies might contribute accurate assessment of various pathologies of ossicular fixation by performing effective palpation proposed by our research group based on the quantitative measurements, computational modeling and simulation. Furthermore, quantitative assessment of the sense of the feedbacked force and efficacy of training is necessary to applicate in the real world.

M180. Detection of Bacterial Vs Inflammatory Acute Otitis Media Using Icg-Maltotriose in Short-Wave Infrared (SWIR)

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Category: Middle & External Ear
Background: Acute otitis media (AOM) is one of the most common diseases in the pediatric patient population around the world, associated with a significant annual cost burden. The current standard of diagnosis for AOM includes direct observation with an otoscope to detect middle ear effusions. However, such methods do not provide information whether pathogenesis is bacterial or nonbacterial, leading to decreased confidence in determining antibiotic necessity. As such, a noninvasive method of distinguishing between bacterial and non-bacterial is critical. Our lab has developed ICG-maltotriose, known to bind specifically to the maltodextrin transporter found in bacterial cells and not mammalian cells. This study aims to demonstrate the utility of ICG-maltotriose in conjunction with short-wave infrared (SWIR) imaging in distinguishing bacterial and non-bacterial AOM in murine models.
Methods: Two groups (N = 10) of Balb/c mice underwent direct inoculation of the middle ear via a transcervical bullostomy with H. influenzae (1x10^8 CFU) or LPS (3 µl of 20 mg/mL). On POD1, the tympanic membranes were observed under a microscope for inflammation and effusion. On POD2, ICG-maltotriose (30 µmolars in 100 µl of PBS) was administered retro-orbitally. In-vivo images of the tympanic membranes were obtained using a custom SWIR otoscope setup with an 1000 nm long pass, with laser excitation at 793 nm. Images were collected at 8 hour intervals from 0 to 24 hours after dye injection. After final imaging, mice were sacrificed and middle ears harvested. Specimens were cryosectioned, gram-stained, immunostained for macrophages with F4/80 mAb, and subsequently imaged using confocal microscopy.
Results: We successfully generated murine infection models for bacterial and non-bacterial acute otitis media. SWIR microscopy images of the tympanic membrane demonstrated that H. influenzae induced AOM models showed an overall signal ratio between the infected to non-infected ear of 3:1, while the LPS models demonstrated no difference. Subsequent confocal imaging of the infected ears corroborated increased presence of macrophages and neutrophils in the infected middle ear. Tissue histology confirmed thickening of the TM and mucosa of the infected left ears with increased fluorescence signaling.
Conclusions: Our study demonstrates that ICG-maltotriose is a viable fluorescent probe for distinguishing bacterial and non-bacterial AOM in murine models. We propose that ICG-maltotriose is an appropriate probe to be used in conjunction with existing tools for objective diagnosis of bacterial AOM. Future studies will aim to demonstrate the utility of ICG-maltotriose in monitoring treatment of bacterial AOM to antibiotics.

M181. A Novel Model of Eosinophilic Otitis Media and Exploration of ILC2 in Middle Ear Mucosa

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Category: Middle & External Ear
Background: Eosinophilic otitis media (EOM) is a type 2-associated refractory otitis media. We have been studying a guinea pig model of eosinophilic otitis media conducted with ovalbumin (OVA). In this study, we tried to create a new EOM model using Papain, a plant protease,

administered intratympanically daily. We also examined the expression of innate lymphoid cell type 2 (ILC2) in the middle ear mucosa of the model animals.
Methods: Hartley Guinea pigs were injected with 25 μg of papain into the tympanic cavity 12 times from Day 0 to Day 11. The temporal bone was removed and fixed within 24 hours of the last challenge. Specimens were observed by HE staining, Alcian blue staining, and immunohistochemistry (fluorescent antibody method).
Results: Significantly more eosinophils were observed in the submucosa of the Papain-stimulated group. The distribution of eosinophils in three specific areas was examined, but no significant differences were observed. Mucin was observed around the eustachian tube, and many eosinophils were contained in the mucin. Hyperplasia of goblet cells did not show a significant difference between the control and stimulated groups. The number of submucosal ILC2 in the tympanic chamber was analyzed in the stimulated and control groups, and a significant increase in ILC2 was observed in the stimulated group.
Conclusions: There have been reports of Papain-induced allergic disease models in asthma and eosinophilic sinusitis. However, there have been no reports of eosinophilic otitis media models. In the present study, intratympanic administration of Papain alone caused eosinophil migration into submucosal tissues, hyperproduction of mucin with eosinophils, and increased ILC2. Thus, this model may contribute to the elucidation of the pathogenesis of EOM, a type 2 inflammation.

M182. Nonlinear Displacement of the Tympanic Membrane in Response to Static Pressure and Low Frequency Tones: A Computational Study

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Category: Middle & External Ear
Background: The effect of geometric and material nonlinearities on responses of the tympanic membrane (TM) to pressure and loud sounds remains insufficiently studied. The primary goal of the present work is to develop a nonlinear finite element (FE) model of the TM capable of simulating mechanical responses to high static pressures and low-frequency acoustic tones commonly used in clinical tympanometry.
Methods: A subject-specific, three-dimensional nonlinear FE model of the TM, along with the ME's ossicles, ligaments, and joints, was constructed based on human ME micro-CT scans and tuned using tympanometry data. An orthotropic material model was applied to the TM to capture the influence of radial and circumferential collagen fibers, while an isotropic elastic model represented the other ME structures. A discrete dashpot was introduced to simulate the input impedance of the cochlea. This preliminary model consisted of 204267 second-order tetrahedral elements (TET10). For the boundary conditions, the outer rim facets of the TM, ligaments, and tendons connected to the temporal bone were fixed in three dimensions (x, y, and z). Pressure loading conditions were applied to simulate tympanometry, with static pressures ranging from -200 to 200 daPa and a 226 Hz, 80 dB sinusoidal pressure superimposed. All simulations were performed using the implicit nonlinear FE solver, FEBio. Future model improvement includes

refining the current mesh, particularly on the TM, and exploring the most suitable constitutive model for the TM.
Results: Simulated tympanograms show the application of the static pressure affects TM mobility by reducing the peak-to-peak displacement amplitude in response to sinusoidal pressure. This closely follows the behavior observed in clinical tympanometry and indicates lowering admittance as TM is pressurized while the peak admittance occurs at zero applied static pressure. Additionally, results suggest a difference in the TM geometric stiffening for negative versus positive applied static pressure.
Conclusions: This research aims to enhance clinical diagnostics and therapeutic strategies by offering a more detailed and physiologically accurate computational model of the ME. Our validated model can be employed to study how variations in ME anatomy and pathology influence sound transmission under both normal and potentially hazardous acoustic conditions including blast. Our findings are expected to improve the interpretation of clinical assessments of ME function in response to high static pressures and loud sounds.

M183. Characteristics of Frequency- and Temporal Resolutions, and Speech Perception by Bone-Conducted Stimuli Presented to the Facial Parts

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Category: Middle & External Ear
Background: Bone-conducted (BC) sounds are typically presented to the mastoid or condyle processes. However, BC-sound presentations to face such as the nasal and zygomatic bones have also recently been investigated. Because the face has complex structures, BC sounds presented to facial parts are likely to show different characteristics from those presented to conventional parts.
Methods: Here, to investigate frequency- and temporal resolutions, and speech perception characteristics when BC sounds were presented to facial parts (nasal bone, infraorbital region, zygomatic, jaw angle, and chin) in normal-hearing subjects, we conducted psychoacoustical measurements of difference limens for frequencies (DLFs), temporal-modulation transfer functions (TMTFs), and Japanese-monosyllable articulation.
Results: DLFs were found to be 0.2–0.3% at 250-4000 Hz, increasing to 0.4–0.6% at 125 Hz, and increasing to 0.3–0.5% at 8000 Hz significantly at each stimulated part. Moreover, no significant differences were observed among conventional and facial parts at any frequency. A two-way analysis of variance (ANOVA) revealed a significant effect of the frequency (p LESS THAN 0.01), but no effects of stimulus site was observed.
TMTFS leveled off at 23–26 dB at 10–100 Hz and then decreased with increasing frequency up to 150 Hz regardless of the stimulus type or stimulated parts. Moreover, the modulation thresholds for each facial part had been confirmed to be approximately equivalent to those for conventional parts and air-conducted (AC) sounds. A two-way ANOVA revealed a significant effect of the frequency (p LESS THAN 0.01), but no effects of stimulus site was observed.

The percent corrects for Japanese monosyllables for each stimulated part tended to be lower than those of the AC sounds. In the BC presentation conditions, the percent corrects were found to be

more than 50% for the facial parts. In addition, a relatively high percent corrects was obtained in the conventional parts (mastoid and condyle processes), zygomatic and jaw angle. In particular, the zygomatic showed the highest percentage (approximately 70%). A one-way ANOVA showed a significant effect of the stimulus site (p LESS THAN 0.001). In the facial parts, the confusion of voiced consonants was slightly increased, particularly in the nasal, infraorbital region, and chin, and more so than in the zygomatic and jaw angle. A one-way ANOVA showed main effects of the stimulus site for /sj/, /g/, and /j/ (/sj/: p LESS THAN 0.05, /g/: p LESS THAN 0.01, /j/: p LESS THAN 0.05).

Conclusions: The results indicate that frequency- and temporal resolutions, and intelligibility obtained from the measured facial parts are similar to or close to conventional parts. In particular, the zygomatic (cheek bone) is capable of practical speech perception equivalent to that of AC sounds. These results indicate that BC devices that use facial presentation can provide practical frequency- and temporal resolutions, and speech perception that are not inferior to conventional BC devices.

M184. Admittance at the Eardrum Estimated From Canal Measurements

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Category: Middle & External Ear

Background: Wideband absorbance of sound power by the ear is typically calculated from the acoustic impedance measured at a single location in the ear canal. However, the complex and varying shapes of individual ear canals complicate the interpretation of middle-ear input and its ability to serve as a diagnostic tool for middle-ear disorders. To understand the effect of the canal on measurements of absorbance and admittance, we measured these quantities with the probe coupled to multiple 3D-printed replicas of human ear canals generated from high-resolution CT scans and terminated by the same physical artificial ear that models the acoustic impedance at the eardrum (Voss et al, 2024). This method allows direct comparisons between anatomical canal features and acoustic absorbance measures. Additionally, we assess middle-ear admittance as an alternative diagnostic to absorbance for middle-ear function, following the approach of Lewis and Neely (2015) to minimize ear-canal contributions.

Methods: Admittance measurements were made with a custom software system, the Etymotic ER-10X probe system, and a 3D-printed artificial ear coupled to one of several loads. The loads included 3D-printed cylinders (30–80 mm in diameter, 30 mm in length), and three 3D-printed ear canal replicas. Measurements were made at multiple probe insertion depths, and absorbance was calculated from the admittance at the probe. Additionally, middle-ear admittance – the admittance at the connection between the 3D printed canal and the artificial ear -- was calculated from the probe measurement.

Results: Cylinders: While standing-wave frequencies varied with insertion depth as expected, absorbance remained largely independent of depth below 6 kHz, indicating that plane wave assumptions hold reasonably well up to at least 6 kHz. Absorbance decreased with increasing cylinder diameter, consistent with the reduced surge impedance of the cylinders coupled to a single fixed ear coupler. The middle-ear admittance calculations removed this diameter

dependence, confirming the accurate acoustic characterization of the cylindrical “canal” segment.
3D-printed ear canals: Absorbance and middle-ear admittance were similar in two of the three canals, while the third showed signs of a potential leak, likely between the canal and the artificial ear. The admittance magnitude showed more variability at higher frequencies (GREATER THAN 6-8 kHz). The estimate of surge impedance worked well when the anatomical area function was not known, but the results are similar if a constant area of 44 mm2 is assumed.

Conclusions: Absorbance is a commonly used clinical measure of middle-ear status, but middle-ear admittance is potentially better because (1) its magnitude is independent of cross-section area and (2) its phase may inform differential diagnosis of middle-ear defects. These preliminary results are encouraging, but further refinements in the methodology are needed. The measurements at high frequencies show promise despite variability in this frequency range, which may be due to imperfect characterization of high-order evanescent modes.

M185. Restoration of Middle Ear Function in Partial Ossicular Discontinuity: A Basic Science and Clinical Correlation

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Category: Middle & External Ear
Background: Partial ossicular discontinuity in temporal bones (TBs) produces a greater loss in stapes velocity (SV) at high frequencies (HF) than low frequencies (Farahmand RB 2016), which is clinically recognized as a primarily HF conductive hearing loss (CHL); however, the influence of joint reestablishment on HF hearing has not been widely studied. This study describes measurements of middle ear transfer function in cadaveric TBs with 1) induced incudostapedial (IS) and incudomalleolar (IM) joint partial disarticulation and 2) surgical repair of manipulated joints. We correlate experimental results with pre and post-operative audiometric results from patients with surgically confirmed partial ossicular discontinuity who underwent repair.
Methods: Fresh, previously frozen human TBs from donors with no known history of ear disease were used to study the mechanical effects of partial ossicular discontinuity before and after repair. Dual laser Doppler vibrometry were used to simultaneously measure stapes and umbo velocities (UV). A TDH speaker with a short silicone tube was coupled to the external ear canal to deliver pure tones 0.2-20 kHz at 9 points per octave. A PCB piezotronics probe microphone was inserted into the ear canal to record sound pressure near the tympanic membrane. Measurements were taken with the ossicular chain intact, partial IS or IM disarticulation, complete disarticulation, following repair with alginate (Jeltrate®) and then dental cement. To correlate with clinical findings, post-surgical audiometry from two patients with pre-operative HF CHL (4kHz air-bone gap ≥25dB), and clinical indications of a hypercompliant middle ear on tympanometry were reviewed. Patients underwent endoscopic middle ear surgery revealing IS or IM discontinuity which was repaired with otomimix bone cement.

Results: Experimental outcomes show a decrease in SV with a partial joint disarticulation that typically begins 2-3kHz and is primarily recorded 4-20kHz. SV on average drops by an order of magnitude and the magnitude of change depends on the degree of ossicular loosening. Repair of the joint with Jeltrate, simulating a fibrous connection, restores SV typically through 3kHz, but not above 4kHz. Repair with dental cement, forming a hard connection, restores SV to baseline across all frequencies. UV exceeds baseline values LESS THAN 1kHz with partial joint disarticulation or Jeltrate consistent with a hypercompliant system. Repair with dental cement restores UV.
Clinically, both patients had improvement of HF ABG by ≥25dB, with complete closure at 4kHz in one patient. HF air conduction thresholds at 6 and 8kHz improved to mild hearing loss or normal levels, suggesting correctable hearing loss GREATER THAN 4kHz. Postoperative tympanometry showed normalization of tympanogram wave forms. These findings are consistent with the SV changes observed experimentally.

Conclusions: Experimental observations and clinical outcomes demonstrate repair of partial ossicular discontinuity with a hard-drying substance can return HF ossicular function and audiometric outcomes to normal, suggesting some HF hearing losses are surgically correctable.

M186. Osteoprotegerin Deficiency in the Human OTIC Capsule as a Potential Driver of Otosclerosis

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Category: Middle & External Ear
Background: Otosclerosis features pathologically accelerated bone remodeling in the otic capsule. Previous research in mice suggests osteoprotegerin (OPG), an inhibitor of bone remodeling, governs the naturally low level of bone remodeling in the otic capsule. Absence of OPG thus may drive otosclerosis. This study explores OPG distribution pattern in the human normal and otosclerotic otic capsule, its possible anatomical distribution routs, and its potential role in otosclerosis.
Methods: (i) OPG immunolabeling on human temporal bone sections from individuals with and without otosclerosis, focusing on cochlea and otic capsule bone. Confocal microscopy was used to examine OPG distribution. (ii) Fresh human temporal bone samples were perfused with a fluorescent tracer for different incubation times and confocal microscopy on non-decalcified, plastic-embedded tissue sections evaluated tracer distribution. (iii) Lacuno-canalicular network pores surface area from the cochlear wall were evaluated using scanning electron microscopy.
Results: OPG labeling was strongest in cochlear supporting cells and showed a radial gradient in the surrounding otic capsule. In the otic capsule, OPG labeling was localized in osteocyte lacunae and extracellular clefts within globuli interossei. The fluorescent tracer outlined osteocyte lacunae and clefts within globuli interossei similarly to OPG-immunolabeling, indicating diffusion into the otic capsule via a continuous lacuno-canalicular network.
Conclusions: Our findings support OPG's role in inhibiting bone remodeling in the human otic capsule. Cochlear supporting cells are a probable major OPG source, likely secreting it into inner ear fluids, where it diffuses through the otic capsule via a continuous lacuno-canalicular network.

Disruption of this network may lead to localized OPG-deficiency, potentially driving otosclerotic bone remodeling.

M187. Intratympanic Dexamethasone Administration Reduces Radiation-Induced Middle Ear Mucosal Damage

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Category: Middle & External Ear

Background: Radiotherapy (RTx) is a highly effective treatment for head and neck cancer that can cause concurrent damage to surrounding healthy tissues. In cases of nasopharyngeal carcinoma (NPC), the auditory apparatus is inevitably exposed to radiation fields and sustains considerable damage, resulting in dysfunction. To date, little research has been conducted on changes induced by RTx in the middle ear and the underlying mechanisms. Dexamethasone (DEX) is widely used in clinical practice due to its immunosuppressive and anti-inflammatory properties.

Methods: The present study investigated the effects and underlying mechanisms of DEX delivered via intratympanic administration on RTx-induced damage to the middle ear and human middle ear epithelial (HMEE) cells. Sprague-Dawley (SD) rats were exposed to fractionated RTx (6.6 Gy/day for 5 days) and middle ear samples collected at 1 and 4 months.

Results: Rats receiving RTx showed a significant increase in submucosal layer thickness in the middle ear and disorganization of ciliated epithelium in the Eustachian tube (ET) mucosa. Importantly, intratympanic administration of DEX 30 min before RTx resulted in a lower degree of damage compared to the control group. Furthermore, DEX pretreatment induced downregulation of cell death pathway markers in HMEE cells.

Conclusions: Our collective results indicate a therapeutic role of DEX against RTx-induced middle ear damage and support its application in prospective measures to prevent radiation-mediated injury.

M188. Effects of Characteristics of Exposed Noise on the Intelligibility of Bone-Conducted Speech With Earplugging

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Category: Middle & External Ear

Background: Bone-conducted (BC) sound can be applied to speech-communication devices in intense noise environments because BC sounds can be heard even when wearing earplugs. However, the actual perception of BC sound is affected by temporal- and frequency-characteristics of ambient noise, the noise-attenuation rate of earplugs, and even the “occlusion effect” (a phenomenon in which the loudness in the low-frequency range is increased by wearing earplugs), and the mechanisms in detail remain unclear.

Previous reports on the effect of noise-type showed different results; one showed the largest effect in white noise and another in babble noise. The problem of these previous studies seems to lie that the noise characteristics and experimental conditions differed somewhat in each report.

This research aims to investigate the effects of several types of noises on the intelligibilities of Japanese monosyllables presented by BC with earplugging (Experiment 1). We also investigated the contribution of each BC component to speech intelligibility under noise conditions by physioacoustical measurements on/around the head (Experiment 2).

Methods: Seven adults participated in experiment I. The BC vibrator was located on the left mastoid on the temporal bone. One hundred Japanese monosyllables spoken by one male and one female, set to the clearest sound level for each participant were used as stimuli. White noise, pink noise, and babble noise were exposed from a loudspeaker set 1.0 m in front of the participants. Measurements were conducted under ten different noise conditions (quiet, white, pink, and babble noises, each noise set three levels: 60, 70, and 80 dB A(A-weighting)). The conditions of experiment 2 were the same as those of experiment 1. A probe microphone was used to measure ear-canal sound pressure (ECSP). The ECSP data were converted to a spectrogram.

Results: The percent correct for the female voice was: silence GREATER THAN babble noise GREATER THAN GREATER THAN pink noise GREATER THAN white noise. The percent correct for the male voice was: silence GREATER THAN babble noise GREATER THAN GREATER THAN white noise GREATER THAN pink noise. According to spectrograms, it was indicated that some of the spectral information of BC speech remains even under air-conducted noise. Under babble noise, speech spectral information was relatively well remained than white noise and pink noise.

Conclusions: The results of the intelligibility test mean that intelligibilities were more significantly reduced for the noise with high-frequency components. The reduction of high-frequency components in BC speech during its propagation through the body, in addition to the masking of high frequency, may have degraded the hearing of consonants cued in the high-frequency range. Moreover, the results were validated by physical measurement. The results of ECSP measurement mean that the amount of remaining speech spectral information decreased with noise that has a higher frequency. These results provide useful information for the development of BC communication devices.

M189. Middle Ear Transfer Functions: High-speed Measurement and Analysis at Moderate to High-Intensity Sound Levels

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Category: Middle & External Ear

Background: The middle ear transfer function (TF) is the ratio of tympanic membrane (TM) or ossicular chain (OC, e.g. umbo or stapes) motion to the sound pressure of the ear canal stimulus, and is a measure of the excitatory signal to the inner ear. In non-pathological middle ears at

sound pressures below ~120 dB SPL, this system behaves linearly. At higher intensity sounds, such as blasts, non-linear behavior occurs in the TM and OC, but has not been rigorously described. In this work, we apply a novel integrated high-speed optical imaging system combining High-Speed 3D Digital Image Correlation (HS 3D-DIC) and High-Speed Schlieren imaging (HS-SI) with a custom-designed shock tube to quantify full-field TM and OC TFs in response to various levels of impulsive sound, including blast, in real time.
Methods: To obtain TFs of human middle ear at moderate levels of impulsive sound (~130 dB SPL), acoustic clicks were generated by a loud speaker and delivered to cadaveric human TBs. Transient input sound pressures near the TM were monitored with a microphone. Sound-evoked motions, such as TM and stapes vibrations, were measured with HS 3D-DIC and Laser Doppler Vibrometry (LDV). For high-intensity levels, a shock tube generated peak pressures ranging from 160 to 180 dB. A high-speed pressure sensor and HS-SI characterized the blast overpressure near the TM including shock wave propagation and interaction with the TM. Full-field TM responses were measured using HS 3D-DIC at 100,000 frames per second, providing detailed descriptions of TM surface motions. Fourier transform was applied to the recorded time-domain data-sets to compute middle ear TFs in the frequency domain. As the DIC technique requires painting the TM, LDV measured responses of painted and unpainted TMs were compared to assess the impact of the paint.
Results: Middle Ear TFs at the umbo and stapes, as well as full-field TM mode shapes were determined at various stimulus levels and compared to assess the onset of middle ear non-linear responses. TFs at moderate stimulus levels show some similarities with those in the literature, while TFs and novel full-field TM responses at high level pressures improve our understanding on how the TM and middle ear transmit high-level acoustic impulses from the environment to the cochlea.
Conclusions: Middle ear TFs in response to impulsive sounds were successfully obtained across a range of stimulus pressure levels. Our preliminary results suggest the paint required for 3D-DIC measurements affects the response, highlighting the need for thinner layers or paint-free approaches. Preliminary analyses also suggest multiple non-linear processes, which operate in different frequency and level range, exist within the middle ear. We are working on further measurements across broader pressure ranges to quantitatively describe these processes.

M190. Effects of Auricular Size and Hardness on Propagation Components of Cartilage Conduction: Comparison Among Auricular-hematoma, Child and Normal-auricle Subjects

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Category: Middle & External Ear
Background: Cartilage conduction (CC) is a method of perceiving sound via biological tissues by presenting a transducer to the auricular cartilage (pinna). Unlike conventional bone conduction (BC) through the skull, conduction through the lightweight pinna requires a weak force, thus reduced discomfort. This feature has been applied in hearing aids for ear canal atresia, smartphone screens, and earphones.

CC is also effective for children and patients with auricular hematoma, who often experience difficulties using conventional earphones or hearing aids. However, auricular characteristics of those individuals, such as size and hardness, differ from those of normal-hearing adults and that difference may affect CC perception.

In this study, we investigated relationships among auricular size, hardness, and CC perception. Three types of subject group, auricular hematoma subjects (Hematoma), 5-9-year-old children (Children), and normal-hearing adults (Normal) were measured and compared to clarify effects of auricular size and hardness on CC perception.

Methods: We measured auricular size and hardness and investigated how these factors affect the CC perception. We measured hearing threshold, ear canal sound pressure (ECSP) and pinna/head vibration.
Results: The hearing thresholds for Hematoma were significantly higher/lower than Normal at 250-1000/2000-4000 Hz (p LESS THAN 0.05). The thresholds for Children were higher than Normal at 500 and 1000 Hz and lower at 250 and 2000 Hz. Auricular size significantly decreased in Children compared to Normal. Hematoma/Children's pinna were harder/softer than Normal.
In Hematoma and Normal group, there were positive/negative correlations between the threshold/ECSP and the hardness at 250-1000 Hz. At 4000 Hz, the threshold was negatively correlated with the pinna/head vibration. The effect of hardness on vibration varied depending on the parts of the auricle.

In Children and Normal group, positive correlations were observed between the threshold and some auricular size parameters at 250 and 2000 Hz. The threshold was negatively correlated with the hardness at 500 and 1000 Hz and positively correlated at 2000 and 4000 Hz.

Conclusions: The results obtained showed that CC perceptions in Hematoma and Children differ from that of Normal. Auricular size and hardness affect CC perception; however, the effect varies by frequency and between subject groups. In the Hematoma and Normal group, softer auricles led to an increase in the air-conduction component and improved hearing at 250-1000 Hz, likely due to a reduced distance between the eardrum and a transducer. At 4000 Hz, although hearing improved with increased pinna/head vibration, the effect of hardness on vibration varies depending on the points on the auricle. In the Children and Normal group, children with smaller ears heard CC better at 250 and 2000 Hz, while adults with stiffer ears hear CC more efficiently at 500 and 1000 Hz.

M191. Origin and Function of Tissue-Resident Macrophages in Postnatal Development of the Eardrum

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Category: Middle & External Ear

Background: Background: Tissue-resident macrophages (TRMs), the first line of immune defense, are also central to organ development and homeostasis. We discovered the abundance of CX3CR1+ neonatal macrophage cells resident in the TM of a Cx3cr1EGFP reporter mouse line. However, it is unclear of either the origin of the neonatal TRMs in the TM or what function is served by the early 'wave' of TRMs after birth is unclear.
Methods: Methods: Genetic fate mapping is employed to trace the origin of neonatal TRMs after birth and their dynamics in adults, combined with sc-RNA seq and macrophage depletion approaches, to determine the functionality of neonatal macrophages.
Results: Results: Using a Cx3cr1EGFP reporter mouse line, we first demonstrated a unique pattern of TRMs in the tympanic membrane (eardrum) and the characteristics of their dynamics from neonatal (postnatal day 1) to adulthood (1 month). In neonatal mice, most macrophages are unpolarized, branched in morphology, and primarily situated in regions where blood vessels and peripheral nerve fibers are richly intermixed. By the time the animals mature, the TRMs are noticeably elongated, polarized, and situated radially toward the nerve fibers and surrounding blood vessels and nerve fibers. The density of the TRMs declines as the animals reach adulthood. Single-cell RNA-sequencing of the tympanic membrane from young mice reveals a highly diverse set of TRMs. Using a fate mapping approach on different transgenic mouse lines, we then identified the neonatal TRMs predominantly derived from the fetal liver, with a minor contribution of yolk sac origin. In adults, the TRMs are gradually replaced by circulating monocytes derived from bone marrow-derived hematopoietic stem cells. In further studies using a macrophage suicide model, we demonstrated the 'early wave' neonatal TRMs are essential for the postnatal development of the tympanic membrane and maturation of the vessels and peripheral nerve fibers. Depletion of the neonatal TRMs results in a smaller size tympanic membrane and abnormal vascular and neuronal development.
Conclusions: Conclusion: Taken together, the new findings uncover the critical role of TRMs in the structural and functional development of the eardrum after birth. TM abnormalities have long been noticed in humans, although the underlying mechanisms are often unknown. Our new findings may provide new insight into how eardrum structure and function are shaped by the innate immune system.

M192. Monocyte-Derived Macrophages, Signaled by TRPV1, Promote Angiogenesis and Wound Healing in the Tympanic Membrane

Xiaorui Shi*[1], Lingling Neng[2], Kushal Sharma[2], Allan Kachelmeier[2], Xiaorui Shi[2]

[1]*Oregon Health and Science University,* [2]*Oregon Hearing Research Center, Oregon Health and Science University*

Category: Middle & External Ear
Background: Background: The tympanic membrane (eardrum) is a thin and sensitive tissue. Its vibratory characteristics and transmissibility of the sound signal to the inner ear are critical for hearing. Perforation of the eardrum often causes conductive hearing loss, a common clinical problem, affecting millions of people worldwide. However, the knowledge of how TM is naturally repaired after injury is still limited.
Methods: Methods: We used a 26-gauge syringe needle to perforate the anteroinferior region of the pars tensa (where the TM is frequently damaged by traumatic injury) in combination with

other research approaches, including EdU pause labeling, a bone marrow transplant chimeric animal model, bulk gene analysis, and genetically mutated potent nociceptive tachykinin regulator1 (TRPV1) mouse model, we determine how wound tympani membrane healed via monocyte-derived macrophages.
Results: Results: we found that following injury, macrophages massively accumulate in the perforated area of the acutely wounded tympanic membrane. We identified that the majority of recruited macrophages were not locally proliferated tissue-resident macrophages but rather monocyte-derived macrophages, as only a small number of the CX3CR1+ macrophages had incorporated nuclear EdU. Paralleling the macrophage recruitment, we noticed early vascular inflammation and later angiogenesis in the vicinity of the wound. Angiogenesis was initiated by day 3 after perforation, which further progressed by day 7, and diminished by day 14. We found angiogenesis is strongly associated with monocyte-derived macrophages. Depletion of macrophages causes reduced angiogenic activity and significantly delayed wound healing. Further bulk RNA sequencing showed a rather remarkable upregulation of tac1 genes. The tac1 gene encodes neuropeptide substance P (also known as neurokinin 1), associated with TRPV1 of immune-inflammatory reactions. Genetic mutation of the TRPV1 channel was shown to significantly reduce monocyte recruitment and delay wound healing.
Conclusions: Conclusion: Taken together, the new findings reveal the essential role of monocyte-macrophages, mediated by TRPV1 signaling, in wound healing of the eardrum.

M193. An Open Source Hearing Research Platform

Odile Clavier*[1], Joshua Alexander[2], Mattheus Ueckermann[1], WIlliam Audette[1], Véronique Archambault-Léger[1], Christopher Brooks[1], Brian Graybill[1], Michael Heinz[2]

[1]*Creare LLC,* [2]*Purdue University*

Category: Other
Background: As researchers focus on improving the diagnosis of individual hearing deficits in diverse populations, they need to reliably and cost-effectively collect multiple measures on individuals to overcome variability, and to collect the same measures in coordinated cross-species studies for insight from animal studies. Furthermore, data sharing is imperative to enable reproducibility and quantitative models that capture the relation between underlying pathophysiology and real-world speech intelligibility. We will present our development of a new open-source hearing research platform designed to meet these needs.
Methods: Open Hearing links multiple technologies of varying maturity. TabSINT is a mobile application that lets users administer customizable hearing tests and questionnaires and connects easily to Bluetooth devices for input. Since 2014, it has been used to collect data from thousands of subjects. A newer version of TabSINT now makes it compatible with current mobile operating systems. Tympan is an audio processing platform initially designed for hearing aid research which has been available for a few years. Open Hearing combines TabSINT and Tympan with an open data science repository and a low-cost, open-source ear probe, the “Auren”. The Auren prototype has a small electronic board with multiple low noise MEMS microphones and speakers, and 3D printed calibration tubes. It connects to the Tympan for control through open-source firmware.

Results: Initial data on the usability of the Tympan for audiometry with Sennheiser HDA200 headphones shows maximum output levels ranging from 104 dB SPL at 16,000 Hz up to 115 dB SPL at 500Hz (range 125 - 16,000 Hz), while meeting ANSI S3.6 requirements for maximum distortion. Using the Tympan connected to an off-the-shelf probe (Etymotic ER 10B+), we measured distortion product otoacoustic emissions between 1000 and 8000 Hz of 20 ears from 10 normal-hearing subjects and compared to the same measurement with the Interacoustics Titan clinical system. The data show results were highly correlated between the two systems (r=0.83 and slope = 0.92) with distortion below -13 dB SPL. Calibration of the Auren for wideband acoustic immittance measurements demonstrates that using three microphones and acoustic modeling can reconstruct the 3D plane wave through an ear simulator to within less than ±4 dB up to ~12 kHz. We verify the calibration using additional tubes with ports for the probe mic.
Conclusions: This platform has the potential to increase access to quality hearing research hardware and software for all researchers, and to improve reproducibility and clinical transition in hearing research. Its open-source nature will ensure it remains available regardless of manufacturer commitment.

M194. Refining Convolutional Neural Networks for Temporal Bone Imaging Segmentation Using 3-Dimensional Distance Maps

Andy S. Ding*[1], Manish Sahu[2], Mathias Unberath[2], Russell H. Taylor[2], Francis X. Creighton[1]

[1]*Johns Hopkins School of Medicine,* [2]*Johns Hopkins Whiting School of Engineering*

Category: Other
Background: Three-dimensional (3D) visualization of relevant structures within the temporal bone can be useful for pre-operative planning and image navigation but often requires manual segmentation of patient imaging, which can be tedious and time-consuming. Automated methods using convolutional neural networks (CNNs) for segmenting multiple geometrically complex structures have recently been described, but accuracy particularly for small neurovascular structures, has room for improvement. In this study, we present a novel loss function for neural network training that can provide more anatomically accurate segmentations for smaller structures in the temporal bone.
Methods: Fifteen deidentified, high-resolution cone-beam temporal bone computed tomography (CT) datasets were included in this study. Sixteen anatomical structures, including ossicles, inner ear, facial nerve, chorda tympani, and branches of the vestibular cochlear nerve were manually segmented. Five-fold cross-validation (75-25 train-validation split) was conducted using nnUNet, an open-source 3D semantic segmentation CNN, using the standard combination loss function of cross-entropy and Dice loss. Cross-validation was then repeated on nnUNet using a novel loss function of cross-entropy and distance-weighted Dice loss for training. Predicted segmentations from both models were compared against ground truth manual segmentations using modified Hausdorff distances (mHDs) and Dice scores.
Results: Training for 300 epochs took 4.2 hours per fold on a dedicated 24 GB VRAM GPU workstation. Modified Hausdorff distances and Dice scores between ground truth labels and predictions from standard nnUNet were as follows for select structures: malleus [mHD: 0.044±0.024 mm, Dice: 0.914±0.035], incus [mHD: 0.051±0.027 mm, Dice: 0.916±0.034], stapes [mHD: 0.147±0.113 mm, Dice: 0.560±0.106], inner ear [mHD: 0.038±0.031 mm, Dice:

0.952±0.017], facial nerve [mHD: 0.139±0.072 mm, Dice: 0.862±0.039]. Metrics for distance-weighted nnUNet were similar for these structures: malleus [mHD: 0.046±0.026 mm, Dice: 0.910±0.037], incus [mHD: 0.053±0.028 mm, Dice: 0.911±0.045], stapes [mHD: 0.139±0.096 mm, Dice: 0.565±0.115], inner ear [mHD: 0.039±0.028 mm, Dice: 0.951±0.015], facial nerve [mHD: 0.221±0.280 mm, Dice: 0.858±0.037]. Distance-weighted nnUNet [mHD: 0.474±0.478 mm, Dice: 0.504±0.232] trended toward greater accuracy for labelling the inferior vestibular nerve compared to standard nnUNet [mHD: 1.222±2.458 mm, Dice: 0.471±0.200], though this difference was not significant (p=0.290).
Conclusions: This study sets a foundation for refining an open-source deep learning pipeline for semantic CT segmentation of temporal bone anatomy. By using a distance-weighted loss function, we have demonstrated that nnUNet labels temporal bone CTs with submillimeter accuracy compared to hand-segmented labels for all structures included in this study. This pipeline has the potential to streamline pre-operative planning workflows for a variety of temporal bone procedures and integrate with developing image-guidance and robot-assisted systems for surgical innovation.

M195. Android-Based Mobile Application to Estimate the User's Audiometric Hearing Thresholds and Auditory Temporal Resolution

Ghazaleh Ghaffari*[1], Fredrik Öhberg[1], Mimmi Wernerd[1], Per Hallberg[1], Amin Saremi[1]
[1]*Umeå University*
Category: Clinical Otolaryngology & Pathology
Background: This study presents an Android mobile application developed to perform two key auditory tests: 1) standard pure tone audiometry, which measures the user's audiometric hearing thresholds, and 2) temporal masking (TM) test that assesses the temporal resolution of the user's auditory system. While several mobile applications have been created to automate audiometry for at-home testing, these solutions often lack explicit calibration with the hardware characteristics of connected headphones, leading to uncertainties in the true sound intensities delivered. To address this limitation, the developed mobile app adapts to the specific frequency response of the connected headphones and conducts pure tone audiometry using von Bekesy's method. Additionally, the app performs TM which is a psychoacoustic test for estimating the user's ability to distinguish time-varying (temporal) cues in sound signals. This is an important ability for perceiving sounds properly, especially in the presence of non-stationary noise.
Methods: A total of 48 participants were recruited: 24 with normal hearing (mean age=49.2 years, SD=15.0) and 24 hearing-impaired individuals (mean age=59.0 years, SD=9.0). Clinical tone audiometry was manually conducted on both ears by an experienced audiologist at frequencies between 250 and 8000Hz in a sound booth. Participants then took the app-based audiometry test using SONY WH-1000XM3 headphones in a quiet room. Results from both tests were statistically analyzed using paired t-tests and Pearson correlation (r) in IBM SPSS, with a two-tailed significance level of 0.05. Our TM paradigm consisted of a fixed-intensity narrow-band noise masker at 80dB, followed by a silent gap and a 50ms-long tone (target). During this test, the gap duration varied, and hearing thresholds of the target were measured as a function of gap duration. The TM test was conducted on all test participants at gap durations of 5, 10, 20, and 80ms and tone frequencies of 0.5, 2, and 4kHz.
Results: The app-based audiometry results closely matched clinical outcomes. The mean absolute error (MAE) between the two methods was 5.1dB for normal-hearing participants and

7.3dB for hearing-impaired participants, averaged across all frequencies. Paired t-tests revealed no significant differences between the two methods at any of the eight tested frequencies. The correlation between the two methods was highly significant ($p<0.05$) and consistently strong across all frequencies ($r=0.90$ on average). For the TM test, One-Way ANOVA showed that the slope of the TM function was significantly ($p<0.05$) steeper in the normal-hearing group (-0.22 at 2kHz, -0.22 at 4kHz) than in the hearing-impaired group (-0.14 at 2kHz, -0.10 at 4kHz).
Conclusions: These findings indicate that our mobile app can accurately estimate the clinical audiogram. Furthermore, the TM results showed that hearing-impaired individuals gain only minimal benefit from the temporal cues, represented by the gap duration, in the signal.

M196. Evaluating the Consistency of Otoacoustic Emission Measurements With Self-Inserted Probes

Krzysztof Kochanek[1], W. Wiktor Jedrzejczak*[1], Marta Malina[2], Aleksandra Kowenia[2], Henryk Skarzynski[1]
[1]Institute of Physiology and Pathology of Hearing, Warsaw, Poland, [2]The University of Maria Curie-Sklodowska in Lublin
Category: Otoacoustic Emissions
Background: Otitis media (OM), commonly referred to as an ear infection, is the one of the most common clinical diagnoses, particularly in children. OM results from many infectious agents, both viral and bacterial. The disease is usually associated with ear pain and a transient conductive hearing loss (CHL), however, neither are necessary pre-requisites for OM. Importantly, middle ear effusions (MEE) from fluid and mucus accumulation can occur without the presence of an active infection. A sterile MEE will present with a transient CHL like seen in OM but rarely presents with the same discomfort in absence of an inflammatory condition. A timely diagnosis of OM and MEEs is important to prevent structural damage in the middle ear cavity and, most importantly, irreversible hearing loss. Furthermore, hearing is pivotal for speech and language development, highlighting the need for early detection in children. This is particularly true in those with genetic conditions with increased risk for OM and MEE, like in Down Syndrome (DS).
Current methods used within the clinic include otoscopic examinations and tympanometry. Although the standard of care, their use is often reliant on clinician experience. This difficulty has led to high rates of misdiagnoses and unnecessary antibiotic usage from both otolaryngologists and pediatricians.
Methods: Recently, optical techniques have been explored for diagnosing OM and MEE. Highly sensitive optical techniques, like speckle contrast imaging (SCI), may open the door towards earlier, more accurate diagnostics. SCI characterizes patterns of reflected coherent light on biological surfaces to characterize feathers like morphology and relative blood flow. Research has been conducted towards creating a device that uses this technique within a comfortable, fully functional device to diagnose OM and MEE. Initial prototypes contain 1) a nano-camera for imaging the (tympanic membrane) TM, 2) optical fiber connected to coherent light source, and 3) micro-LED providing white light. The device will take two images of the TM, one with white light and the other with the coherent light source. Speckle contrast values will be calculated using custom written MATLAB codes to determine differences in cases of OM, MEE, or healthy controls.
Results: We have successfully shown proof of principle towards characterizing patterns of the TM in OM and MEE using SCI within microfluidic channels. Upcoming studies are planned to

take images of human TMs to be analyzed and coupled with AI algorithms with the hope of creating a tool that can help practitioners within the clinic and parents/caregivers to determine when clinical evaluation is warranted.
Conclusions: By creating a comfortable, accurate device, this research has the potential to make a large impact on the development of children and maintaining ear health, particularly in DS.

M197. Optical Method of Diagnosing Middle Ear Effusions

Jordan Villa*[1], Joaquin Cury[1], Claus-Peter Richter[1]
[1]*Feinberg School of Medicine, Northwestern University*

Category: Middle & External Ear
Background: Otitis media (OM), commonly referred to as an ear infection, is the one of the most common clinical diagnoses, particularly in children. OM results from many infectious agents, both viral and bacterial. The disease is usually associated with ear pain and a transient conductive hearing loss (CHL), however, neither are necessary pre-requisites for OM. Importantly, middle ear effusions (MEE) from fluid and mucus accumulation can occur without the presence of an active infection. A sterile MEE will present with a transient CHL like seen in OM but rarely presents with the same discomfort in absence of an inflammatory condition. A timely diagnosis of OM and MEEs is important to prevent structural damage in the middle ear cavity and, most importantly, irreversible hearing loss. Furthermore, hearing is pivotal for speech and language development, highlighting the need for early detection in children. This is particularly true in those with genetic conditions with increased risk for OM and MEE, like in Down Syndrome (DS).
Current methods used within the clinic include otoscopic examinations and tympanometry. Although the standard of care, their use is often reliant on clinician experience. This difficulty has led to high rates of misdiagnoses and unnecessary antibiotic usage from both otolaryngologists and pediatricians.
Methods: Recently, optical techniques have been explored for diagnosing OM and MEE. Highly sensitive optical techniques, like speckle contrast imaging (SCI), may open the door towards earlier, more accurate diagnostics. SCI characterizes patterns of reflected coherent light on biological surfaces to characterize feathers like morphology and relative blood flow. Research has been conducted towards creating a device that uses this technique within a comfortable, fully functional device to diagnose OM and MEE. Initial prototypes contain 1) a nano-camera for imaging the (tympanic membrane) TM, 2) optical fiber connected to coherent light source, and 3) micro-LED providing white light. The device will take two images of the TM, one with white light and the other with the coherent light source. Speckle contrast values will be calculated using custom written MATLAB codes to determine differences in cases of OM, MEE, or healthy controls.
Results: We have successfully shown proof of principle towards characterizing patterns of the TM in OM and MEE using SCI within microfluidic channels. Upcoming studies are planned to take images of human TMs to be analyzed and coupled with AI algorithms with the hope of creating a tool that can help practitioners within the clinic and parents/caregivers to determine when clinical evaluation is warranted.
Conclusions: By creating a comfortable, accurate device, this research has the potential to make a large impact on the development of children and maintaining ear health, particularly in DS.

Symposium 5: Across Species: The Functional Role of Cochlear Synaptopathy for Speech Coding in the Brain (CoSySpeech)

3:00 p.m. - 5:00 p.m.

Ocean Ballroom 1 - 4

Across Species: The Functional Role of Cochlear Synaptopathy for Speech Coding in the Brain (CoSySpeech)

Ocean Ballroom 1 - 4

Chair: Marlies Knipper*, University Hospital Tuebingen*

Co-Chair: Sarah Verhulst*, Ghent University*

Symposium Description: Despite the high prevalence of hearing disorders and their contributions to speech comprehension deficits, our knowledge of how cochlear synaptopathy contributes to speech perception is still limited. In a consortium, CoSySpeech funded by the ERA-NET NEURON 2020, scientists from clinical labs and animal physiology labs combined their expertise to develop a computer-based model of speech coding in the brain (SCB-model). Combining research studies from different brain areas (periphery, brainstem, midbrain, and cortex) analyzing various research modalities (histology, physiology, computational behavior, and imaging technology), the consortium has provided new insight in speech coding strategies throughout the auditory system and across species. Our findings will be first presented to the public in this symposium. Specific designed phoneme stimuli, which was first used to investigate temporal fine structure (TFS) and temporal envelope (TENV) coding were used in animal and human studies through psychophysical experiments. These exact stimuli were then used in electrophysiological experiments: single-unit recordings from the inferior colliculus in gerbils, in vivo direct recordings from both animal and human auditory nerves, and EEG/MEG imaging in thalamic and cortical structures in humans. Cross-species results were tied together using short-pulsed distortion product otoacoustic emissions (DPOAEs), fine structure audiometry, auditory brainstem responses (ABRs) and cortical auditory evoked responses (EEG, MEG) in humans. Finally, we will report how these parallel experiments have progressed and how they will be developed to generate a new SCB-model.

Theoretical and Empirical Approaches to Understand Consequences of Auditory Synaptopathy

Andrew Oxenham*, University of Minnesota*

Individual Abstract: Since the ground-breaking discovery of auditory synaptopathy in non-human mammals more than 15 years ago, considerable effort has gone into determining whether noise-induced and/or age-related auditory synaptopathy occurs in humans, whether it can be reliably measured, and whether it affects of our perception of sound in general, and speech in particular. This talk will provide an overview of work from our lab that has addressed these issues from a theoretical, physiological, and perceptual perspective. From a theoretical perspective, it is possible to consider the information carried by the auditory nerve in response to

auditory stimuli, including speech, and by making assumptions about the independence of that information between auditory nerve fibers to predict the perceptual effects of losing a proportion of functional auditory nerve fibers to synaptopathy. Such predictions can be compared with empirical results and expected levels of synaptopathy based, for instance, on age. Although a "gold standard" for diagnosing auditory synaptopathy in humans remains elusive, combinations of theory, physiological, and perceptual findings can be combined to provide insights into the interactions between peripheral and central factors governing the changes in hearing that occur due to age and hearing loss.

Using Morphing Phoneme Stimuli to Study Speech Coding in The Brain

Deniz Başkent*, University of Groningen, University Medical Center Groningen*

Individual Abstract: Language comprehension is dependent upon correct discrimination of vowels (Won JH et al. Svirsky 2016 , 84) and consonants (Hornickel J et al. Kraus N 2009) that, in turn, require precise temporal fine-structure coding (below the human phase-locking limit (PLL), under 1500 Hz) and temporal envelope coding (above PLL, over 1500 Hz) (Verschooten E et al. Plack CJ 2019; Weiss TF and Rose CA 1988, Hear Res). Aiming to test the consequences of stimuli-induced phoneme discrimination tasks in animals and humans we generated specific computer-generated phonemes enabling the differentiation of TFS and TENV coding: Towards this aim vowels pairs /o/u/ that differed by their first formant located well below the supposed PLL in humans (~1.5 kHz) were synthesized that similar to /du/-/bu/ syllable pair only differed at frequencies below the PLL, and distinguished from the first only within the first 100 ms. In addition vowel pairs /i/ y/ only differed in their second and third formants, which were above the PLL, with the result that encoding of this /i/-/y/ contrast could not rely on temporal fine structure but on envelope coding. These /i/-/y/ vowel contrast had the same duration and ramps as the other vowel pair presented above. The /di/-/bi/ syllable pair was also built to only differ in frequencies above the PLL, and within the spectral power of the first 100 ms. We here provide new insight of how – using these stimuli in animal and human study – new insight in the molecular basis of TNF and TENV coding during speech comprehension could be achieved.

Work supported ERA-NET NEURON JTC 2020, FWO G0H6420N; IZKF Promotionscolleg, VICI Grant (Grant No. 918-17-603), NOW, ZonMw.

Vowel Encoding in The Gerbil Auditory Nerve

Daniil Kiselev*, Institut National de la Santé et de la Recherche Médicale*

Individual Abstract: Introduction: Given its low-frequency hearing sensitivity, the gerbil is a valuable model for examining the mechanisms of vowel encoding in the auditory nerve. Methods: Single-fiber recordings from the auditory nerve were conducted in normal-hearing gerbils in response to four synthetic vowels: /o/, /u/, /i/, and /y/. These vowels were designed to have identical durations (170 ms) and identical fundamental frequencies (116 Hz). Each vowel was presented 20 times to each fiber, maintaining consistent polarity and sound level throughout the presentation.

Results: The responses to vowels were recorded in more than 100 ANFs with characteristics frequency (CF) covered the full hearing range of gerbils (0.5-45 kHz). In response to vowels

presented at 70 dB SPL, 70% of fibers elevate significantly their firing rate above the fiber spontaneous rate of discharge. Surprisingly, a significant proportion of fibers with CF above 8 kHz demonstrated responsiveness to the vowels. This activation of high-CF fibers can be attributed to the presence of a prominent "tail" in their tuning curve. At a lower presentation level of 50 dB SPL, the proportion of responsive high-CF fibers decreased markedly. This decrease provides further support for the critical role of the "tail" in the neural coding of vowels.

Conclusion: This study in gerbils provides compelling evidence that auditory nerve fibers with CF above 8 kHz and characterized by prominent “tail” in their tuning curve, may play a significant role in coding vowels. The input from these high-CF fibers could potentially complement the information provided by low-CF fibers, thereby enriching the overall neural representation of vowels in the auditory nerve.

Work supported by ERA-NET Neuron grant CoSySpeech (ANR R21034FF).

Phoneme Encoding in the Inferior Colliculus of Gerbils, With and Without Noise Induced Cochlear Synaptopathy

Warren Bakay*, Institute of Neuroscience Castilla y Leon, University of Salamanca*

Individual Abstract: Several studies describe how peripheral processing of auditory information changes after synaptopathy, but only for basic acoustic sounds (e.g., transients, noise, tones). Studies rarely consider speech stimuli, even though this is necessary to ultimately relate speech intelligibility deficits to synaptopathy. Here we used computer-generated phonemes that enabled the differentiation of temporal fine structure (TFS) and temporal envelope (TENV) coding (see Gaudrain and Baskent) as stimuli to record responses from individual neurons in the inferior colliculus of gerbils, both controls and animals with noise-induced cochlear synaptopathy (CoSy). The cochlear synaptopathy has been quantified using a variety of non-invasive measures, in parallel with the human studies, including auditory brainstem responses (ABR), distortion product otoacoustic emissions (DPOAE), cortical auditory evoked potentials CAEP), and validated by immunohistochemistry to identify the number of sensory cells and synapses in the cochlea. Awake, freely moving gerbils were exposed to an octave band of noise (2.6–5.2 kHz) at 100 dB SPL, for 2h, in an antiparallel chamber. During exposures, animals were unrestrained within small cells in a subdivided cage (1 animal/cell). Here, we present findings categorizing the responses to different speech stimuli from individual neurons in the inferior colliculus of anaesthetized gerbils and relate it to the frequency response areas of each neuron. We further present how various masking conditions affect these responses and present the efficacy of the coding to represent the acoustic stimuli. Finally, we present how noise induced cochlear synaptopathy affects these coding mechanisms and their efficacy.
Work supported ERA-NET NEURON JTC 2020 (CoSySpeech Project) and the Consejería de Educación y Cultura de la Junta de Castilla y León (grants SA252P20)

Examination of Speech Coding in the Human Auditory Nerve Using Intracranial Recordings

Xavier Dubernard*, University Hospital of Reims*

Individual Abstract: Early intracranial recordings of the auditory nerve, conducted by Møller et al. in the 1980s, explored how the nerve encodes simple sounds, such as clicks or tone bursts. We aimed to extend on this work by investigating human auditory nerve responses to phonemes, sounds crucial for speech perception. Our study focused on the relative importance of temporal fine structure and temporal envelope. Recordings were performed at the University Hospital of Reims on patients undergoing microvascular decompression surgery (NCT03552224) for trigeminal neuralgia or hemifacial spasm. This approach provided access to the human auditory nerve. Both normal-hearing and high-frequency hearing loss patients participated. Phonemes (/o/, /u/, /i/, /y/ vowels and /du/, /bu/, /di/, /bi/ syllables) were delivered via Etymotic ER1 earphones at 70 dB SPL in quiet or noisy environments. Auditory nerve activity was measured using a ball electrode placed on the nerve during surgery (see Huet et al. J Neurosci. 2022 Mar 16;42(11):2253-2267). Our results revealed a remarkable preservation of neural responses reflecting temporal fine structure in both normal and hearing-impaired patients. However, responses related to the temporal envelope were significantly reduced in those with hearing loss. This suggests that the basal region of the cochlea, which is often affected in hearing loss, plays a key role in processing the temporal envelope of sound. Intracranial recordings offer a promising way to study how the human auditory nerve encodes complex sounds. This technique has the potential to significantly improve our understanding of how hearing loss disrupts speech processing at the neural level.
Work supported by ERA-NET Neuron grant CoSySpeech (ANR R21034FF).

Stimulus Onset Contributions to Speech Comprehension
Marlies Knipper*, University Hospital Tuebingen*

Individual Abstract: Slowing and reduction of auditory responses over age, promoting speech processing and cognitive deficits, are currently controversially discussed as being either related to central brain atrophy or slowing of neural processing from the periphery. We examined young, middle-aged, and older individuals with and without hearing threshold loss using pure-tone (PT) audiometry, short-pulsed distortion-product otoacoustic emissions (pDPOAE), auditory brainstem responses (ABR), auditory steady state responses (ASSR), speech comprehension (OLSA), and syllable discrimination in quiet and noise; amplitudes and latencies of speech EEG responses to syllables, and 4-5 word discrimination tasks using MEG. Speech comprehension deficits were identified dependent or independent of pure tone threshold (PTT) and age. Not only was poor speech comprehension independent of age and PTT linked with differences in cochlear amplifier performance and ABR wave latency shift but also phoneme induced thalamic delay (EEG) and altered attention requiring word discrimination responses in cortical regions (MEG). We furthermore discuss differential changes in amplitude and delays of thalamic or cortical activity in the context of differences in responses to phonemes requiring either temporal fine structure (TFS, LESS THAN phase locking limit (PLL) or temporal envelope (TENV, GREATER THAN PLL) coding. Data may suggest possible new predictors for altered speech comprehension in quiet and ipsilateral noise that link deficits of the non-adapted, pre-neural input signal to the IHCs stimulus onset to neocortical activity.
Work supported by the DFG KN 316/13-1, DFG RU 713/6-1, KL 1093/12-1; ERA-NET NEURON JTC 2020: BMBF 01EW2102 CoSySpeech and FWO G0H6420N; IZKF Promotionscolleg, VICI Grant (Grant No. 918-17-603), NOW, ZonMw.

Across Species Modeling Insights Into Hearing-Impaired Speech Coding
Sarah Verhulst, *Ghent University*

Individual Abstract: Animal models are well suited to connect the pathophysiology of hearing damage to neural recordings, whereas in humans we only have access to indirect neural population responses (EEG or round-window potentials). Because different species are adopted in research studies, it is difficult to translate specific outcomes to implications for human speech processing. To bridge this translational gap, we developed a model of the auditory periphery that can be adjusted to the cochlea of the species investigated. The models generate single-unit auditory-nerve-fiber and brainstem responses as well as the generators of auditory evoked potentials and otoacoustic emissions. We calibrated the model based on physiology and speech data collected in Gerbils and applied it to study (hearing-impaired) human speech processing. Human and Gerbil model simulations/experiments adopted the same auditory stimuli, and a human collected EEG dataset in response to the stimuli adopted to evaluate the quality of the translational approach.

A one-dimensional human nonlinear cochlear transmission-line model (Verhulst et al., 2018) was used as a starting point to adjust the processing to the Gerbil using the cochlear scaling-symmetry principle and available data on cochlear and middle-ear mechanics. The double-pole of basilar-membrane admittance was set to match the frequency selectivity and compressive responses of each species' cochlea and the 2-D pressure focusing technique was adopted to yield sufficient compression. The models were calibrated based on collected DPOAE and ABR data, and various hearing-impairment profiles (synaptopathy and outer-hair-cell damage) were simulated. We specifically focused on simulating the neural coding of four 70-dB-SPL phonemes (/du/, /bu/, /di/, /bi/) and vowels (/o/, /u/, /i/,/y/) that were adopted in both neural (EEG, neurograms) as well as psychoacoustic discrimination experiments. For pairs /du/-/bu/ and /o/-/u/ the discrimination cue targets temporal-fine-structure (TFS) coding whereas the /i/-/y/ and /di/-/bi/ pairs (energy above 1.5 kHz) temporal envelope (TENV) cues are adopted.

Both simulations and recordings show easy neural discriminability of the TFS-cue based /u/ - /o/ vowel contrast, while the /i/ - /y/ neural discriminability was difficult and behavioral discriminability easy. The hearing-impairment simulations could account for phenomenological differences in the experimental recordings, and the simulations confirm that altering the cochlear mechanics of the model accounted for the main differences in normal hearing responses. Taken together, our across-species modeling approach gives an interesting and translational pathway into fully understanding how hearing-impairment affects the dominant features associated with speech coding in the brain.

Work supported by ERA-NET Neuron grant CoSySpeech (FWO G0H6420N).

Podium 11: Advances in Vestibular Science and Rehabilitation: From Cellular Mechanisms to Clinical Innovations

Moderators: Taha Jan and Divya Chari

3:00 p.m. - 5:00 p.m.

Ocean Ballroom 5 - 8

Characterization of Viral Transfection of Human Vestibular Epithelial Tissues in Vitro

Chisako Tanaka*[1], Sushobhan Biswas[1], Tian Wang[2], Micheal Freeman[1], Anika Patro[1], Elizabeth Perkins[1], Kareem Tawfik[1], Aaron Moberly[1], Matthew O'Malley[1], Marc Bennett[1], David Haynes[1], Alan G. Chang[2], Taha A. Jan[1]

[1]*Vanderbilt University Medical Center,* [2]*Stanford University*

Background: Loss of sensory hair cells in the human utricle can lead to vestibular dysfunction. We previously demonstrated limited spontaneous hair cell transdifferentiation in vivo in utricles harvested from vestibular schwannoma (VS) patients. By comparing this model to organ donor tissues, we demonstrated that VS utricles serve as a damage model (Wang et al., 2024). The aim of our experiments here is to characterize an in vitro model of human VS utricles that allows the characterization of cell viability and degree of transfection using adeno-associated viruses (AAV).

Methods: IRB approval was obtained at the Vanderbilt University Medical Center (VUMC, IRB#230391). We harvested utricles from 20 patients that have undergone translabyrinthine resection of vestibular schwannoma tumors between August 2023 - March 2024 at VUMC. Six were processed for cryosectioning and histological analysis. Seven were processed for whole mount immunostaining and quantification. Images were captured using a NikonA1R confocal microscope and number of cells was quantified. Six additional samples were cultured. De-identified clinical data from patients' medical records were also collected to correlate with histological analyses. For AAV experiments, we tested tropism of 3 vectors: AAV1, 8, and 9 carrying CMV-GFP. Utricles were incubated with AAV (1.05x1011, 1.74x1011, 2.0x1011 vg/ml respectively) for 3 days of transfection and cultured for another 4 days without AAV prior.

Results: We show that human utricles from VS patients have 48.58±25.26 Myo7a+ hair cells per 10,000 μm^2 and 177.34±31.74 supporting cells per 10,000 μm^2. These data were correlated with a variety of clinical factors. Our preliminary data indicates that AAV8 transfects epithelial cells most efficiently among the three tested vectors.

Conclusions: We have established a pipeline from the operating room to the laboratory for harvesting of high-quality utricles. Our data demonstrate that these samples can undergo a variety of histological and in vitro experiments. Based on our findings, we aim to explore additional viral vectors to achieve high transfection rates with the ultimate goal of pursuing regenerative mechanism to promote human hair cell regeneration in vitro.

Blast Exposure, Vestibular Sequelae, and the Role of Therapeutic Hypothermia in Mitigating Blast-Induced Vestibular Dysfunction

Pavan Krishnan*[1], Federica M. Raciti[2], Yuan Gao[2], Megan Barber[3], Rachele Sangaletti[3], Suhrud Rajguru[3]

[1]*University of Miami/Jackson Health System,* [2]*University of Miami Miller School of Medicine,* [3]*University of Miami,*

Background: Audiovestibular symptoms, including hearing loss, dizziness, and balance issues, are among the most common and long-lasting effects reported after blast exposure. However, there is limited research on the impact of blast exposure on the vestibular system in preclinical models. Moreover, there are no established therapeutic approaches to mitigate blast-induced vestibular dysfunction. In this study, we aim to characterize vestibular functional and behavioral outcomes post-blast exposure, ascertain mechanisms underlying the vestibular sequelae, and evaluate the efficacy of mild therapeutic hypothermia (MTH) as a potential intervention for dysfunction.

Methods: An ecologically validated, oxyacetylene-driven blast-tube was designed to deliver consistent exposures in rodent models. We have recently developed a novel methodology for non-invasive delivering of MTH to the inner ear via neck collar. We performed auditory brainstem responses (ABRs) and cervical myogenic-evoked potentials (cVEMPs) in four cohorts (No blast (NB), Blast (B), No blast with MTH (NBwMTH), Blast with MTH (BwMTH)) of Brown Norway rats to establish a baseline. Baseline measurements were also collected for the balance beam. These tests were repeated on days 1, 3, and 7 post-blast. Non-invasive delivery of MTH was implemented within 24 hours post-blast for control and blast groups. Mechanistic studies were carried out at 24 hours post-blast.

Results: We developed a blast-driven shock tube that reliably produces pressure waves of 5-8 psi. These did not lead to tympanic membrane perforations in rats. ABR thresholds at across the tonotopic map significantly increased post blasts (p LESS THAN 0.03). At 4kHz, thresholds increased from 32.5□5.00 dB at baseline to 67.5 at Day 1 post-blast to 71.3 dB at Day 3 post-blast. At 24 kHz, thresholds increased from 37.5 dB at baseline to 72.5 dB at Day 1 post-blast to 71.25 dB at Day 3 post-blast. cVEMP thresholds at 1 and 8 kHz frequencies also significantly increased after blasts (p LESS THAN 0.04). At 1 kHz, thresholds increased from 53.8 dB at baseline to 85.0 dB at days 1 and 3 post-blast. At 8 kHz, thresholds increased from 50.0 dB at baseline to 83.8 dB at day 1 post-blast to 82.5 dB day 3 post-blast. Balance beam testing revealed an increase in time-to-finish from 6.4 seconds to 18.7 seconds on day 1 post-blast, with partial recovery to 9.2 seconds by D3 post-blast. We observed was significant changes in the cochlea as well as brainstem and cortex of pyroptosis related mechanisms.

Conclusions: The blast paradigm presented induces acute audiovestibular functional changes and contributes to measurable behavioral deficits associated with vestibular dysfunction, making it a valuable model to explore potential therapeutic effects of MTH.

Optimizing Postural Stability Clinical Assessment

Talah Wafa*[1], Christopher Zalewski[2], Carmen Brewer[2], Gayla Poling[1]

[1]*National Institute on Deafness and other Communication Disorders,* [2]*National Institute on Deafness and Other Communication Disorders/NIH*

Background: Ineffective postural control contributes to imbalance and unsteadiness that may result in falls if an individual is destabilized. Falls are especially prevalent in the aging population, with morbidity costs exceeding $50 billion annually.
The sensory organization test (SOT) is the gold standard for assessing postural stability. Interpretation of the SOT is based on equilibrium scores derived from the relationship between an individual's sway and a fixed theoretical limit of stability (tLOS). However, determining an individual's postural stability and fall risk based on this one-size-fits-all theoretical approach has the potential to overestimate functional equilibrium and in turn underestimate fall risk.

Objective: To investigate whether personal LOS (pLOS) measured from healthy adults differs from the tLOS, and whether SOT equilibrium scores are significantly different when calculated based on pLOS instead of tLOS in healthy adults.

Methods: Participants were 60 healthy volunteers comprising three age-groups: young (18-39), middle-aged (40-64), and elderly (65-80), each with equal numbers of males and females. All had normal age-based hearing and a negative history of vertigo and imbalance. SOT scores derived using pLOS were compared to tLOS.
Results: The pLOS from the healthy volunteers was consistently and significantly lower than tLOS across both sexes and all age groups (p LESS THAN .0005); however, no aging effect was observed. SOT equilibrium scores calculated using pLOS were significantly lower and more variable than scores derived using tLOS. A case study is used to demonstrate the impact of tLOS versus pLOS on the interpretation of an individual patient's SOT.
Conclusions: Individual measures of LOS are significantly lower than theoretical estimates of the LOS in healthy adults. This suggests that use of tLOS in the calculation of SOT equilibrium scores often overestimates postural stability and may have implications for the determination of fall risk.

Increasing Access to Vestibular Rehabilitation Using Machine Vision-Based Automation

Erin Williams*[1], Felipe Echeverri Tribin[1], Luis Rodriguez-Diaz[1], Blaine Ayotte[2], Christopher McKenna[2], Odile Clavier[2], Michael Hoffer[1]

[1]*University of Miami Miller School of Medicine,* [2]*Creare LLC*

Background: Vestibular rehabilitation therapy (VRT) aims to reduce or eliminate symptoms of dizziness by enhancing vestibular adaptation and substitution. Early therapy typically focuses on gaze stability via the vestibulo-ocular reflex, with exercise progressions incorporating increased head rotation speeds, varied background distractions, positional changes (i.e., sitting, standing, or walking), and increased daily dosages or target distances. Unfortunately, limited availability of specialized physical therapists can lead to significant delays in care. These delays may negatively affect patients, particularly in light of recent evidence suggesting early intervention with VRT improves outcomes. To address this unmet need, we developed an automated vestibular rehabilitation system (AVRS) to administer basic VRT.
Methods: The AVRS (Creare LLC, [Hanover, NH]) utilizes a stereo vision system for monitoring eye and head movements and a computer display to provide participants instructions

and audio/visual indicators. For this study, exercise progression was administrator-guided with a wireless and dynamically coupled portable tablet. Following written informed consent (#20200839), participants performed VRT exercises consisting of two eye-tracking exercises (X1/X2) with the head moving side-to-side in the horizontal plane while keeping the eyes fixed on a visual target (dot). These tasks were performed sitting and standing at a selection of physiologically relevant frequencies (0.5, 1.0, 1.5, and 2.0 Hz) for approximately 60s. For each exercise, gain was measured as the ratio of the relative combined eye speed to the head speed when the head angle was near zero (center). To mitigate the influence of extreme measurements during head overshoot, gain was averaged by test using median values and the 10-90 interpercentile range (IPR).
Results: Overall, n=40 aged 36±11 (range: 23-62) years and sex-matched (21F/19M) healthy individuals were enrolled. Participants were primarily White (87.5%), with half identifying as Hispanic (50%). Nearly all participants were right-handed (97.5%). The AVRS was well-tolerated across VRT exercises. For seated X1 exercises, the average median gain observed was -0.96 (IPR: 1.47), -0.97 (IPR: 0.17), -1.00 (IPR: 0.20), and -0.99 (IPR: 0.21) at 0.5, 1.0, 1.5 and 2.0 Hz, respectively. Similar trends were observed during standing X1 exercises, with an average gain of -1.00 (IPR: 0.94), -0.94 (IPR: 0.32), -1.00 (IPR: 0.05), and -1.01 (IPR: 0.08) at 0.5, 1.0, 1.5, and 2.0 Hz, respectively. During seated X2 exercises, participants had a mean gain of -1.12 (IPR: 2.04), -0.90 (IPR: 1.71), and -0.90 (IPR: 0.86) Hz at 0.5, 1.0, and 1.5 Hz, respectively. Finally, during standing X2 exercises, we observed an average gain of -1.14 (IPR: 4.89) at 0.5 Hz, -0.90 (IPR: 0.88) at 1.0 Hz, and -1.01 (IPR: 0.08) at 2 Hz.
Conclusions: The AVRS effectively administered basic VRT in healthy controls, demonstrating its viability as a tool for early therapeutic intervention. This normative data will help guide therapeutic progression in symptomatic individuals using the AVRS.

Bone-Conducted Vibration Reduces Vestibulo-Ocular Reflex Time Constant and Motion Sickness During Step Velocity Testing

Didier Depireux*[1], Daniel Stolzberg[1], Eve Mnatsakanyan[2], Tin Truong[2], Samuel Owen[1], Chelsea Nava[2], Brooke Pearce[2]

[1]*OtolithLabs,* [2]*Dizzy and Vertigo Institute of Los Angeles*

Background: This study investigated the effects of bone-conducted vibration (BCV) on vestibular function in healthy participants with variable susceptibility to motion sickness. Our previous research has shown BCV can reduce motion sickness symptoms, but the impact of BCV on the vestibulo-ocular reflex (VOR) is unknown. We hypothesized that BCV would decrease the VOR time constant, suggesting modulation of the central velocity storage mechanism, and is associated with an improvement in motion sickness.
Methods: The study included 38 healthy participants divided into experimental (n=29) and control (n=9) groups. Participants underwent step velocity testing with velocity of 60°/s in a clinical rotary chair in a dark booth while wearing a BCV device over their right mastoid. The BCV device operated at a single frequency in a range from 52 Hz to 60 Hz. The experimental group experienced the device at three power settings—inactive, followed by random ordering of low (112 dB re 1 μN) and high (118 dB re 1 μN) force levels.The control group wore the device powered off for all rotations. Eye movements were tracked to measure VOR. Participants also

rated their motion sickness using the Fast Motion Sickness (FMS) scale before and after each rotation sequence.
Results: Linear mixed effects models show that BCV significantly reduced the VOR time constant in the experimental group compared to controls and when evaluated independently. Both low and high power settings were effective and order of power setting did not contribute statistically. FMS ratings indicated that most participants in the experimental group experienced reduced motion sickness symptoms with BCV, especially at the high power setting. In contrast, control group participants generally reported worsening symptoms across testing rounds.
Conclusions: We observed a statistically significant decrease in VOR time constant during BCV application, both compared to the control group and when evaluated within the experimental group. This effect was accompanied by improvements in subjective motion sickness ratings, consistent with previous studies on BCV and motion sickness.
This study provides evidence that BCV can modulate vestibular function by reducing the VOR time constant, which may explain BCV's previously observed effectiveness in mitigating motion sickness symptoms. These findings have potential implications for developing non-invasive treatments for other vestibular disorders as well as for motion sickness.

Sex-Specific Impairment in Spatial and Episodic Memories and Vestibular Function in Aging Shank3 KO Mouse Model of Autism

Nelson Shi[1], Patrick Wu[1], Dylan Arevian[1], Soroush Sadeghi[1], Tara Deemyad*[1]

[1]*Johns Hopkins University*

Background: The vestibular system, especially the otolith organs (saccule and utricle), play a crucial role in episodic memory by providing cues for spatial orientation and balance, as episodic and spatial memories are closely interconnected through their shared spatial context. While vestibular dysfunction is commonly seen in individuals with Autism Spectrum Disorder (ASD), there is a significant gap in understanding its specific impact on spatial memory, particularly in aging ASD populations. This study explores the temporal correlation between vestibular dysfunction and impairments in episodic and spatial memory in the Shank3 KO ASD mouse model. Given that ASD phenotypes are more pronounced in males, we specifically focused on the influence of sex in our findings.
Methods: We assessed episodic memory in male and female Shank3 KO mice on a C57BL/6 background across three different age groups: adolescent (2 – 3 months old), young adult (4 – 6 months old), and mid adult (7 –12 months old). For episodic memory, we used the Novel Object Recognition test to evaluate recall and the Y- and Barn Mazes to evaluate spatial memory. We used contact righting reflex as a test of the function of the saccule and utricle.
Results: We found that Shank3 knockout mice in the 7-12 month age group displayed impaired performance on the Novel Object Recognition test when compared to age-matched wild type mice, indicating impaired recall memory. Our data currently shows that male mice exhibit a larger impairment compared to female mice. Furthermore, we found that the Shank3 knockout mice likely could not differentiate between the different arms of the Y-Maze, indicating impaired spatial memory. Moreover, only male mice show impairment in contact righting reflex as an indicator of otolith organ dysfunction.

Conclusions: Our results show a sex-specific disruption in both episodic and spatial memories in Shank3 ASD mouse model, starting around 7-12 months of age. Interestingly, we also observed a decline in otolith function in the same age and sex group. This correlation suggests that early impairments in vestibular performance may contribute to declines in episodic memory and spatial orientation. These findings are in line with previous reports indicating that autistic adults around 30 years of age experience impaired episodic memory compared to age-matched neurotypical individuals. Therefore, vestibular otolith tests (e.g., VEMP) could serve as valuable early detection tools for identifying individuals at risk.

The Relationship Among Vestibular, Hearing, and Balance Outcomes in Individuals With Down Syndrome

Casey Vandervelde[1], Jessie Patterson[2], Heather Porter[2], Gabrielle R. Merchant[2], Kristen Janky*[2]

[1]*University of Utah,* [2]*Boys Town National Research Hospital*

Background: Despite the reported high incidence of hearing loss and balance dysfunction in individuals with Down syndrome (DS), almost nothing is known regarding the contribution of the vestibular system to balance and its relationship to hearing loss. The purpose of this study was to determine the prevalence of vestibular dysfunction in individuals with DS and its relationship with balance and audiometric outcomes. It was hypothesized that there would be a higher prevalence of vestibular dysfunction in individuals with DS with sensorineural hearing loss (SNHL) than those without. It was further hypothesized that greater severity of vestibular dysfunction would be related to greater severity of SNHL and greater functional impairments. This study represents an important first step in characterizing the relationship between vestibular dysfunction, balance, and hearing loss in individuals with DS.
Methods: Twenty-seven participants with DS (mean age: 23.3; range: 7 to 38) and twenty neurotypical controls (mean age: 23.3; range: 7 to 47) participated. All participants completed otoscopy, audiometry (pure-tone average (PTA) of 0.5, 2, and 8 kHz thresholds), tympanometry, wideband acoustic immittance (WAI), air- and bone-conduction cervical and ocular vestibular evoked myogenic potential (VEMP) testing, and video head impulse testing (vHIT). Balance and gait testing included gait speed, Timed-Up-And-Go (TUG), and the Single Leg Stance (SLS) with eyes open and closed.
Results: In the 54 ears of participants with DS, 15 ears had normal hearing (mean PTA: 13.4), 8 ears had SNHL (mean PTA: 43.5), 15 ears had conductive hearing loss (CHL, mean PTA: 30.4), and 16 ears had mixed hearing loss (MHL, mean PTA: 36.4). In participants with DS, results showed present cervical VEMPs in 88% of ears (44/50), present ocular VEMPs in 80% of ears (40/50), and normal vHIT in 87% of ears (45/52). Overall, in participants with DS, 35.2% (19/54) of ears demonstrated some degree of vestibular dysfunction (i.e., absent cervical or ocular VEMP, abnormal vHIT). Compared to neurotypical controls, individuals with DS had significantly longer TUG scores ($p = .006$), slower gait speed in the fast condition (p LESS THAN .001), and poorer balance in the SLS eyes open (p LESS THAN .001) and closed (p LESS THAN .001) conditions. vHIT abnormalities were more likely to occur with SNHL.
Conclusions: In this cohort of individuals with DS there was a large degree of variability in both the severity (normal to profound) and type (normal, SNHL, CHL, and MHL) of hearing loss. VEMP response rates increased with bone conduction stimuli, supporting the high prevalence of

conductive components in individuals with DS and suggests that bone conduction stimuli should be used when assessing VEMP. Overall, one third of individuals with DS in this cohort had some degree of vestibular dysfunction with a higher likelihood in those with SNHL.

Role of Transcription Factor Six2 in the Development of Vestibular Epithelia

Sumana Ghosh*[1], Punam Thapa[1], Vineel Vanga[1], Kaylee Zettler[1], Steven Gressett[1], Garner Fincher[1], Beth Baker[1], Bradley Walters[1]

[1]*University of Mississippi Medical Center*

Background: The transcription factor Six2 is known to function in the embryonic development and patterning of many tissues including kidney and heart. Previous studies suggest the highly related Six1 protein plays an important role in the development of the inner ear. However, little is known about the role of Six2 in this context. Here, we investigated the expression pattern and function of Six2 during inner ear development.
Methods: : Six2 expression was mapped by RNAScope in-situ hybridization using CD-1 mice at different stages of development (E10.5, E12.5, E14.5, E17.5, P0, and P21). We used a germline knockout (Six2-/-) mouse line and a Six2 overexpression model, Atoh1Cre:H11(Six2/Six2), to study the role of Six2 in the morphogenesis of the vestibular epithelia. Whole-mounted utricles and cristae were immunolabelled with antibodies against SOX2, MYO7A, β-spectrin and espin. The role of Six2 in hair cell fate was examined by immunolabelling with SPP1 and MAPT whereas hair cell orientation and planar cell polarity (PCP) was investigated by immunolabelling with β-spectrin, VANGL2 and Gαi3. We used ImageJ to measure hair cell orientation and Oriana software to analyze the data and plot rose diagrams.
Results: Six2 is expressed in both the pro-sensory and non-sensory cells throughout the development of the otic vesicle. Six2 is expressed in both dorsal and ventral regions of the otic vesicle and as early as E10.5. By E17.5 and at postnatal day 0 (P0) Six2 expression becomes enriched in cochlear outer hair cells and some modest expression persists in supporting cells. In the vestibular tissues, Six2 persists in both the type I and type II hair cells and at lower levels in supporting cells. Germline deletion of Six2 led to increased area of the striola, defined by OCM+ cells in the Six2-/- utricle. However, despite the increase in striolar area, the number of OCM+ve cells was decreased. Reduced numbers of OCM+ cells were also noted in the central region of the lateral cristae. Additionally, our data suggests increased numbers of immature hair cells and some misorientation of the hair cells in the utricles of Six2-/- animals, though the overall orientations of VANGL2 and Gαi3 were largely preserved. Using Sox2+ cells to draw the boundaries of the utricles and cristae, we detected no significant differences in the overall sizes of the vestibular epithelia with either deletion or overexpression of Six2. However, the density of MAPT+ve cells and SPP1+ve cells were decreased at P3-P4 with overexpression of Six2.
Conclusions: Overall, our data indicate that Six2 influences vestibular development, potentially influencing hair cell differentiation and/or maturation. Future directions seek to further characterize effects of Six2 manipulation on differentiation and cell fate, and to investigate molecular mechanisms of Six2- mediated phenotypes.

Podium 12: OTOF Success and GJB2 Progress

Moderators: Yen-Fu Cheng and Hela Azaiez

3:00 p.m. - 5:00 p.m.

Ocean Ballroom 9 - 12

Safety and Efficacy of DB-OTO Gene Therapy in Children With Profound Deafness Due to Otoferlin Variants: Data From the Chord Phase 1/2 Open-Label Trial

Jay Rubinstein*[1], Manohar Bance[2], Lawrence Lustig[3], Akira Ishiyama[4], Robert Nash[5], Ruben Polo[6], Manuel Jesus Manrique[7], Evie Landry[8], Margaret A Meredith[9], Tera Quigley[10], Jason Riggs[10], Eduardo Corrales[10], Jonathon Whitton[10], Jeffery Anderson[10], Vassili Valayannopoulos[10]

[1]*Virginia Merrill Bloedel Hearing Research Center,* [2]*Cambridge University,* [3]*Columbia University,* [4]*UCLA,* [5]*Great Ormond Street Hospital,* [6]*Hospital Universitario Ramon y Cajal,* [7]*Clinica Universidad de Navarra,* [8]*Nemours Children's Health,* [9]*Seattle Children's Hospital,* [10]*Regeneron Pharmaceuticals*

Background: Otoferlin is critical for inner hair cell (IHC) signal transmission to auditory nerve fibers. Biallelic otoferlin gene (OTOF) variants typically cause severe-to-profound deafness. Preclinical data showed that DB-OTO gene therapy promotes IHC-selective otoferlin expression from the human OTOF gene, which may provide high-quality hearing. In this ongoing, first-in-human, multicenter Phase 1/2 open-label clinical trial (CHORD, NCT05788536), the safety, tolerability, and efficacy of DB-OTO (administered by intracochlear injection using a typical facial recess approach through the round window) is being evaluated in pediatric patients with profound OTOF-related deafness.
Methods: Early safety and efficacy data in patients who received a single, unilateral intracochlear injection of DB-OTO (7.2 x 1012 vector genomes) have previously been shared. To date, surgical delivery has been uneventful in all patients. No dose-limiting toxicities or DB-OTO-related adverse events have been reported, and no persistent vestibular manifestations have been observed.
Results: Updated data from the trial, including Week 48 efficacy data and speech perception outcomes, will be presented at this meeting.
Conclusions: Early results demonstrate a positive safety and tolerability profile and suggest that DB-OTO gene therapy may significantly improve hearing in patients with profound deafness caused by OTOF variants. Patient enrollment is ongoing.

OTOV101 Gene Therapy for Autosomal Recessive Deafness 9: A Multicenter, Open-Label, Single-Arm, Investigator Initiated Intervention Study

Renjie Chai[1], Jieyu Qi*[2], Liyan Zhang[3], Ling Lu[4], Fangzhi Tan[3], Cheng Cheng[5], Wenxiu Dong[6], Yinyi Zhou[2], Lulu Jiang[6], Chang Tan[6], Shanzhong Zhang[6], Huaien Song[7], Maoli Duan[8], Xia Gao[5], Dingjun Zha[9], Yu Sun[10], Lei Xu[11], FanGang Zeng[12]

[1]*Southeast University ; Nantong University ; Beijing Institute of Technology ; Sichuan Provincial People's Hospital,* [2]*Beijing Institute of Technology,* [3]*Southeast University,* [4]*Zhongda Hospital ; Southeast University,* [5]*Nanjing Drum Tower Hospital,* [6]*Otovia Therapeutics Inc,* [7]*Fosun Health Capital,* [8]*Karolinska University Hospital,* [9]*Xijing Hospital,* [10]*Huazhong University of Science and Technology, Union Hospital,* [11]*Shandong Second Provincial General Hospital,* [12]*University of California Irvine*

Background: We reported the safety and efficacy of AAV-OTOF in eight DFNB9 children for the first time. However, there have been no clinical studies in older participants.
Methods: This is a multicenter, open-label, single-arm, intervention trial. Nine DFNB9 participants (1.6-23.9 years) with severe-to-complete bilateral hearing loss were recruited from 4 centers. Seven participants received unilateral AAV-OTOF treatment due to the contralateral cochlear implant. Two received bilateral treatment. The dosage was 8.4E11 to 11.2E11 vg per ear. Primary and secondary outcomes were safety /tolerability within 2-12 months and corresponding auditory function assessments on the efficacy, respectively.
Results: No serious adverse events (SAE), nor AEs leading to discontinuation, nor death occurred. A total of 97 treatment-emerged AEs were observed and graded as I or II. Hearing was restored after treatment for all participants, including the adolescent and adult. Averaging over the 9 participants, the pure-tone-average (PTA) thresholds were improved from 105 dB at baseline to 64- and 62-dB HL at 1 and 2 months, respectively. Gene therapy effect was rapid, taking one month to restore most hearing. Objective measures like Click- and TB-ABR could reliably predict the PTA thresholds after 4 months (R2=0.85 and 0.75, respectively). An age-dependent therapeutic effect was observed, with participants of 5-8 years old showing the most hearing restoration.
Conclusions: AAV-OTOF rapidly and effectively restores hearing in DFNB9 patients from toddler to adults. with the degree of restoration being dependent on age. (Funded by National Key R and D Program of China and others; Otovia Therapeutics ClinicalTrials.gov number, NCT05901480).

Effectiveness of Gene Therapy in Patients with DFNB9: Evidence From Cortex and Development

Jiajia Zhang*[1], Zengzhi Guo[2], Changjie Pan[3], Chunchun Hu[4], Xinyang Weng[5], Bing Chen[5], Zheng-Yi Chen[6], Shan Sun[1], Xiu Xu[4], Huawei Li[5], Fei Chen[3], Yilai Shu[5]

[1]*Fudan University Eye Ear Nose and Throat Hospital,* [2]*Northeastern University,* [3]*Southern University of Science and Technology,* [4]*Children's Hospital of Fudan University,* [5]*Eye and ENT Hospital, Fudan University,* [6]*Graduate Program in Speech and Hearing Bioscience and Technology and Program in Neuroscience, Harvard Medical School; Eaton-Peabody Laboratory, Massachusetts Eye and Ear Infirmary*

Background: Autosomal recessive deafness 9 (DFNB9) is caused by mutations in the OTOF gene and is characterized by severe to complete congenital or pre-lingual bilateral hearing loss. The safety and efficacy of gene therapy for DFNB9 in children have been confirmed recently. Congenital deafness patients receiving gene therapy represent a unique group of individuals who

experience hearing deprivation to recovery, speech function from absent to present, and subsequently how the hearing-related cortex changes is unknown.
Methods: We aimed to study the neural processing in these patients after gene therapy, especially in the auditory and speech cortex. Functional near-infrared spectroscopy (fNIRS) and electroencephalogram (EEG) were used to evaluate auditory cortex function and cognitive function. All tests were performed before and after gene therapy.
Results: Ten patients (3 girls and 7 boys) aged from 1 to 11 year were included in this study. Patients receiving binaural or unilateral gene therapy showed significant activation in the auditory speech cortex during music or speech stimuli compared to pre-treatment, with activation occurring as early as 4 weeks, and auditory cortex function continued to improve. In addition, the activation in the prefrontal cortex was involved in sound perception. EEG data showed significant mismatch negativity (MMN) as early as 4 weeks postoperatively in patients receiving binaural gene therapy (patient11), and the power of the resting-state EEG beta band was significantly increased in all three groups of patients after gene therapy (p LESS THAN 0.05). The developmental level of the patients increased after gene therapy.
Conclusions: This study for the first time depicts the auditory pattern transition from hearing loss to auditory function reconstruction in patients with congenital deafness, elucidating the resting state brain spectrum and neural processing of various sound stimuli in patients with congenital deafness at different stages after gene therapy. In addition, our study provides a reference on the developmental levels of patients after gene therapy.

Rapid Emergence of Cortical Sound Processing and Auditory Perception Following Otoferlin Gene Transfer Therapy in Young Adult Otof-/- Mice

Kameron Clayton*[1], Korey Sudana[1], Jennifer Zhu[1], Elizabeth Norris[1], Evan Hale[1], Myunghoon Yoo[1], Artur Indzhykulian[1], Daniel Polley[1]

[1]*Eaton-Peabody Laboratories, Massachusetts Eye and Ear*

Background: Otoferlin is a calcium sensor protein that enables synaptic transmission between inner hair cells and the afferent fibers of the spiral ganglion. OTOF gene mutations result in profound hearing loss and virtual elimination of the auditory brainstem response (ABR) in both mice and humans. Clinical trials are now underway to study the recovery of speech and hearing in children with OTOF gene mutations after OTOF gene transfer. Initial findings from several clinical trials show tremendous promise, yet functional testing in mice with otoferlin gene transfer has not gone beyond the ABR. Here, we studied the emergence of sound awareness and higher-level central processing in mice with genetic deafness following OTOF gene transfer in young adulthood.
Methods: Research-grade (i.e., non-clinical) hybrid AAV vectors encoding myc-tagged mouse Otoferlin protein were injected into the left posterior semicircular canal of 6 mice with an OTOF missense mutation (Otof deaf5, Otof-/-) in postnatal week 6. Over the following 6 weeks, we performed quantitative facial videography (Clayton et al., Curr Biology, 2024), single unit recordings from the auditory cortex of awake, head-fixed mice, and operant behavioral testing.
Results: Otof-/- mice had no measurable ABR and no behavioral evidence of sound awareness prior to gene therapy. Three weeks after OTOF gene transfer, quantitative videography revealed the emergence of sound-evoked facial movements in response to broadband noise. Over the

following week, facial movements could be entrained by sequences of pitch-shifted English phonemes with a comparable accuracy to Otof+/- controls. Single unit in the contralateral auditory cortex of treated mice were well-tuned to the full range of sound frequencies with comparable thresholds to wild type mice. Using an operant Go-NoGo behavioral paradigm, we found that Otof-/- mice were unable to detect any sounds prior to treatment but rapidly developed normal sensitivity to sounds in quiet and varying levels of background noise.
Conclusions: Within just a few weeks, the auditory system of young adult mice can transition from profound deafness to relatively normal sensitivity in silence and background noise. Our results highlight the plasticity and resilience of the central auditory pathway long after developmental critical periods for activity- and experience-dependent maturation have ended. These findings provide new insights into the awakening of higher-order regions of the central auditory pathway that may be related to the recovery of sound awareness and discriminative auditory processing in children with DFNB9 mutations receiving AAV-based otoferlin gene transfer therapy.

Preclinical Studies Using a Novel Gene Therapy Show Robust Rescue of Hearing for a Common Hereditary Deafness

Andre Landin Malt[1], Felicia Gilels[1], Ashley Hinton[1], Maryna Ivanchenko[2], Jason Farnsworth[1], Yaqiao Li[2], William Neidermyer[1], May Wang[1], Tian Yang[1], Jessica Chiang[1], Marc Johnson[1], Casey Maguire[3], David P. Corey[2], Will McLean[1], Shawn Harriman[1], Jodi Cook[1], K. Domenica Karavitaki*[1]

[1]*Skylark Bio, Inc.,* [2]*Harvard Medical School,* [3]*Massachusetts General Hospital, Harvard Medical School*

Background: The most common human hereditary deafness, DFNB1, is caused by GJB2 mutations. Currently, there is no cure for GJB2-related hearing loss.
GJB2 encodes the gap-junction protein connexin26, which mediates transport of potassium and metabolites between inner ear cells. The GJB2 coding sequence easily fits in an AAV viral vector, making gene addition an attractive therapeutic approach.

Here, we optimize the vector design to achieve robust expression of human GJB2 protein in the appropriate cells of mouse and nonhuman primate (NHP) cochleae, and greatly improving mouse hearing capabilities.

Methods: Human GJB2 coding sequence was packaged in the AAV-S capsid, which shows high transduction in native GJB2-expressing cells in neonatal mouse and adult NHP cochleae. Expression was driven by GJB2 human gene regulatory elements and the human coding sequence. Vectors were injected into P1 mouse cochleae and adult NHP via the round window membrane (RWM).
Results: To understand the vector transduction efficiency and expression specificity, wild-type (WT) mice were injected with an AAVS-GJB2 HA-tagged vector. After ~30 days, cochleae were processed for histology. Cochlear cells natively expressing Gjb2 produced the HA-tagged GJB2 protein, but the sensory hair cells did not, demonstrating targeted, cell-specific vector expression.

To test the efficacy of the AAVS-GJB2 vector, we used a Sox10-Cre, Gjb2flox conditional knock-out (cKO) mouse model, notoriously difficult to obtain hearing rescue using prior AAV gene therapy strategies. Animals were tested by auditory brainstem response (ABR) ~30 days after injection. Functional rescue was dose-dependent, with an increasing response rate and magnitude of response as the doses increased. Similarly, dose-dependence was observed in hair cell survival, a secondary histological effect of the Gjb2 mutation, with 100% survival at the highest dose tested.

We also assessed the efficacy of AAVS-GJB2 in the Gjb6 KO model, which has hearing loss caused by a 90% GJB2 reduction. We and others observed that this model has substantial hearing loss but lacks the severe histological phenotype of the Gjb2 cKO model. The Gjb6 KO showed greater incidence, magnitude, and potency of efficacy compared to the Gjb2 cKO model, demonstrating WT hearing thresholds at the highest dose, and nearly complete hair cell preservation.

Finally, to assess potential efficacy in humans, the AAVS-GJB2-HA vector was injected through the RWM of cochleae of cynomolgus monkeys (M. fascicularis). As in mouse, HA labeling demonstrated cell-specific expression.

Conclusions: The novel AAVS-GJB2 vector demonstrated cell-specific expression of human GJB2 in mice and NHPs. Auditory sensitivity testing demonstrated the best rescue of hearing thus far reported in a mouse model of Gjb2 deletion and a milder Gjb2 downregulation model, improving auditory sensitivity to wild-type levels. This vector is now in toxicology studies. These data support development of this proprietary vector as a promising therapeutic for treatment of DFNB1 in human subjects.

Development of AAV Gene Therapy Targeting GJB2 Related Hearing Loss by Capsid and Promoter Modification

Kazusaku Kamiya*[1], Daisuke Arai[1], Takao Ukaji[1], Makoto Matsuyama[1], Hidekane Yoshimura[2], Shin-ya Nishio[2], Sho Kanzaki[1], Yutaka Takumi[2], Shin-ichi Usami[2], Katsuhisa Ikeda[1]

[1]*Juntendo University Faculty of Medicine,* [2]*Shinshu University School of Medicine*

Background: Mutation of GJB2 (encoding Gap Junction Beta 2) is the most frequent cause of hereditary deafness worldwide and accounts for up to 50% of non-syndromic sensorineural hearing loss. GJB2 encodes connexin 26, a component of cochlear gap junctions that help maintain ion balance in the cochlea. We previously demonstrated that GJB2 deficiency in mice impairs auditory function (Kamiya, J. Clin. Invest., 2014) and that immediate postnatal delivery of wild-type GJB2 to the inner ear via adeno-associated virus (AAV) vector restored the hearing loss in mice (Iizuka, Hum. Mol. Genet., 2015). However, delivery of GJB2 to the mature cochlea did not restore hearing. The early postnatal period in mice corresponds to the embryonic period of inner-ear developmental in humans. Thus, to develop a feasible clinical approach for human gene therapy, mature mice should be used for delivery of AAV to the inner ear.

Methods: We generated the several AAV capsids that effectively infect cochlear supporting cells by shuffling capsid sequences between wild serotypes or directed evolution with AAV library. And the promoters were modified to express only in GJB2 expressing cells and form proper gap junctions. Then the vectors were tested with adult GJB2 deficient mice (Kamiya, J. Clin. Invest., 2014) and our supporting cell model derived from human patient iPS (induced pluripotent stem) cells with typical GJB2 mutation (Fukunaga, Stem Cell Reports, 2016, Hum. Mol. Genet. 2021)
Results: Here we report the development of AAV-Sia6e, a representative vector suitable for rectifying GJB2-related hearing loss, by shuffling capsid sequences between wild serotypes and selecting AAV vectors that effectively infect inner-ear supporting cells. To avoid transgene expression in untargeted cell types such as hair cells, we developed a specific promoter for GJB2-expressing cells (GJS promoter). AAV-Sia6e-mediated delivery of GJB2 (including the GJS promoter) significantly restored hearing loss in mature GJB2-deficient mice. Gap junction recovery was confirmed in both animal (GJB2 deficient mice) and cellular models (Patient iPS derived model cells).
Conclusions: These results suggest that this capsid/promoter modified AAV–mediated delivery of functional GJB2 could potentially restore hearing to patients with GJB2-related hearing loss. We are currently conducting preclinical studies of this modified AAV-GJB2 in animal models including non-human primate.

From Cells to Cures: hIPSC-Derived Inner Ear Organoids and RNA Therapy to Resolve Genetic Inner Ear Diseases

Esther Fousert*[1], Winnie van den Boogard[1], Wouter van der Valk[1], Amy Lucassen[1], John de Groot[2], Peter Paul van Benthem[2], Hannie Kremer[3], Erik de Vrieze[3], Erwin van Wijk[3], Heiko Locher[1]

[1]*Leiden University Medical Center, The Novo Nordisk Foundation Center for Stem Cell Medicine, reNEW,* [2]*Leiden University Medical Center, Leiden, the Netherlands,* [3]*Radboud University Medical Center*

Background: Genetic hearing loss affects millions of people globally, yet no effective biological treatments are currently available. As a result, patients must rely on technological aids such as hearing aids or cochlear implants. A key barrier to developing therapies is the lack of accurate in vitro models of the human inner ear that can simulate genetic inner ear diseases and support treatment validation. In this study, we introduce a novel approach using human inner ear organoids as genetic models to address this limitation. Beyond characterizing the model, we reverse the disease phenotype in vitro using antisense oligonucleotides (ASOs). ASOs are designed to specifically target and modify RNA transcripts, potentially slowing or stopping the progression of genetic disorders. With this approach we demonstrate the clinical significance of human inner ear organoids.
Methods: Our research focused on two genes associated with significant auditory impairments: USH2A, with early-onset hereditary deaf-blindness, and COCH, linked to late-onset genetic hearing loss. We differentiated human induced pluripotent stem cells (hiPSCs) into three-dimensional self-organizing inner ear organoids. Using patient-derived, isogenic mutant, and

healthy hiPSC lines, we generated inner ear organoids to compare the effects of these mutations. We analyzed the diseases phenotypes using immunohistochemistry and PCR, and applied ASOs via gymnotic delivery to vibratomed late-stage, disease-specific organoids. The impact of the ASOs was assessed through immunohistochemistry, RNAscope, and PCR analysis.
Results: We successfully generated disease-specific inner ear organoids of two genetic inner ear diseases, USH2A and COCH. Immunohistochemistry confirmed the presence of key cell types associated with each condition: hair cells with stereocilia for USH2A and peri-otic mesenchymal cells for COCH. Molecular comparisons between disease-specific and healthy organoids showed mutant transcripts in the patient-derived and isogenic mutant models. Immunohistochemistry and RNAscope confirmed the effective distribution of ASO therapy throughout the organoids and demonstrated that it reached the targeted cell types. With PCR analysis we demonstrated that ASO treatment resulted in reduced mutant transcript expression in the disease inner ear organoids.
Conclusions: This study highlights the potential of human inner ear organoids as a platform for modelling genetic inner ear diseases and evaluating potential therapeutic interventions. Our findings offer promising new avenues for expanding treatment options for individuals affected by genetic hearing loss, offering hope for improved outcomes and quality of life.

Novel Large Animal Model for Human Inner Ear Gene Therapy: Transgene Expression of Viral Vectors in Pigs

Erdem Yildiz[1], Till Buschhorn*[1], Caroline Sesztak[1], Anselm Joseph Gadenstaetter[1], Matthias Gerlitz[1], Clemens Honeder[1], Hinrich Staecker[2], Christoph Arnoldner[1], Lukas Landegger[1]

[1]*Medical University of Vienna,* [2]*Univeristy of Kansas Medical Center*

Background: The recently published data on the clinical application of viral vectors in human patients with congenital hearing loss show the potential of inner ear gene therapy. For the first time, children affected by autosomal recessive otoferlin mutations were treated by delivering adeno-associated viruses (AAVs) into the inner ear. An in-depth investigation of the respective efficacy of the applied viruses has so far only been carried out in a limited number of large animal models. In this study, AAVs were injected into the cochleae of pigs to investigate transduction patterns of the inner ear tissue.
Methods: In three pigs, AAV2 with a CMV promoter was injected via the round window membrane using an established surgical procedure under general anesthesia. Pre- and postoperative brainstem audiometry was performed. After a follow-up of one week, inner ears and other organs relevant for systemic biodistribution were extracted and analyzed by means of immunofluorescence and confocal microscopy.
Results: Successful transduction was identified in approximately 50% of inner and outer hair cells. In addition, a relatively uniform transduction pattern was observed in the immunofluorescent images in all tonotopic regions throughout the cochlea, including the apex. A unilateral injection showed no transduction in the contralateral ear. In addition, no threshold changes were observed in the injected ears when measuring auditory brainstem click responses.
Conclusions: Due to the large dimensions of the inner ear (most comparable volume to the human cochlea), the pig represents a suitable alternative large animal model for hearing research and in particular inner ear gene therapy. The above-mentioned findings confirm that AAV2 is a

reliable vector for targeting sensory hair cells and that future gene therapies can be investigated preclinically in pigs to optimize vector delivery, distribution and corresponding transduction patterns, all of which could result in translational benefits by developing more effective human therapies.

Tuesday, February 25, 2025

Young Investigator Symposium 2: Bridging the Senses: Lessons Learned at the Intersection of Audition and Vision

Chair: Malinda McPherson*, Purdue University*
Co-chair: Abigail Noyce*, Carnegie Mellon University*
8:00 a.m. - 10:00 a.m.

Ocean Ballroom 1 - 4

"Hearing With the Eyes": Visual Perception for Auditory Scientists

Abigail Noyce*, Carnegie Mellon University*

Individual Abstract: Vision and audition differ first in straightforward ways. Although both allow us to perceive objects at some distance, they utilize different sense organs that respond to different forms of energy in the surrounding environment. These simple differences compound into differences in the timescales of perception, the features that are most salient, the principles that yield perceptual organization, and the semantic labels we assign to objects. This introduction to our Bridging The Senses symposium will first review the mechanisms of the human visual system, from properties of light, through transduction and early processing in the retina, to the lateral geniculate nucleus of the thalamus, to cortical processing pathways. Then, we will discuss the most important features for visual perception, including spatial location, edges and spatial frequency, color, and onsets and motion. For each, attention will be given to possible corresponding features in auditory perception, such as pitch, timbre, and temporal modulations. At a more cognitive level, the senses continue to diverge. The capability for eye movements lets us foveate different portions of the visual world, affording a larger number of discriminable objects within a scene compared to audition. Conversely, memory limits for words are much higher than those for visual features. Finally, we will compare what is known about the cortical organization of the visual system to that of the auditory system. Although there are parallels, there are a number of striking differences that should be considered in multisensory investigations.

Spatiotemporal Neural Dynamics of Cross-Modal Integration in Audiovisual Perception

Yalda Mohsenzadeh*, The University of Western Ontario*

Individual Abstract: Our brain can integrate different sensory information, such as what we see and hear, to form a coherent perception despite their different physical properties. However, the specific brain regions and timings involved in processing and integrating these different levels of information are not well understood. To investigate this, we curated 60 naturalistic videos with matching visuals and sounds and recorded functional magnetic resonance imaging (fMRI) and electroencephalography (EEG) data from 22 participants as they watched these videos. We found the low-level acoustic information was represented not only in auditory areas but also in early visual regions, suggesting the early cross-modal interaction and its role in combining low-level acoustic features with visual features. However, the visual information was only represented in

visual cortices, indicating that the early cross-modal interaction is asymmetrical. The low-level visual and auditory features were processed with similar onset but different temporal dynamics. The high-level categorical and semantic information was identified in high-order and multi-modal areas and resolved later in time, demonstrating the late cross-modal integration and its distinct role in converging conceptual information. We further compared the neural representations with a two-branch audio-video deep neural network model and observed the mismatch in early cross-model interaction, suggesting the need of early fusion to build a biologically plausible model for audiovisual perception. By linking the multivariate response patterns of fMRI and EEG with an advanced analytical technique termed EEG-fMRI fusion, we characterized neural processes in the whole brain at each voxel and each millisecond. Together, our results revealed functional roles of distinct cross-modal interaction stages and spatiotemporal dynamics of neural representations during the multisensory perception of naturalistic audiovisual events.

Salient Sounds Boost Visual-Cortical Processing and Enhance Visual Perception
Viola Stoermer*, Dartmouth College*

Individual Abstract: Visual and auditory perception are often studied in isolation, and while this approach has been exceptionally successful, in the real world, sounds and sights co-occur and need to be integrated to form coherent, multimodal representations of the world around us. In this talk, I will review studies from my lab showing that a sound can have strong effects on visual perception by facilitating perceptual processing of co-localized visual stimuli. Using EEG, I will show that these sound-induced effects on visual perception are accompanied by enhanced neural responses over the visual cortex and that sounds alone – in the absence of visual stimuli – can trigger spatially selective activations in visual cortex, suggesting a close link between spatial processing across audition and vision. Finally, I will discuss to what extent these sound-induced changes in visual-cortical activity represent facilitatory vs. inhibitory effects in neural processing, and how they relate to theories of spatial attention across modalities.

How the Visual Domain Might Elucidate the Fidelity of Auditory Working Memory
Jamal Williams*, University of California, San Diego*

Individual Abstract: Memory is characterized by both remarkable precision and a disheartening fragility. What factors determine the quality of remembered information? Recent work in the visual domain has demonstrated that memory errors are straightforwardly predicted when the perceptual similarity of a stimulus space is mapped. Here, we explore whether the link between perceptual similarity and memory is a distinct feature of the visual system or whether these insights are a fundamental aspect of memory, irrespective of modality. To explore this question, we used two distinct stimulus spaces: a linear pure tone space and a circular Shepard tone space. To map the perceptual similarity of these spaces, participants rated the perceived similarity of two tones on a 1-7 scale. The pure tone space consisted of two octaves separated by 1/10 semitone intervals (240 tones) while the circular Shepard tone-space was made up of 360 tones. First, we mapped the psychophysical similarity function of tones in each of the unique tone spaces, validating the circularity of the Shepard tone-space, and demonstrating that reported similarity decreases with pitch distance for both spaces. Next, a new set of participants were randomly assigned to a memory experiment with either pure or Shepard tones. Participants

maintained one or three randomly sampled tones in working memory across a delay (2.5s), before performing a change detection task. Participants were presented with a single tone and reported whether this tone was the same/different as one in memory on a six-point confidence scale. Critically, "different" (foil) tones were systematically related to a tone in memory. This allowed us to probe how similarity between a memory tone and a test tone affected performance. For both tone spaces, we find that the perceptual similarity of that space directly predicted how likely participants are to falsely endorse an incorrect foil, based on how similar that foil was to an item in memory. This work suggests that auditory memory is tightly linked to perception: the perceived similarity of any given pitch space can predict memory errors regardless of memory load. This is particularly notable since the motivation for this work derives from recent findings in the visual literature and suggests that perceptual similarity uniquely describes memory errors regardless of modality. In sum, a model of memory first characterized in vision also accurately captures features of auditory memory.

Memory Performance in Hearing and Vision is Differentially Impacted by Task Structure and Stimulus Similarity

Malinda McPherson*, Purdue University*

Individual Abstract: While there is evidence that auditory memory, such as memory for songs, is robust, several experimental results have suggested that auditory memory is still far inferior to visual memory. It is unclear whether these results are the consequence of experimental paradigms which may favor vision. In this talk, I will discuss new experiments that tested various approaches to fairly comparing visual and auditory memory performance. A classic metric for measuring memory in vision is to show viewers a set of objects, then probe their memory with two alternatives, one previously seen target and one new object foil. The choice of the foil can make the task harder or easier. For example, if the target item is a picture of a pen and the foil is a different pen, choosing the correct pen is more difficult than if the foil is an apple or table. Recent work in vision has shown that maximizing dissimilarity between a target and foil in a forced choice task is necessary when comparing distinct stimulus sets. The extent to which this is likewise true in hearing was unclear. To examine this, we use developments in deep convolutional neural networks to select foils ranging from maximally similar to maximally dissimilar for both visual and auditory stimuli. Additionally, we modified stimulus presentation parameters to differentially alter performance across domains. We hypothesized that visual stimuli may be more inherently dissociable (dissimilar) than auditory stimuli, suggesting that with randomly selected foils, auditory memory performance would appear worse than visual memory. We also predicted that the presentation mode (simultaneous, sequential, etc.) would differentially impact auditory vs. visual memory. We found that hearing is more sensitive to similarity structure than vision: auditory performance worsens more rapidly than visual performance as foil similarity increases. Still, by changing experimental paradigms, we could observe better overall auditory memory performance than visual performance, vice versa, or comparable performance. Therefore, when comparing vision and audition, or even when examining memory for different stimuli within audition, the choice of stimuli and experimental parameters can drastically change the ultimate conclusions about memory capacity and ability. Overall, information is retrieved differently in vision and hearing: vision is inherently spatial, and hearing is intrinsically temporal, and it is critical to account for these and other differences when working across the senses.

Model-Brain Comparisons in the Visual and Auditory Domains
Jenelle Feather, *Flatiron Institute*

Individual Abstract: In recent years, deep artificial neural networks have emerged as leading models of sensory system responses. These models can perform complex tasks such as speech recognition or object classification, contributing to their use in both the auditory and visual domains. Various architectural constraints and training environments have been proposed in each modality to improve model-brain alignment. By investigating whether modifications that improve models for one modality can also be applied to models in the other, we can gain inspiration for creating better models, and also identify training constraints or architectural motifs that may be generally applicable to modeling problems. In this talk, I will present a set of experiments demonstrating that both visual and auditory models have idiosyncratic invariances, suggesting a common problem across many deep neural network models. I will also detail improvements that can be made to both auditory and visual models and highlight how insights from one modality can inspire improved models of the other.

Automatic Perceptual Segmentation Results in Biased Acuity in Audition and Vision
Linda Garami, *Central European University*

Individual Abstract: At each level of abstraction, the brain performs the same operation: transforming the incoming information by chunking and extracting patterns to obtain more adequate and efficient representations that can support its goals. Despite the prevalence of such processes across sensory modalities, investigations into the organizing principles of segmentation (chunking) typically focus on individual modalities separately. We hypothesize that at least some basic chunking principles and corresponding perceptual biases are akin across modalities with tractable neural correlates in the primary sensory cortical areas. To test this hypothesis, we focused on the auditory segmentation principle called the Iambic-Trochaic law (ITL). Established in language processing, ITL posits that longer syllables in a sequence signal word-ends while an increase in intensity signals the beginning of a word. Moreover, as a behavioral relevance, such chunking leads to decreased accuracy in detecting perceptual changes at perceived chunk boundaries compared to that within the segment itself. Importantly, ITL biases have been found unrelated to linguistic content and across multiple species, but never tested in other modalities, we implemented a stream segregation go/no-go paradigm for human participants in audition and vision to explore the generality of the phenomenon. Participants' task was to identify unexpected gaps in a structured stream in an identical manner in the visual and auditory modalities. The stream had 3-element intensity or duration patterns to probe segmentation biases. We found that variability in sensitivity to gap deviations showed similar, pattern-derived biases across the two modalities. This sensitivity bias could be explained neither by the repetition of individual elements nor by the absolute feature value (e.g. duration, intensity) of the individual elements alone. Instead, this bias depended on the internal repeating structure of the stream and it had an effect even when conscious recognition of the structure itself did not occur. We also analyzed neuronal activity in the auditory cortex (AC) of awake, head-fixed mice passively exposed to similar acoustic stimuli to see how AC neurons respond to changes within a continuous stream. We found that AC activity significantly increased in response to stimuli featuring unexpected gaps, again, as a function of their position in the pattern. By employing consistent paradigms across sensory modalities (auditory/visual) and experimental models (human/mouse), our results support the idea of domain-general non-linguistic grouping

principles and raise well-testable further questions that have the capacity to lead to a domain-independent model of sensory processing.

Podium 13: Cochlear-Specific Genomics and Gene Regulation

Moderators: Matthew Kelley and Jingyun Qiu

8:00 a.m. - 10:00 a.m.

Ocean Ballroom 5 - 8

The Genetic Landscape of Hearing Loss: Insights From a Multiethnic Cohort of Over 7,700 Cases

Hela Azaiez*[1], Amanda Odell[2], Estella Roster[2], Diana Kolbe[2], Donghong Wang[2], Maria Wong[2], Carla Nishimura[2], Kathy Frees[2], Amanda Taylor[2], Daniel Walls[2], Elisabeth A Black-Ziegelbein[2], Adela Mansilla[2], Joseph Chin[2], Kevin T Booth[2], Miles Klimara[2], Kiersten Knobbe[2], Luke Hovey[2], Erika Renkes[2], Paige Harlan[2], Cathy Feng[2], Jori E. Hendon[2], Amy E. Weaver[2], Richard JH Smith[2]

[1]*University of Iowa,* [2]*Molecular Otolaryngology and Renal Research Laboratories, University of Iowa Hospitals and Clinics*

Background: Hereditary hearing loss (HL) is characterized by significant genetic and phenotypic diversity. Advances in next-generation sequencing have enhanced our understanding of the genetic basis of HL and revolutionized its diagnosis and management. This study presents findings from the genetic screening of over 7,700 individuals, offering new insights into the genetic landscape of HL.

Methods: We used a multigene panel to analyze single nucleotide variants and copy number variants (CNVs) across all known deafness-associated genes. Based on genetic findings, gene-variant maps and gene-variant-phenotype maps were developed by a multidisciplinary team to correlate genotypic data with clinical outcomes.

Results: The overall diagnostic yield was ~44%, however the precise percentage was influenced by factors such as inheritance pattern, phenotypic presentation, onset and severity of HL, and ethnicity. Six genes accounted for half of all diagnoses, with 10 genes being implicated in over 75% of autosomal recessive HL and 8 genes being implicated in 50% of autosomal dominant HL.

CNVs were detected in more than 70 genes and accounted for ~18% of diagnosed cases, making them significant contributors to HL. De novo variants were identified in 1% of cases with this proportion ranging from 0.1% to 6% based on inheritance patterns and phenotypic presentations. Dual genetic diagnoses occurred in 1 out of 250 patients, underscoring the importance of comprehensive testing and the need to consider multiple genetic factors in affected individuals. Gene-variant and gene-variant-phenotype maps uncovered phenotypic variability associated with certain genes, even among individuals with identical pathogenic variants.

Conclusions: Our study underscores the importance of comprehensive genetic testing in the diagnosis of HL. It also highlights our evolving understanding of the genetic landscape of HL and the implications this understanding has for future research and clinical practice. The complexities and nuances of the genetic architecture of HL must be appreciated to accurately diagnose and effectively manage HL, particularly as gene therapy options evolve. Gene-specific therapies, such as gene replacement, editing, or silencing, require precise knowledge of the underlying mutations, their pathomechanisms and their phenotypic consequences.

Big Data to Precision Medicine for Hearing Impairment

Zippora Brownstein*[1], Lara Kamal[1], Inbar Blech[1], Yazeed Zoabi[1], Shadi J. Khoury[1], Tal Patalon[2], Asaf Peretz[2], Juan Fernandez-Recio[3], Xavier de La Cruz[4], Fabian Glaser[5], Noam Shomron[1], Karen B. Avraham[1], Lara Kamal[6]

[1]*Tel Aviv University,* [2]*Maccabi Healthcare Services, Tel Aviv, Israel,* [3]*Instituto de Ciencias de la Vid y del Vino (ICVV), Logroño, Spain,* [4]*Vall d'Hebron Institute of Research (VHIR), Barcelona, Spain,* [5]*THHI, Technion Human Health Initiative, Haifa, Israel,* [6] *, Faculty of Medical and Health Sciences, Tel Aviv University*

Background: The rapid advancements in next-generation sequencing (NGS) have ushered in a big-data revolution, enabling the swift detection of pathogenic variants. Hearing loss presents significant challenges, with at least 224 genes implicated, many containing hundreds of pathogenic variants. Despite this knowledge, it is estimated that up to half of inherited cases of deafness worldwide remain unsolved. NGS enables simultaneous gene detection across many samples, but bioinformatics analysis remains challenging, often requiring individual patient or family analysis and multi-family segregation for variant validation. In our study, we analyzed electronic medical records (EMRs) of 1,038 hearing-impaired patients to identify the underlying causes of deafness through whole-exome sequencing (WES). Audiograms were available, but data on onset, family history, or inheritance were missing. The large cohort and lack of key information complicated bioinformatics, leading to the creation of a script for big-data analysis of WES results across hundreds of patients. We adapted the script for incomplete medical histories. We developed PredHL, a protein modeling tool to identify novel variants and functionally characterize new deafness genes for precision medicine in hearing loss.
Methods: Using the Tipa Biobank, which included 60,635 samples from the Israeli Jewish population, we performed WES on 1,038 deaf individuals. We developed a specialized pipeline for variant detection in a hearing-impaired population with limited background information. After identifying variants through a comprehensive bioinformatics mega-analysis, we evaluate their pathogenicity. We created an AI-based methodology, PredHL, for novel variants to predict their functional impact at the protein level. Additionally, we are functionally investigating these novel variants using CRISPR/Cas9 gene editing and gene therapy approaches.
Results: Our bioinformatics meta-analysis pipeline for WES of 1,038 samples solved 12% of cases, with an additional 8% showing inconclusive variants. Many of the detected variants were either known or novel variants in established deafness genes. Furthermore, in 8% of cases, we identified homozygous variants in novel genes.
Conclusions: Our study shows that NGS effectively determines disease etiology in large populations, even with incomplete data. Using protein modeling and AI, we identified key

molecular causes in the diverse Israeli Jewish population. This approach advances precision medicine in hearing loss and can be applied globally.

Research Support: The Israel Precision Medicine Partnership Program (IPMP) 3499/19 and the NIH/NIDCD R01DC011835.

Cell Death; Type Dependent Interactions Between Immune Cells and Sensory Hair Cell Regeneration Programs

Daniela Muench[1], Shiyuan Chen[1], Elizabeth Ellis[1], Nicolas Denans[2], Mark Lush[1], Tatjana Piotrowski*[1]

[1]*Stowers Institute for Medical Research,* [2]*Janelia Research Campus*

Background: Tissue regeneration following injury requires the concerted response of multiple cell types, including immune cells and stem cells. The cellular and molecular components of regeneration programs following injury can be affected not only by the severity of the injury and tissue identity, but also the type of cell death. Determining the individual contribution of these parameters has remained challenging, largely due to the variable nature of mechanical injury paradigms.
Methods: Here, we established a comparative approach of regeneration programs to induce either necrosis or apoptosis in zebrafish lateral line hair cells (HCs), respectively. Keeping the identity and quantity of the ablated cells consistent, this approach allows us to specifically interrogate the influence of the cell death modality on regeneration.
Results: Using high resolution live imaging we visualized the rapid recruitment of tissue-resident macrophages to the site of cell death, uncovering intricate differences in their phagocytic behavior depending on the cell death modality. Single-cell RNA sequencing revealed that these cellular differences were accompanied by distinct transcriptional signatures in both phagocytosing macrophages and lateral line support cells. While HC necrosis triggered a robust injury response in support cells, it was greatly diminished following apoptosis. Lastly, blocking recruitment of immune cells using a dominant-negative approach not only increased injury response gene expression but also injury-induced proliferation of support cells in response to apoptosis.
Conclusions: Our findings have important implications for our understanding of the context-dependent earliest transcriptional responses to hair cell death and how these could be harnessed to trigger regeneration in mammals.

Comparative Maturation and Sensory Hair Cell Regeneration Potential in the Inner Ear

Marcela Lipovsek*[1], Rachel Williams[1], Jimena Perez Lloret[1]

[1]*University College London*

Background: The inner ears of vertebrates house the peripheral structures of the vestibular and auditory systems. Although responsible for different sensory modalities, head movement and sound detection, the vestibular and auditory end organs share tightly knit developmental and evolutionary paths. Inner ear sensory epithelia are mainly composed of mechanosensory hair cells, that transduce mechanical (head movement or sound) input into electrical signals, surrounded by supporting cells, that provide mechanical, trophic and metabolic support. Auditory hair cell loss is irreversible in adult mammals, while in the vestibular system there is minimal hair cell production throughout life and in response to damage. During early postnatal development, significant hair cell regeneration occurs in response to damage in the vestibular utricle. This is in stark contrast with the widespread regeneration capabilities of avian auditory and vestibular sensory epithelium.

It is generally accepted that the loss of regeneration potential in mammals relates to the degree of differentiation reached by supporting and hair cells, indicating a divergence of the maturation trajectories of mammalian and avian sensory epithelia.

Methods: To test this hypothesis, we have obtained single nuclei transcriptomes from mouse and chick vestibular utricles, from embryonic to adult stages. We prepared the sequencing libraries using combinatorial barcoding from densely sampled timepoints.
Results: After reads mapping and quantification, cell calling and quality controlled, we obtained 190,883 cells. We used TopOmetry to perform dimensionality reduction and visualisation followed by leiden clustering. This workflow, that makes no assumptions on the linearity and uniformity of the data, allowed us to thoroughly identify cell types, subtypes and states, and obtain accurate maturational trajectories.
Conclusions: The comparison between the maturational profiles of identified cell types and states from the chick and mouse utricles is revealing the gene expression patterns and signalling pathways relevant for the loss of hair cell regeneration potential in mammals.

Six1 is Essential for the Maturation and Homeostasis of the Auditory Sensory Organ in Adult Mice

Ting Zhang[1], Xiaohui Ma[1], Jinshu Xu[1], Jun Li[1], Pin-Xian Xu*[1]

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Background: The auditory sensory epithelium is a highly specialized, two-cell-layered structure, composed of hair cells (HCs) and supporting cells (SCs). These cells are precisely organized in a mosaic pattern, where SCs are interspersed between HCs and span the full depth of the sensory epithelium. While HCs are responsible for mechanosensory transduction essential for hearing, SCs play a vital role in maintaining the function and structural integrity of the sensory epithelium. During inner ear development, Six1 is indispensable for the formation of sensory organs and the commitment of progenitor cells toward the HC lineage. However, the potential involvement of Six1 in the maturation and maintenance of the sensory epithelium postnatally remains unclear.
Methods: To explore this, we employed distinct Cre lines to conditionally delete Six1 specifically in SCs or HCs in postnatal cochlea. To identify Six1 targets, we carried out ChIP-seq analysis using chromatins prepared from mature cochlea.

Results: Our findings reveal that the loss of Six1 in either cell type results in significant cellular depletion within the organ of Corti, leading to profound hearing loss. A genome-wide analysis of Six1 binding sites further identified a broad spectrum of regulatory targets, including genes involved in the assembly and function of the hair bundle's actin cytoskeleton, as well as SC-specific genes crucial for the structural stability of the mature sensory epithelium. These results underscore the pivotal role of Six1 in both HC and SC physiology within the auditory sensory epithelium.
Conclusions: These results underscore the pivotal role of Six1 in both HC and SC physiology within the auditory sensory epithelium.

Characterizing Gene Regulatory Networks in Mouse Developing Hair Cells Using Bioinformatic Tools and Omics Integration Strategies

Celia Bloom*[1], Tuba Ege[1], Mi Zhou[1], Guanfang Xie[1], Litao Tao[1]

[1]*Creighton University*

Background: Alone, transcriptomic, epigenomic, and other types of omics data can offer important insights into cellular states, identities and responses. However, single data modalities such as RNA expression lack important information linking genes to each other. Advances in both multimodal data acquisition and integrative strategies to combine different data modalities are making it possible to generate stronger predictions of the complex gene regulatory networks (GRNs) that underlie cell identities and states. Currently, very little is known about the GRNs of cochlear hair cells, and the scope of omics studies in the inner ear remains limited. To understand the drivers of hair cell identity, we need a more enriched mapping of their underlying GRNs, integrating many data modalities including transcriptomes, chromatin accessibility profiles, histone modification landscapes, transcription factor binding sites, and chromatin interaction architecture. Assembling these data together, we can infer networks that underlie hair cell differentiation and identity.
Methods: We collected samples of both unsorted cochlear cells (whole sensory epithelium) and sorted hair cells from E15 mouse embryos and performed single-cell ATAC-seq and single-cell RNA-seq. We also have supporting data from varying ages and other data modalities. In total, we have the following datasets to build or support our GRNs: scATAC and scRNA for E15 unsorted cochlea and sorted hair cells; CUT and RUN for SOX2 transcription factor for P0/P1 unsorted cochlea; CUT and RUN for ATOH1 and POU4F3 transcription factors and epigenetic histone modifications for E17/P1 sorted hair cells; scMultiomics (ATAC and RNA) for P1/P8 unsorted cochlea; and Micro-C for P1 unsorted cochlea. We have used these data with multiple computational tools, including CellOracle, SCENIC+, and others, to construct GRNs.
Results: We have generated mouse hair cell GRNs from E15 ATAC and RNA data, with other data modalities integrated as support. We show well-known transcription factors and other elements centered in our GRNs, including Atoh1 and Pou4f3. We observe key enhancer-promoter relationships in our Micro-C data. As expected, our transcription factor footprinting analysis shows strong occupancy of known hair cell gene promoters. We are also able to distinguish inner and outer hair cells. Importantly, we show homogeneity among the results of our GRN building tools, which adds robustness and reliability to our observations.

Conclusions: Here we present comprehensive gene regulatory network analysis of developing hair cells in mice. We included many different datasets, data types, time points, and computational methods to add strength to our findings. True biological GRNs are vast and complex, and using many data types and bioinformatic tools enables us to resolve these GRNs in greater detail. We are thus able to decipher important links within the networks. This will guide future studies and encourage progression toward translational research to target key GRN elements to treat and prevent hearing loss.

Ligand Dependent Function of the Retinoic Acid Receptor Alpha Complex During Cochlear Organogenesis

Saikat Chakraborty[1], Shuze Wang[1], Jack Ruhala[1], Jie Liu[1], Joerg Waldhaus*[1]

[1]*University of Michigan*

Background: Retinoic acid (RA), a vitamin A derivative, signals through binding to its nuclear receptor (RAR/RXR hetero dimer). In the developing nervous system, the RA receptor complex controls gene expression via induction and repression. In absence of RA, RAR-RXR recruits co-repressors such as NCOR1, and functions as transcriptional repressor. Upon binding of the ligand, co-repressors are replaced with co-activators such as NCOA1, rendering the receptor complex into a transcriptional activator. Previous studies identified RAR-alpha (RARA) to mediate RA signaling in the cochlea; however, molecular mechanisms and target genes remain to be determined.
Methods: To study the function of the RARA complex in cochlear development, we used antibodies raised against RARA, RXR, NCOA1, and NCOR1 in immunohistochemistry and co-immunoprecipitation at embryonic day (E)14.5 and postnatal day (P)2. Next, we predicted Lfng as RARA target gene using scRNA/ATAC-seq data and identified RA regulatory elements (RAREs) in putative Lfng enhancer elements. ChIP-qPCR was used to test for binding of the RARA receptor complex to the RAREs. Finally, RA levels were modulated in organ culture and ChIP-qPCR and fluorescent in situ hybridization (FISH) were used to monitor for changes in the complex composition and target gene expression, accordingly.
Results: The findings of the study show that RARA, RXRs, NCOA1, and NCOR1 form a receptor complex in the cochlear floor at E14.5 and in P2 hair cells. All components of the RARA receptor complex bind to RAREs present at the Lfng locus as confirmed via ChIP-qPCR experiments. While NCOR1 binds to the Lfng-locus at E14.5 and P2, binding of NCOA1 was only determined for the embryonic stage. Next, modulations of the RA levels in E14.5 cochlear explants indicated a ligand dependent function for RARA. Briefly, ectopic RA significantly increased the amount of DNA precipitated from the Lfng locus using the NCOA1 antibody. On the other hand, culture with AGN193109, a pan-RAR-blocker, increased the amount of DNA precipitated with the NCOR1 antibody. Finally, FISH experiments indicated that modulations of RA levels not only changed the amount of DNA precipitated by the activator and repressor components of the RARA complex, but also concurrent changes in Lfng-mRNA expression were observed.
Conclusions: In this work, we identified opposing functions of RA signaling during cochlear development that are mediated by different levels of the ligand. In embryonic development, when RA is present, RARA and NCOA1 function together to induce supporting cell specific

genes in cochlear floor cells. This likely represents a critical step in inducing prosensory progenitors that gain the potential to differentiate into hair cells and supporting cells. Later during postnatal development, when RA is absent, NCOA1 is replaced by NCOR1, and the complex functions to repress supporting cell specific genes in hair cells to aid in differentiation of the immature cell type.

Cochlear Mesenchyme Consists of Four Cellular Subtypes Regulated by Distinct POU3F4 Related Transcriptional Pathways

Wei Song*[1], Kevin Rose[2], Beatrice Milon[2], Yang Song[3], Thomas Coate[4], Ran Elkon[5], Ronna Hertzano[2]

[1]*National Institute on Deafness and other Communication Disorders, National Institutes of Health,* [2]*Neurotology Branch, National Institute on Deafness and other Communication Disorders, National Institutes of Health,* [3]*University of Maryland, Baltimore,* [4]*Georgetown University,* [5]*Tel Aviv University*

Background: Otic mesenchyme cells (OMCs) represent the predominant cellular population within the cochlea and play crucial roles in formation of cochlear structures. OMCs can be classified into four heterogeneous subtypes (Types I-IV) based on spatial and functional characteristics, including the basilar membrane, spiral limbus, modiolar osteoblasts, and lateral wall. The absence of the OMC-specific transcription factor POU3F4 leads to various cochlear abnormalities associated with specific OMC subtypes, suggesting distinct regulatory programs driven by POU3F4 within each subtype. However, the identification of key regulators specific to each cell type and the mechanisms by which POU3F4 deficiency disrupts their functionalities remain insufficiently elucidated.
Methods: To explore the diverse regulatory networks orchestrated by POU3F4 across OMC subtypes, we employed single-cell RNA sequencing (scRNA-seq) and single-cell ATAC sequencing (scATAC-seq) on cochlear tissues from postnatal day 7 wild-type and Pou3f4 knockout (KO) mice. Comprehensive transcriptomic and epigenomic analyses were conducted to identify altered biological processes, diminished signaling pathways, and disrupted binding of key transcription factors across OMC subtypes in both wild-type and Pou3f4-KO mice.
Results: Integrative analysis of gene expression and epigenomic profiles revealed OMC subtype-specific transcriptional programs, allowing for systematical identification of marker genes that delineate molecular identities and putative regulatory elements that govern their expression. Motif analysis of differentially active ATAC-seq peaks among OMC subtypes highlighted specific key regulators, including JUN in Type I, FOX in Type II, RUNX and DLX in Type III, and NFIC in Type IV. Notably, binding sites of these transcription factors exhibited significant disruption in peaks differentiating wild-type and Pou3f4-KO samples, confirming their cooperative role with POU3F4 in regulating OMC subtype-specific networks. For instance, in Type I OMCs, the depletion of transcription factor JUN and downregulation of the gene Emilin2 in Pou3f4-KO might correlate with impaired collagen fiber alignment and altered mechanical properties in the cochlear basilar membrane. In Type III OMCs, the diminished binding of transcription factors RUNX2 and DLX5 in Pou3f4-KO is consistent with the decreased signaling in pathways associated with bone sialoprotein, as well as significant alterations in biological processes related to ossification and bone mineralization.

Conclusions: This analysis provides a comprehensive dissection of POU3F4-mediated transcriptional networks that regulate OMC subtypes, which sheds light on its downstream target genes and signaling pathways essential for cochlear development and functionality. The distinct regulatory pathways of POU3F4 in the four mesenchymal subpopulations highlights its cooperative role in governing inner ear morphogenesis. Finally, these results are foundational as we consider studies of gene therapy for patients with mutations in Pou3f4.

Podium 14: New Advances in Tinnitus: Humans and Animal Models

Moderators: Daniel Polley and Joel Berger

8:00 a.m. - 10:00 a.m.

Ocean Ballroom 9 - 12

Effects of Lifetime Occupational Noise Exposure on Tinnitus in Older Adults With Hearing Loss

Sabina Storbjerg Houmøller*[1], Li-Tang Tsai[2], Sreeram K Narayanan[2], Carl Pedersen[2], Dan Dupont Hougaard[3], Michael Gaihede[3], Christian Godballe[2], Jesper Hvass Schmidt[2]

[1]*University of Southern Denmark,* [2]*Research Unit for ORL – Head and Neck Surgery and Audiology, Odense University Hospital and University of Southern Denmark, Odense, Denmark,* [3]*Aalborg University Hospital, Aalborg*

Background: Tinnitus is a prevalent condition affecting millions worldwide, posing significant societal and healthcare challenges. Advancing age and noise exposure are major risk factors for tinnitus. Previous studies have focused on adults over 18 years, with limited research in older populations with accumulated noise exposure. This study investigates the effects of lifetime occupational noise exposure on tinnitus prevalence and severity in older adults with hearing loss, along with long-term changes in tinnitus percept following rehabilitation with hearing aids (HA).
Methods: The study was based on a cross-sectional and longitudinal analysis of data from the Danish Better-Hearing Rehabilitation (BEAR) project cohort. Adults aged 60 or older (n=1514) with bilateral sensorineural hearing loss referred for hearing alleviation were included. Tinnitus was a secondary complaint and managed with HA amplification only. Participants were divided into two groups based on noise exposure status. Tinnitus severity and occupational noise exposure were assessed with self-reported outcomes. The Tinnitus Handicap Inventory (THI) was answered at baseline, two months following HA fitting, and at long-term follow-up (698±298 days). Analyses were stratified by sex due to significant differences in noise exposure levels. Stepwise linear regression with backward elimination was used to analyze differences in tinnitus severity. Mixed-effects models were applied to explore long-term differences in tinnitus percept following rehabilitation with HAs. A control group of 105 older adults (mean age: 66.5 ± 9.3 years) from the User-operated Audiometry project (UAud) was included for baseline THI score comparison.
Results: Tinnitus was significantly more prevalent in occupationally noise-exposed men and women compared to the non-exposed group. Noise-exposed men reported on average 4.1 scale points higher THI score at baseline than men without an occupational noise exposure history

(95%CI: 0.1; 8.2, p LESS THAN 0.05). Baseline THI scores were comparable to the control group (t[105]=-0.01, p GREATER THAN 0.05). No effect of noise exposure status was found in women, likely due to occupational differences in noise exposure levels. Both men and women reported a significant reduction in their tinnitus percept two months following HA fitting (men: -2.8 [95%CI: -4.1; -1.6], p LESS THAN 0.001; women: -2.2 [95%CI: -4.3; -0.2], p LESS THAN 0.001) with sustained improvement at long-term follow-up (men: -7.2 [95%CI: -8.5; -5.9], p LESS THAN 0.001; women: -8.3 [95%CI: -10.4; -6.2], p LESS THAN 0.001). Noise-exposed men consistently reported higher THI scores than non-exposed men (3.3 [95%CI: 0.2; 6.4], p LESS THAN 0.05). No significant difference was found between the noise exposure groups in women

Conclusions: Lifetime occupational noise exposure was linked to a higher prevalence of tinnitus in older adults with hearing loss, and more severe tinnitus in older men. Tinnitus improved following hearing rehabilitation in both men and women, with sustained effects at long-term follow-up. Our study underlines the importance of acknowledging previous occupational noise exposure in older adults as a risk factor for tinnitus and rehabilitation outcomes.

Genetic Architecture of Tinnitus: A Genome-Wide Association Study Among Women

Nan Lin[1], Raji Balasubramanian[2], Heather A. Eliassen[3], Konstantina M. Stankovic[4], Gary Curhan[1], Sharon Curhan[1], Oana Zeleznik*[5]

[1]*Brigham and Women's Hospital and Harvard Medical School,* [2]*University of Massachusetts, Amherst,* [3]*Brigham and Women's Hospital and Harvard T.H. Chan School of Public Health,* [4]*Stanford University School of Medicine,* [5]*Harvard Medical School*

Background: Tinnitus affects more than 740 million adults globally with over 120 million reporting disabling symptoms. Persistent tinnitus is associated with greater risk of anxiety, depression, suicidal ideation, sleep disturbances, and reduced quality of life. Previous genome-wide association studies (GWAS) have identified 34 unique tinnitus loci at a genome-wide significance level. Importantly, only some of the identified loci were validated in a male-predominant veteran population. Sex differences in tinnitus severity and associated symptoms have been reported.

Methods: We included 27,560 female participants in the Nurses' Health Study (NHS) and Nurses' Health Study II (NHSII) with genotyping data. The quality control, imputation (with Haplotype Reference Consortium) and calculation of principal components of genotyping data were conducted previously. Single nucleotide polymorphisms (SNPs) with minor allele frequency GREATER THAN 0.01 and imputation r^2 GREATER THAN 0.3 were included in the analysis. In our primary analysis, persistent tinnitus was defined as tinnitus experienced several days per week or more. GWAS was conducted adjusting for the top 10 principal components using RVtest software, and a two-stage meta-analysis was performed using METAL. SNPs with p-value LESS THAN 1e-5 were further included in the downstream functional mapping and annotation with FUMA pipeline, and gene-based and tissue-enrichment analysis with MAGMA pipeline. In additional analyses, we conducted GWASs that examined persistent tinnitus defined as tinnitus experienced daily and, similar to the approach used in a previous GWAS, tinnitus categorized as a 5-level ordinal outcome.

Results: We identified 17 novel SNPs associated with persistent tinnitus at a genome-wide significance level (p LESS THAN 5e-8). Of the 22 genome-wide tinnitus-associated SNPs identified in previous studies, 20 showed the same the same direction of association in our study. In particular, rs2749882 (mapped to gene NID2) was significantly associated with persistent tinnitus [beta=7.5e-2,padj=8.1e-3]. We mapped SNPs related to persistent tinnitus with p-value LESS THAN 1e-5 to 129 protein-coding genes. Gene-set analysis results suggest persistent tinnitus-associated SNPs were enriched in GWAS catalog reported gene sets related to fish/iron consumption (e.g. fish- and plant-related diet, oily fish consumption, iron status biomarkers, and liver iron content), and brain functions and disorders (e.g. autism spectrum disorder or schizophrenia, brain morphology, and general cognitive ability). Additionally, we identified 3 novel SNPs associated with tinnitus experienced daily at a genome-wide significance level, which shared similar functional annotations as those SNPs associated with persistent tinnitus. However, none of the investigated SNPs were significantly associated with ordinal tinnitus.
Conclusions: Our study indicates there may be genetic underpinnings that contribute to tinnitus susceptibility and these may be potentially related to fish, iron and polyunsaturated fatty acid metabolism and brain functions consistent with previous epidemiologic and metabolomic findings. Further studies that investigate the relation of dietary intake, metabolism and the development of persistent tinnitus could reveal potential targets for treatment and possibly prevention of this challenging disorder.

Hearing Damage Due to Use of Radio Ear-Pieces in the United Kingdom Police

Hannah Guest[1], Christopher Plack*[1]

[1]*Manchester Centre for Audiology and Deafness, The University of Manchester*

Background: Research into the long-term effects of noise exposure on hearing is often confounded by lack of control for other lifestyle and general health differences between individuals. For the past two decades, UK police officers have used TETRA radio ear-pieces to communicate. The devices are capable of high sound levels (typically ~101 dBA at maximum volume) and crucially are used in one ear only, allowing between-ear comparisons which control for confounding endogenous and exogenous factors. No previous research has determined the volume-control settings that officers select. No previous research has tested for resulting tinnitus or hearing deficits.
Methods: Participants were police staff and ex-staff enrolled in the Airwave Health Monitoring Study. A web survey gathered detailed data on ear-piece usage, temporary threshold shifts, diagnosed hearing loss, and persistent tinnitus. Digits-in-Noise (DIN) thresholds (left ear, right ear, and antiphasic) were gathered using an online instrument via the participant's own headphones or earphones. A comprehensive study protocol, including full specification of analysis models, was pre-registered (https://osf.io/2bh6z).
Results: Over 4000 participants provided hearing data. Results revealed that: (i) Volume settings for most TETRA users resulted in estimated weekly-averaged exposure levels (Control of Noise at Work Regulations, 2005) GREATER THAN 85 dBA; (ii) Almost half of users had experienced signs of temporary hearing changes (dulled hearing / tinnitus) after a shift, and 7% experienced them after at least half their shifts; (iii) Temporary hearing changes were related to

volume settings (p LESS THAN 0.00001) and prevalence of permanent spontaneous tinnitus (p LESS THAN 0.00001); (iv) Risk of permanent tinnitus was 1.7 times higher in TETRA users than non-users (p LESS THAN 0.00001); (v) For users with asymmetric tinnitus, the predominant location of the tinnitus corresponded to the ear of exposure (p LESS THAN 0.00001); (vi) For users with asymmetric hearing loss, the poorer-hearing ear corresponded to the ear of exposure (p LESS THAN 0.00001); (vii) There were no significant differences in DIN thresholds between exposed and non-exposed ears, or between users and non-users.
Conclusions: Permanent tinnitus is a significant risk for TETRA users. However, despite high statistical power, no effects on speech-in-noise perception were observed. Findings have significance beyond police health, due to use of ear-pieces in myriad professions and also to widespread recreational noise exposure, especially from similarly powerful consumer headphones and earphones.

Suppressing Distracting Sounds: Neurophysiological and Behavioral Assays to Distinguish Between Benign and Bothersome Tinnitus

David Sorensen*[1], Jenna Sugai[2], Kenneth Hancock[2], Daniel Polley[2]

[1]*Harvard Medical School,* [2]*Eaton-Peabody Laboratories, Massachusetts Eye and Ear*

Background: Individuals with chronic tinnitus either hear an indefatigable and irrepressible phantom sound every hour of their waking day or a phantom sound that benignly fades into the background when unattended. The difference between benign and burdensome tinnitus may be explained by a combination of perturbed sensory areas (e.g. excess cortical gain), limbic arousal, or executive ability to suppress the sound. Previous results from our lab show that the degree of central gain does not correlate with tinnitus burden, but that pupil dilations and involuntary facial movements provide indices of affective processing that can predict tinnitus severity. To test the hypothesis that individuals with burdensome tinnitus exhibit broader deficits in inhibitory control over external auditory sounds, we developed a paradigm to probe the neural and behavioral effects of auditory distraction.
Methods: Subjects were presented with a target stimulus organized along four nested timescales, including temporal fine structure (~500 Hz), envelope (~25-80 Hz), envelope changes (~5 Hz), and embedded context (~0.5 Hz). EEG was recorded to capture following responses as participants reported perceptual judgments about the embedded context. The target was paired with two sets of competitor stimuli—melodies and matched noise—which share low-level features but differ in their level of distraction.
Results: Results in participants with normal hearing showed that synchronization to rapid features was insensitive to the difference in distraction, whereas synchronization to the slower envelope changes was reduced when the target was accompanied by the more distracting melodies. Additionally, the envelope change following response (ECFR) in trials where participants answered incorrectly was reduced relative to trials where participants answered correctly. Together, these results suggest the ECFR is sensitive to distraction.
Participants with tinnitus behaviorally perform in line with participants with normal hearing. Preliminary evidence suggests that the ECFR in participants with low Tinnitus Handicap Inventory scores is in line with participants with normal hearing: the ECFR is reduced with more distracting melodies compared to less distracting matched noise. The ECFR in participants with

higher Tinnitus Handicap Inventory scores, however, does not differ between the two competitor conditions.

Conclusions: Our results build on work that shows that individuals with tinnitus perform as well as individuals with normal hearing on listening tasks in noisy environments and expand the work into the neural representation of sounds in distracting environments. We also demonstrate that our novel paradigm for measuring auditory distraction can be applied in clinical populations.

Hyperexcitability in the Central Auditory System Caused by Chronic Noise Exposure

Fei Xu[1], Guangdi Chen[1], Wei Sun*[1]

[1]*University at Buffalo*

Background: Noise exposure is one of the most common causes of hearing loss and hyperacusis. Studies have shown that noise exposure can induce a cortical gain to compensate for reduced input of the cochlea, which may contribute to the increased sound sensitivity. However, many people with hyperacusis have no measurable cochlear lesion after being exposed to loud sound.
Methods: In this experiment we studied the neurological alterations in the cortical and subcortical areas following a prolonged moderate level of noise exposure (84 dB SPL, 8 h/d for 4 weeks) in the CBA mice. The cochlear function was monitored by auditory brainstem responses (ABR). The behavioral auditory sensitivity and temporal processing were evaluated using the acoustic startle response (ASR) and gap-induced prepulse inhibition (gap-PPI). The central auditory functions were determined by electrophysiological recordings of the IC and the AC, and the residual current source density (CSD) analysis was used to dissociate intracolumnar and horizontally relayed corticocortical input of the auditory cortex (AC).
Results: Our results showed that although there was no significant difference in the ABR thresholds, the noise group showed enhanced ASR and gap-PPI compared to the control group. Increased neural activity in both the IC and the AC were recorded in the noise exposed mice compared to the control group, suggesting a central gain in both the subcortical and cortical regions. The CSD analysis revealed an increased columnar excitation and reduced corticocortical projection in the noise group, which results are different from the central gain model of noise induced hearing loss.
Conclusions: Our results suggest that chronic “nondestructive" noise can increase the gain of the central auditory system by altering the balance of auditory thalamocortical and intracortical inputs, which may contribute to the increased sound sensitivity in people with normal hearing.

Tinnitus is Associated With Greater GABA(A) Receptor Availability in the Human Primary Auditory Cortex

Pim Van Dijk*[1], Marc Thioux[1], Emile de Kleine[1], Sonja Pyott[1], Antoon Willemsen[1], Erik de Vries[1]

[1]*University Medical Center Groningen*

Background: Subjective tinnitus is characterized by the perception of a sound in the absence of external stimulation. The prevalence is estimated between 6 and 30% in the normal adult population (Schubert et al., 2021).
Animal studies suggest that GABA plays an important role in the pathophysiology of tinnitus. In fact, pharmacological reduction of GABAergic neurotransmission in the auditory cortex is sufficient to induce tinnitus in rodents (Miyakawa et al. 2019, Hayes et al. 2021). Furthermore, pharmacologically increasing GABAergic neurotransmission in rodents that developed tinnitus following noise exposure can abolish the tinnitus behavior (Lu et al. 2011, Yang et al, 2011).

So far only two studies using magnetic resonance spectroscopy have investigated GABA levels in the auditory cortex of humans with tinnitus (Sedley et al. 2015, Isler et al. 2022). They concluded that GABA concentration is lower in individuals with tinnitus.

Here, we utilized 11C-Flumazenil dynamic PET scans to investigate the binding potential of GABA(A) receptors in the whole brain with high resolution.

Methods: Thirty-four participants were included in this ongoing investigation (18 with and 16 without tinnitus, age range 39-75 years). Pure tone audiometry was obtained (0.125 - 8 kHz). Participants underwent a dynamic PET scan (26 frames, 60 min) after injection of the 11C-Flumazenil PET tracer, which selectively binds to available GABA(A) receptors.
Preliminary analyses were conducted using PMOD-3. Time activity curves were obtained for cortical regions of interest (ROIs) defined by the AAL-3 atlas in subject space. Then kinetic modeling was conducted using the Simplified Reference Tissue Model with a reference in the pons to compute the binding potential for GABA in those regions.

Results: GABA(A) receptor availability was significantly higher in the left (P LESS THAN 0.001) and right (P=0.001) primary auditory cortex of participants with tinnitus compared to participants without tinnitus. There was no significant relation between binding potential and hearing thresholds (rho = 0.1 left and right). Remarkably, no other region showed such a large group difference and only 9 out of 74 tested ROIs showed a similar trend (Wilcoxon rank sum test P LESS THAN 0.01 uncorrected).
Conclusions: In line with animal research, our results in humans confirm the key role played by GABAergic neurotransmission in the pathophysiology of tinnitus. The observed differences may be explained by lower GABA concentrations and/or the upregulation of GABA(A) receptors on the neuronal membrane. These results motivate renewed consideration of clinical trials targeting GABAergic neurotransmission in humans with tinnitus.

Tinnitus is Associated With Reduced Spontaneous Spiking Activity in Auditory Nerve Fibers

Imme IJsseldijk[1], Amarins Heeringa*[1]

[1]*Carl Von Ossietzky University*

Background: Tinnitus is often initiated by damage to the peripheral auditory system, for example by overexposure to loud sounds. Animal studies have shown that such noise-induced tinnitus is related to aberrant spontaneous activity in the cochlear nuclei as well as further along

the central auditory pathway. However, the role of the auditory nerve, connecting the peripheral and central auditory systems, in tinnitus development remains unknown. Therefore, we here studied the spontaneous activity of auditory nerve fibers in animals with and without noise-induced tinnitus a few days after noise exposure.
Methods: Anesthetized Mongolian gerbils were unilaterally exposed to narrowband noise (115 dB SPL, centered at 4 kHz). After one day of recovery, animals were behaviorally tested for tinnitus using a gap-prepulse inhibition of the acoustic startle reflex (GPIAS) paradigm. Three days after noise exposure, animals were anesthetized again and spiking activity from single-unit auditory nerve fibers was recorded using high-impedance glass electrodes. Fibers were characterized according to their best frequency, threshold, and spontaneous rate (SR).
Results: Recordings from a total of 47 fibers in tinnitus animals (n = 2) and 121 fibers in no-tinnitus animals (n = 6) were obtained so far in the ongoing study. Mean spontaneous rate was significantly lower in fibers recorded from tinnitus animals (33 spikes/s) compared to fibers from no-tinnitus animals (58 spikes/s; p LESS THAN 0.001). Accordingly, when fibers were categorized in low- and high-SR fibers (cut-off at 18 spikes/s), the proportion of high-SR fibers decreased substantially in tinnitus animals (45%, p LESS THAN 0.001 following bootstrap analysis) compared to that in normal-hearing controls (78%), whereas the proportion of high-SR fibers in no-tinnitus animals did not change significantly (73%, p GREATER THAN 0.05). Ongoing analysis focuses on a long recording in silence (3 min), to probe for aberrant patterns in spontaneous activity.
Conclusions: These preliminary results suggest that tinnitus perception, and its associated central changes, might be triggered by reduced spontaneous spiking activity in the auditory nerve. As the changes were observed within days after noise exposure, loss of auditory nerve spontaneous rate may be involved in the initiating mechanism for tinnitus development.

Ebselen Permanently Reverses Noise-Induced Tinnitus in Young and Older Mice With Age-Related Hearing Loss

Annie Jia[1], Kushal Sharma[1], Rende Gu[1], Ryan Longenecker[1], Jonathan Kil*[1]

[1]*Sound Pharmaceuticals, Inc.*

Background: Tinnitus is a significant inner ear disorder with no FDA approved therapies. Exposure to loud sounds and noise-induced hearing loss (NIHL) is a major risk factor for noise-induced tinnitus (NIT). Prior human studies have shown that 4-days of ebselen can prevent acute NIHL in young adults with normal hearing and 21-28 days of ebselen can improve hearing and tinnitus in aged adults with Meniere's disease. We previously showed in young and old mice that 4 or 14-days of ebselen treatment can temporarily reduce or partially reverse NIT. The goal of this current study was to determine if 28-days of ebselen treatment can permanently reverse NIT in young (3 to 4-month) and aged mice (12 to 15-month) where hearing loss and tinnitus are more prevalent.
Methods: Two ages of CBA/CaJ mice (N=30) were utilized: 3 to 4-month-old (N=20) and 12 to 15-month-old (N=10). Each age was divided into two groups: Group 1: 3-6 month (N=4) and 12-15 month (N=2) served as an unexposed controls with no ear occlusion; Group 2: 3–4 month (N=16) and 12-15 months (N=8) received a temporary unilateral occlusion of an external ear canal before a brief narrowband noise exposure (awake, 112 dB, two hours, octave-band noise

centered at 8khz). Baseline ABRs and Gap-Induced-Prepulse-Inhibition of the Acoustic Startle Reflex (GPIAS) were assessed prior to and three months after noise to assess gap detection deficits (behavioral marker for NIT). The subset of mice that developed NIT were treated with a 28-day course of ebselen (10/mg/kg/d/ip) and reassessed at 1, 10, and 30-days post-treatment. Additional mice (N=30) of the same age groups are being tested now to increase the number of NIT mice for further comparative analysis including cochlear histopathology.
Results: Three months after noise exposure, permanent threshold shifts were observed in the open ears of all noise-exposed mice across three tested frequencies (8-20 kHz). GPIAS showed frequency specific behavioral evidence of NIT in a subset (N=7) of the noise-exposed mice (N=24).
After 28-days of ebselen treatment, gap detection deficits were significantly reduced to pre-exposure levels in 6 of the 7 NIT mice at 1-day post-treatment, in 4 of 6 NIT mice at 10-days post-treatment, and in 3 of 5 NIT mice at 30-days post-treatment.

Conclusions: A 28-day course of ebselen treatment can permanently reverse gap detection deficits to baseline or before noise exposure in 60% of mice that developed NIT, 3-months after noise exposure. This work is the first demonstration of an anti-inflammatory permanently reversing NIT after the development of age-related hearing loss. These data provide promising implications for ebselen as a treatment for noise-induced tinnitus in young and older adults.

Symposium 6: Electric-Acoustic Interactions Within and Across Ears: Animal, Human, and Computational Models From Periphery to Cortex

Chair: Waldo Nogueira*, Hannover Medical School*
Co-chair: Lina Reiss*, Oregon Health and Science University*
Co-chair: Yang-Soo Yoon*, Baylor University*
10:30 a.m. - 12:30 p.m.

Ocean Ballroom 1 - 4

Symposium Description: Combined electric and acoustic stimulation of the human auditory system can be used to enhance hearing through direct activation of the auditory pathway or through neuromodulation mechanisms. Different forms of electric-acoustic interaction have been reported in the literature from the periphery to the central auditory system in the same ear, across ears or through multisensory integration by stimulating different sensory pathways. For example, advancements in cochlear implant (CI) technology have enabled the combination of acoustic and electric hearing, both within the same ear (known as electric-acoustic stimulation or EAS) and across ears (referred to as bimodal listening). With EAS, electric hearing via a CI is combined with acoustic hearing in the ipsilateral ear. With bimodal hearing, electric hearing via a CI is combined with acoustic hearing in the contralateral ear. Cochlear implantation combined with acoustic hearing yields significant benefit for speech recognition in complex listening environments for some users. In contrast, others find little-to-no benefit or even experience interference, hindering the benefit. Only few studies have focused on comparing these interactions and their mechanisms between EAS and bimodal stimulation. Moreover, recent studies show interaction effects between non-invasive electric stimulation and acoustic

stimulation, including treatments to reduce tinnitus, a major health issue in society. Understanding mechanisms of electric-acoustic interaction can lead to extended cochlear implantation criteria, novel diagnostics of hearing loss, novel coding strategies to improve hearing as well as novel tinnitus treatments.

The symposium consists of novel contributions investigating electric-acoustic interactions from the periphery to the central auditory system and ranging from fundamental research to clinical application.

The symposia will cover the following topics related to electric and acoustic interaction within and across ears:

- Animal models
- Computational models
- Psychoacoustic and electrophysiologic methods
- Speech integration
- Multisensory integration
- Novel fitting and sound coding approaches

Outcomes of Electric-Acoustic Stimulation in the Same Ear: Insights From Electrophysiological Measures in Animal Models and Cochlear Implant Users

Viral Tejani*, Case Western Reserve University, University Hospitals*

Individual Abstract: Cochlear implantation (CI) has evolved in the past two decades such that residual hearing in the implanted ear can be preserved and utilized clinically. The combination of a hearing aid for low-frequency acoustic stimulation and electrode array for high-frequency electrical stimulation in the same ear (electric-acoustic stimulation - EAS) confers benefits for speech understanding in background noise, music perception, localization, and overall sound quality. Clinical data shows 30-40% of EAS CI users lose some residual hearing post-surgery. Though this partial loss of residual hearing does not necessarily preclude use of acoustic amplification, maximization of hearing preservation is the goal.
Electrically evoked compound action potentials (ECAPs), acoustically evoked auditory potentials (electrocochleography – ECoG) and electrode impedances are used in both animal models and human EAS CI users to study hearing/structural preservation. These electrophysiologic measures yield insight into cochlear physiology, particularly if residual acoustic hearing is compromised.

Early animal studies provide a basic understanding of the contributions of cochlear hair cells, auditory nerve, and cochlear site of stimulation, to the overall composite ECoG response and help with interpretation of ECoG responses in human EAS CI. Additionally, normal animal models of electrode impedance highlight their relationship to intracochlear fibrosis / osteogenesis, which has been a theorized contribution to compromised residual hearing in EAS CI based on pathologic animal models and post-mortem human histology.

ECoG measures have been incorporated in CI surgeries to minimize cochlear trauma and maximize hearing preservation, though results are mixed. Longitudinal post-operative ECoG measures of cochlear mircophonics (hair cell responses) and auditory nerve neurophonics (neural responses), as well as longitudinal ECAP and electrode impedance measures, revealed that these measures are stable for EAS CI users with stable acoustic hearing and correlated with audiometric thresholds. In cases of complete loss of residual hearing, residual cochlear microphonic with no remaining auditory nerve neurophonics is seen, implying synaptopathy. Rises in electrode impedances seen in loss of residual hearing implies intracochlear fibrosis as well. These interpretations are based on animal electrophysiologic models. Measures of ECoG tuning curves, rate adaptation, and electric/acoustic masking will also be discussed.

These animal and human studies highlight how electrophysiologic recordings reveal cochlear status in EAS CI. Animal histologic data allows human electrophysiologic data to be interpreted appropriately. Some of these electrophysiologic recordings can readily be incorporated into a clinical battery to provide objective markers of residual hearing and cochlear function.

Simulating Intracochlear Electrocochleography With a Combined Model of Acoustic Hearing and Electric Current Spread in the Cochlea

Margriet van Gendt, *Leiden University Medical Center*

Individual Abstract: Background: Intracochlear electrocochleography (iECochG) recordings have gained a lot of attention as an assessment tool to reduce surgical trauma during cochlear implantation surgery. A model of iEcochG with biophysical as well as phenomenological components has been proposed. To validate the performance of the model, it is compared against the previously used phenomenological model and animal experimental data. The effect of different biophysical factors of the auditory periphery on the various model outputs is explored, thereby simulating the effect of different pathologies.
Methods: The proposed computational model combines different stages. The middle ear filter and the basilar membrane are modelled using filters whereas the inner hair cells and the outer hair cells are modelled biophysically, via a circuit representation. The outputs compared include: input-output curves of the middle ear to the basilar membrane, frequency responses of the hair cells at different sound levels, saturation behavior of the hair cells, auditory nerve fiber responses and tuning curves, and intracochlear cochlear microphonics simulations at different electrode locations.

Results: Outputs showed similarities to experimental animal data and better followed the physiological behavior compared to the previous model. The proposed model better simulates the saturation behavior of the hair cells compared to the previous model. The frequency response of the hair cells in the new model show similar levels compared to the experimental data whereas the levels in the previous model are one order of magnitude higher. Electrode frequency mapping was more accurate in the proposed model. Different pathologies; e.g. differences in hair cell count, endocochlear potential, stiffness of tip links in stereocilia, affect CM and SP amplitudes throughout the cochlea in varying degrees.

Conclusion: The proposed model follows physiology more closely compared to the previous phenomenological model. When comparing outputs of the models to the experimental data, the proposed model matches the level of the experiments more closely compared to the previous phenomenological model. The proposed model shows the effect of different pathologies on ieCochG responses.

Psychoacoustic Electric-Acoustic Masking With Round Window Extra-Cochlear Electric Stimulation in Cochlear Implant Users With Residual Hearing

Patrick Hinz*, Medical University Hannover and Cluster of Excellence "Hearing4all", Hanover, Germany*

Individual Abstract: Previous studies have shown that low-frequency acoustic stimulation to the cochlear apex can mask electric stimulation from a cochlear implant (CI) at the cochlear base, with this masking effect decreasing as hearing loss increases (Krüger et al. 2017; Imsiecke et al. 2018). However, interactions between electric and acoustic stimulation (EAS) when using an extra-cochlear electrode near the round window (RW) are less understood.
This study measured EAS masking with extra-cochlear electric stimulation in CI users who have partially inserted or short electrode arrays and residual low-frequency hearing. Various electric stimulus parameters were investigated to enhance sound sensations while minimizing side effects, known to occur with basal or extra-cochlear stimulation. Sound sensations were measured using electrodes at the round window, most basal active (MBA), and most apical (MA) positions for comparison, with no side effects expected from intra-cochlear electrodes. Loudness scaling was conducted with different stimulation rates, pulse durations, and biphasic or triphasic stimulation.

The optimal electric stimulus setting was defined as the one eliciting the loudest sound without side effects. EAS masking was then measured using RW, MBA, and MA electrodes with both optimal and clinical settings. The EAS masking experiment used an adaptive three-interval forced-choice psychophysical procedure, with a constant comfort level acoustic stimulus acting as the masker. The acoustic masker's effect on detecting electric stimulation was assessed for each electrode configuration.

Results showed that extra-cochlear RW electric stimulation can elicit sound sensations without side effects, though optimal settings varied across subjects. Masking between low-frequency acoustic sounds and RW extra-cochlear electric stimulation was observed, with variability among subjects. Further research is needed to understand EAS interactions and explore their diagnostic potential for hearing loss.

Effects of Residual Hearing in the Non-Implanted Ear on Device Use and Auditory Development in Children with Bimodal Hearing (Including Single Sided Deafness)

Karen Gordon*, The Hospital for Sick Children*

Individual Abstract: Our research group has been exploring benefits and challenges of bimodal hearing in a large cohort of children. Bimodal hearing, electrical stimulation through a cochlear implant in one ear and acoustic hearing with or without a hearing aid in the other ear, has been provided to children with asymmetric hearing loss to limit auditory deprivation and promote benefits of bilateral hearing. Our group presently follows ~300 children listening bimodally including ~70 children with single sided deafness. Common etiologies of asymmetric hearing loss are congenital cytomegalovirus and genetic mutations. Benefits are auditory development in the implanted ear, especially when delays to implantation are reduced. Further, speech perception in both quiet and noise are typically better in the bimodal than unilateral listening conditions. Nonetheless, challenges particular to children with bimodal hearing have also been identified. Aural preference for one ear is difficult to avoid and depends on degree of residual hearing in the non-implanted ear; preference for the acoustic hearing ear remains common in the presence of normal or mild to moderate hearing loss whereas preference for the cochlear implant often occurs in children with severe to profound hearing thresholds in the non-implanted ear. Better residual hearing in the non-implanted ear is also associated with decreasing daily cochlear implant use (measured by datalogging) and further reductions in implant use during COVID-19 lockdowns. Benefits of spatial separation between target speech and noise are dependent on the degree of asymmetric hearing and spatial hearing of both stationary and moving sound is poor on average. Poor spatial hearing in children with bimodal hearing is not well predicted by degree or asymmetry of hearing loss or by the hearing history. In sum, benefits of bimodal listening are present in children with asymmetric hearing loss but challenges related to the mismatched input and asymmetric function between the two ears remain with implications for bilateral auditory development and binaural/spatial hearing.

Binaural Cue Sensitivity and Spatial Hearing in EAS Candidates: Pre- to Post-Implant Performance and Processing in Adults and Children

Rene Gifford*, Hearts for Hearing*

Individual Abstract: Acoustic hearing preservation theoretically affords cochlear implant (CI) patients access to interaural time differences (ITDs) in low frequencies. Without bilateral acoustic hearing, CI recipients have poor sensitivity to fine structure ITDs due both to envelope-based processing and lack of processor synchronization. Studies have shown significant benefit for combined electric and acoustic stimulation (EAS; CI+bilateral acoustic) as compared to bimodal listening (CI+contra acoustic) for horizontal-plane localization and speech understanding in complex listening scenarios. However, EAS benefit is highly variable. Studies have also shown a significant correlation between acoustic ITD sensitivity and EAS benefit for speech recognition and localization. It is unclear whether pre-implant estimates of ITD sensitivity may be useful in identifying successful EAS candidates. This study prospectively characterizes ITD sensitivity and related spatial hearing abilities pre- and postoperatively for adults and children with ski-slope audiograms. Our hypotheses were 1) cochlear implantation would initially degrade ITD sensitivity relative to preoperative thresholds, and 2) postoperative ITD sensitivity would improve over time due to neuroplastic recalibration of binaural cues following CI-mediated changes in middle ear mechanics.
Behavioral ITD thresholds were obtained for a 250-Hz tone presented to both ears at 90 dB SPL via earphones. Spatial hearing was characterized by minimum audible angle (MAA) for

broadband stimuli, spatial release from masking (SRM; S0N0, S0N90, N0N270), and binaural intelligibility level difference (BILD; N0S0 and N0Sπ) with spondees in broadband noise.

At time of abstract preparation, preoperative data were available for 18 study participants (4 children, 14 adults) with EAS-like audiograms and postoperatively at 1 month for 4 participants. Preliminary data show high variability for all measures with mean pre- and post-implant performance as follows: ITD thresholds (pre: 305, post: 609, range 117-2000+ microsec), BILD (pre: 4.6, post: 1.9; range 0-12 dB), SRM (pre: 5, post: 8; range 3-9 dB); and MAA (pre: 9.4, post: 21.2, range 4-52 deg). The 4 implanted participants demonstrated significant speech understanding benefit from CI, as compared to pre-implant performance as well as variable EAS benefit.

Binaural cue sensitivity and spatial hearing abilities generally worsened following CI activation, consistent with our hypothesis, with exception of SRM which was higher following implantation. Additionally, postop speech understanding in quiet and noise was significantly higher than preop scores, even with just 1 month of CI experience. Though ITD sensitivity may inform long-term EAS benefit for speech understanding in complex listening environments, BILD may be more sensitive and clinically applicable, particularly for children.

Bimodal Neuromodulation for Tinnitus Treatment: Scientific to Real-World Evidence

Hubert Lim, *University of Minnesota*

Individual Abstract: About 10-15% of the population suffers from bothersome or debilitating tinnitus, a phantom sound condition coded within the brain. Although a major health issue in society, there are currently limited treatment options for tinnitus. An emerging approach that can potentially provide long-term benefit of tinnitus symptoms in an accessible way is bimodal neuromodulation, which combines sound therapy with electrical stimulation of the body (e.g., tongue, ear, neck or face regions) that leverages the mechanistic concept of paired plasticity or Pavlovian conditioning. There have been four independent groups across three countries that have consistently demonstrated the ability to reduce tinnitus symptoms with bimodal neuromodulation that can have lasting benefit from several weeks to one year after treatment has stopped; these benefits have shown to be greater and longer lasting compared to what has been observed for sound only approaches in animal and human studies. There are several large-scale controlled clinical trials that have been performed to validate bimodal neuromodulation for the treatment of tinnitus that are paving the way towards building acceptance and confidence for this emerging approach. One particular approach leveraging sound therapy and electrical stimulation of the tongue, known as the Lenire device developed by Neuromod Devices, obtained De Novo FDA approval in 2023 in which already hundreds of patients have been treated with the Lenire device; the real-world data has been consistent with the clinical trial results, supporting successful integration and acceptance of bimodal neuromodulation into a clinical setting. New types of bimodal neuromodulation approaches and algorithms are being investigated to further improve benefit for a larger number of tinnitus patients that will be evaluated in future controlled clinical trials.

Podium 15: Genetics of Hearing Loss: Determining Causation and Function

Moderators: Morag Lewis and Karl Koehler

10:30 a.m. - 12:30 p.m.

Ocean Ballroom 5 - 8

Single Nuclei Rna-Sequencing Reveals Genetic and Cellular Insights Into Cisplatin-Induced Ototoxicity

Deanne Nixie Miao*[1], Emilia Luca[2], Janilyn Arsenion[1], John Pham[1], Alain Dabdoub[2], Britt Drogemoller[1]

[1]*University of Manitoba,* [2]*Sunnybrook Research Institute/ University of Toronto*

Background: Cisplatin, a major chemotherapeutic agent, causes hearing loss (ototoxicity) in up to 80% of patients, with children facing a three-fold higher risk of experiencing this adverse drug reaction. Genetics plays an important role in cisplatin-induced ototoxicity (CIO). Therefore, we hypothesize that genetic variants modulate cisplatin-induced changes in gene expression within the cochlea, leading to CIO. To provide insights into which genes, pathways, and specific cells are involved in CIO, we profiled single-cell gene expression changes in the cochlea.
Methods: Intraperitoneal injections of 3mg/kg of cisplatin or saline were administered to postnatal day 6 (P6) CBA/CaJ mice in the treatment (n=6) and control groups (n=6), respectively. Four-hours post-cisplatin administration, mice were euthanized, and whole cochlear ducts were dissected. Single nuclei were isolated using the 10XGenomics Chromium Nuclei Isolation Kit, followed by single-nuclei RNA-sequencing (snRNA-seq) using the Single Cell Gene Expression + RNA Profiling Kit. Sequencing was performed on the NovaSeq X Plus Sequencing System. snRNA-seq data were processed using CellRanger and analyzed with Seurat, DESeq2 and MILO-R to detect gene expression changes, and identify differences in cell proportions between control and treatment groups.
Results: We sequenced 15,510 and 12,194 nuclei for the control and treatment groups, respectively. Analysis of the snRNA-seq data revealed that cisplatin treatment led to a decrease in the abundance of several cell types within the inner ear, as well as an increase in the abundance of certain clusters of macrophages. The cochlear cells that showed a decrease in abundance post cisplatin treatment included specialized auditory cells (spindle cells, outer hair cells, and type I neurons), supporting cells, immune cells and bone cells (MILO-R: LogFC LESS THAN =-3, SpatialFDR LESS THAN 0.1). Across the cochlear cell types that showed differential abundance, 159 differentially expressed genes (DEGs) were observed (DESeq2: Padj LESS THAN 0.05).
Conclusions: Our pilot study identified key genes, pathways and cell types that are associated with CIO, which is consistent with prior findings. Notably, structures such as the stria vascularis, organ of Corti, and spiral ganglion have been implicated in CIO, with spindle cells, outer hair cells, and type I neurons residing in these structures. The reduced abundance of supporting cells and increased abundance of macrophages following cisplatin treatment underscore the need for further investigation into their roles in CIO. Additionally, cisplatin binds extensively to type I collagen in bone, creating a platinum reservoir that may contribute to ototoxicity. By pinpointing

specific cochlear cells that are significantly associated with CIO, our study provides novel insights into its mechanisms and paves the way for future research. We will conduct a follow-up pilot study focusing on the 1-hour timepoint post-cisplatin administration to investigate gene expression changes preceding cell death. This will inform future snRNA-seq and snATAC-seq experiments examining cisplatin's effect on gene expression and chromatin accessibility in cochlear cells.

Identification of ATP8A2 as a Novel DFNA Gene Associated With Late-Onset Hearing Loss: Insights From Human and Mouse Models

Jing Cheng*[1], Jing Wang[1], Lanchen Wang[1], Wan Hua[1], Libo Liu[1], Yu Huang[1], Guotong Lin[2], Lei Song[2], Huijun Yuan[1]

[1]*West China Hospital,* [2]*Shanghai Jiao Tong University School of Medicine*

Background: Autosomal dominant non syndromic hearing loss (ADNSHL) is genetically diverse, and many causative genes remain unidentified. ATP8A2, a flippase that translocate specific lipids across biological membranes to maintain lipid asymmetry, is essential for numerous cellular processes. While previously linked to neurodegenerative disorders, ATP8A2 has not been implicated in human hearing loss. This study explores ATP8A2 as a novel DFNA gene responsible for late-onset, progressive hearing loss in a Chinese family.
Methods: A three-generation Chinese family with late-onset hearing loss was studied. Genome-wide linkage analysis and whole genome sequencing were employed to identify the causative mutation. A knock-in mouse model was generated to further explore the gene's role. Hearing function was assessed by measuring auditory brainstem response (ABR) thresholds and wave I latencies under normal conditions and after noise exposure. Cochlear pathology was analyzed for hair cell and spiral ganglion neuron loss. In vitro assays using HEK293 cells co-transfected with ATP8A2 and CDC50 plasmids were performed to evaluate protein expression and membrane localization of both the wild-type and mutant ATP8A2.
Results: Linkage analysis mapped the causative gene to chromosome 13 (maximal LOD score = 1.504). Whole genome sequencing identified a heterozygous missense variant, ATP8A2 c.3398T GREATER THAN C (Leu1133Pro), which co-segregated with the hearing loss in the family. The Atp8a2L1093P mouse model exhibited progressive hearing loss mimicking the human phenotype. Noise exposure experiments were performed to address whether mutant ice are more susceptible to environmental insult. 14 days after noise exposure, mutant mice suffered more severe residual hearing losses with significantly elevated ABR thresholds, prolonged wave I latencies and reduced wave I amplitudes compared to wild-type controls. Cochlear pathology revealed progressive loss of outer hair cells and spiral ganglion neurons of all turns. In vitro studies showed that mutant ATP8A2 expression was normal but failed to localize properly to the cell membrane as a lipid transporter.
Conclusions: ATP8A2 is identified as a novel DFNA gene involved in human late-onset hearing loss. The Atp8a2L1093P mouse model recapitulates the human hearing loss phenotype, providing valuable insights into the gene's role in auditory function. These findings suggest ATP8A2 as a potential therapeutic target in rescuing age-related hearing loss phenotype.

Gene Therapy in a Rabbit Model for USH3A

Diane Prieskorn[1], Lisa Beyer[1], Y Eugene Chen[1], Dongshan Yang[1], Yehoash Raphael*[1]

[1]*University of Michigan*

Background: Autosomal recessive pathogenic variants in CLRN1 (clarin 1) cause Usher syndrome type 3A (USH3A), characterized by progressing deafness with post-lingual onset. Animal models are needed to design therapies that reduce or prevent the pathologies associated with USH3A. In mouse models for USH3A, hearing loss is early onset and severe, and hair cells degenerate prior to cochlear maturation, reducing their utility as models for therapy. Here we describe a novel rabbit model for USH3A and attempts for therapies via AAV gene transfer using this model.
Methods: To generate the rabbit model, CRISPR/Cas9-mediated rabbit genome editing was used, with a knock-out approach. For validation, ABRs were measured in anesthetized rabbits using TDT equipment. Tone bursts (15 ms) were delivered with 1 ms rise/fall times, presented 10 per second at 4, 12 and 16 kHz. Age-matched wild-type rabbits served as controls. For histology, bullae were removed, stapes was removed and fresh paraformaldehyde was perfused. To soften the bone for light microscopy sections, bullae were transferred into 5% EDTA with 0.25% glutaraldehyde. Then cochleae were processed for JB-4 embedding, followed by sectioning with a glass knife. For gene transfer experiments, AAV vectors with a GFP reporter gene insert, or AAV.Clrn1-Flag were injected into the perilymph. To assess reporter gene expression, bullae were lightly decalcified, dissected into organ of Corti segments, stained for GFP or FLAG, and for F-actin, and viewed in epi-fluorescence.
Results: ABR thresholds were elevated in 3-month-old CLRN1 rabbits compared to wild types, but the hearing loss was moderate. However, by 14 months of age, no hearing could be recorded at any tested frequencies. Histology showed that the severe hearing loss was accompanied by loss of cochlear hair cells. Injecting AAV.GFP into the perilymph of a wild-type ear resulted in transgene expression in sensory hair cells 14 days post-infusion. In 2 rabbits that received AAV.Clrn1-Flag at 5 weeks of age many surviving hair cells were Flag positive one (N=1) or 2 (N=1) months later, but thresholds did not improve. We are now extending the survival time to determine if viral-mediated expression of CLRN1 can improve hearing thresholds compared to baseline.
Conclusions: The USH3A rabbit model we generated exhibits progressive loss of hearing over several months after birth, presenting a window for testing therapeutics including gene transfer approaches. Surgical injection of AAV vector into perilymph shows transfection of hair cells but hearing did not improve. It is possible that rescue for a mutation involving a developmental gene needs to be performed at an earlier time point.
Support: NIH R21-GM140359-01, 1R42EY035582-01, RO1-DC014832 and the R. Jamison and Betty Williams Professorship.

Functional Results and Implications of the SLC26A5 Genotype R399X/T470N for Genetic Nonsyndromic Hearing Loss DFNB61

Rosemary Kabahuma*[1], Kazuaki Homma[2], Satoe Takahashi[2], ZhengYi Chen[3], Michael Pepper[1], Xue Liu[4]

[1]*University of Pretoria,* [2]*Northwestern University,* [3]*Mass Eye and Ear,* [4]*University of Miami School of Medicine*

Background: Prestin, the cochlear outer hair cell motor protein, is encoded by SLC26A5. Mutations in this gene are associated with a nonsyndromic recessively inherited form of hearing loss, DFNB61. Twenty-five years after the discovery of prestin and its gene, the paucity of families with DFNB61 presents us a conundrum. Whereas prestin's essential role in outer hair cell electromotility has been confirmed and extensive research has elucidated molecular mechanisms underlying electromotility, there is a lag in reported DFNB61 pathogenic variants. To date, only two unrelated individuals carrying compound heterozygous variants, c.209G GREATER THAN A (p.W70X) and c.390A GREATER THAN C (p.R130S), have been confirmed while homozygous c.-53-2A-G prestin variants in two unrelated individuals are yet to be fully characterized.

Methods: We identified compound heterozygous SLC26A5 variants in a prelingually hearing impaired indigenous South African male through massively parallel sequencing and combined electrophysiological measurements (whole-cell patch-clamp technique), immunohistochemical staining and 3D modelling to explore the functional effect of his genotype, R399X/T470N. We established HEK293T-based stable cell lines heterologously expressing the prestin variants in a doxycycline-inducible manner. Expression of wild-type, p.R399X, and p.T470N prestin was examined using N-terminal prestin antibody and the specificity of the prestin antibody confirmed in untransfected HEK293T cells.

Results: The p.R399X mutant prestin had no recordable NLC while the p.T470N mutant recorded severely attenuated NLC (25.1%) and markedly attenuated Qmax (23.3% of WT) and charge density (25.9% of WT), indicating that the p.T470N mutant is a prestin hypomorph. The confocal images of the mutant prestin transfected HEK293T cells against a comparison of the functional effects of these mutations with results from published literature, lead us to predict that the R399X peptide triggers the Nonsense Mediated Decay machinery and that all the available prestin, approximately 12.5% of WT prestin motors, is formed of p.T470N prestin hypomorphs.

Conclusions: Our results suggest the R399X/T470N genotype protein product does not have sufficient motor function to maintain normal hearing sensitivity, cochlear amplification and frequency selectivity, leading us to conclude that this genotype cannot support normal hearing function and is causally associated with DFNB61.

Furthermore, it appears that SLC26A5 not only functions as a recessive loss-of-function dose dependant gene but the functional organization in the cochlea presents an inbuilt fail-safe design which can accommodate large reductions in capacity before hearing function fails, the 'perfect storm' for a DFNB61 phenotype to manifest. We postulate that the determinants of a DFNB61 phenotype include critical levels in the numbers of functional prestin motors and voltage-dependent nonlinear capacitance values, the effect of the different mutations on the molecular structure and conformational properties of the prestin molecule, the interaction of the mutant alleles with each other and with WT prestin, as well as prestin upregulation. This may account for the paucity in reported DFNB61 individuals.

Functional Outcome in a Rationally Designed Genomically Humanized Mouse Model for Dominantly Inherited Hearing Loss DFNA9.

Vincent Van Rompaey*[1], Dorien Verdoodt[2], Erwin Van Wijk[3], Peter Ponsaerts[4], Fien Aben[2], Lize Sels[2], Evi De Backer[2], Hanne Gommeren[2], Krystyna Szewczyk[2], Sanne Broekman[3], Hanka Venselaar[3], Guy Van Camp[5], Erik de Vrieze[3]

[1]*Universitair Ziekenhuis Antwerpen,* [2]*Resonant Labs Antwerp, Faculty of Medicine and Health Sciences, University of Antwerp, Belgium,* [3]*Radboud University Nijmegen Medical Center,* [4]*Laboratory of Experimental Hematology, University of Antwerp,* [5]*Centre of Medical Genetics, University of Antwerp,*

Background: DFNA9 is the most frequent hereditary autosomal dominant hearing disorder in Belgium and the Netherlands causing hearing loss at 20-30 years and evolving towards severe-to-profound sensorineural hearing loss by 60-70 years. Additionally, patients suffer from bilateral vestibulopathy by the age of 40 years. In the Dutch/Belgian population, the c.151C GREATER THAN T founder mutation in the COCH gene is the most prevalent variant.
Methods: A partial (4 exons) genetic humanisation of the COCH gene was generated in C57Bl6 background (corrected for Cdh23), including wt mice, as well as heterozygous and homozygous c.151C GREATER THAN T variants.
Results: At 9 months, all humanized Coch genotypes showed hearing thresholds comparable to wild-type C57BL/6 Cdh23753A GREATER THAN G mice. This indicates that both the introduction of human wildtype COCH, and correction of Cdh23ahl in the humanized Coch lines was successful. Follow-up on ABR and DPOAE up to 24 months will be presented.
Conclusions: Overall, our approach proved beneficial in eliminating potential adverse events of genomic humanization of mouse genes, and provides us with a model in which sequence-specific therapies directed against the human mutant COCH allele can be investigated irrespective of the phenotype.

Critical Challenges in Splicing-Related Variant Analysis for Accurate Pathogenicity Assessment

Yu Lu*[1], Bingqian Yang[2], Linke Li[1]

[1]*West China Hospital, Sichuan University,* [2]*Renmin Hospital of Wuhan University*

Background: Pathogenicity assessment of genomic variants is crucial in precision medicine and the diagnosis of genetic disorders. Splicing variants are common types of mutations that lead to significant gene defects; however, the prediction of how these variants affect gene function can be misinterpreted, resulting in incorrect pathogenicity evaluations and influencing clinical decisions. Through the analysis and experimental validation of splicing-related variants in two hearing loss genes, OTOF and GSDME, we highlight the complexity and challenges of interpreting splicing site variants in pathogenicity assessments.
Methods: We utilized the minigene assay to verify the impact of candidate variants on exon splicing and detect specific changes in mRNA sequences. Based on the experimental results and sequence characteristics, we reanalyzed the pathogenicity of the variants.

Results: In the first case, the splicing variant c.4023+1G GREATER THAN A in the OTOF gene was experimentally validated to cause exon 32 skipping, resulting in a 129 bp exon removal and an in-frame deletion of 43 amino acids. Despite this, the variant was classified as benign due to its high frequency in the population. In the second case, a nonsense variant in exon 8 of the GSDME gene was identified, and experimental validation confirmed its impact on splicing, leading to gene dysfunction.
Conclusions: These cases underscore the importance of accurately assessing the splicing effects of variants in pathogenicity evaluations. Our study advocates for enhanced functional validation of candidate splicing variants in pathogenicity assessments to avoid misinterpretation. This approach is crucial for improving the accuracy and reliability of genetic disorder diagnoses.

Unraveling the Genetic Basis of Autosomal Dominant Hearing Loss

Dominika Oziębło[1], Marcin L. Leja[1], Nina Gan[1], Natalia Baldyga[1], Henryk Skarzynski[1], Monika Ołdak*[1]

[1] *of Physiology and Pathology of Hearing*

Background: Autosomal dominant hearing loss (ADHL) is the second most common form of inherited HL with an onset usually after the first decade of life. It affects mainly high frequencies and progresses over time. Autosomal-dominant genes are responsible for about 20% of cases of hereditary non-syndromic deafness, with 63 different genes identified to date.
Methods: In this study, 110 families with a vertical inheritance pattern of hearing impairment were recruited. Genomic DNA was isolated from peripheral blood samples or buccal swabs of available family members. In all probands targeted next-generation sequencing (NGS) using a targeted multi-gene panel (237 genes) was performed. In 10 largest unsolved families linkage analysis and whole genome sequencing (WGS) were performed. Presence of the selected probably pathogenic variants and their segregation with HL within the family were confirmed by standard Sanger sequencing.
Results: Genetic cause of ADHL was identified in 51% (56/110) of the examined families. Among the 56 identified HL variants only 27% (15/56) have been previously reported and the remaining 73% are novel (41/56). We have identified missense variants (35/56; 62%), splice site variant (8/56; 14%), frameshift variants (6/56; 11%), nonsense variants (6/56; 11%) as well as one synonymous varaint 1/56; 2%). Among the most common causative genes were MYO6 (n=11), TBC1D24 (n=6), WFS1 (n=6), GSDME (n=5), POU4F3 (n=5) and KCNQ4 (n=4). Pathogenic variants causative of HL in the NLRP3, LMX1A, FGFR3, CD164, GRHL2, TMC1, ATP2B2 and CEACAM16 genes were detected in single families. Implementation of linkage analysis and whole genome sequencing (WGS) resulted in the identification of the non-coding variants in the EYA4 and ATP11A genes and novel candidate genes.
Conclusions: Our custom multigene panel has demonstrated good diagnostic performance. Considering frequent identification of novel genetic variants it is necessary to perform thorough clinical examination and variant segregation analysis with ADHL in all available family members. The use of linkage analysis and WGS increases the detection rate of causative variants, especially located in the non-coding regions, and provides the opportunity to identify novel genes.
Grant: 2016/22/E/NZ5/00470 National Science Centre, Poland

Beyond the Gene Panel: Methods to Identify Missing Genetic Causes of Pediatric Sensorineural Hearing Loss

Shelby Redfield*[1], Tieqi Sun[2], Adrian Pastolero[1], Margaret Kenna[2], Eliot Shearer[2]

[1]*Boston Children's Hospital,* [2]*Boston Children's Hospital; Harvard Medical School*

Background: Genetic testing is crucial to the diagnosis and management of pediatric sensorineural hearing loss (SNHL). Estimates of the rates of hereditary SNHL in past literature fall around 50-60%, however diagnostic yields from genetic testing average 43%. This discrepancy suggests a missing heritability in genetic hearing loss using currently available sequencing methods. Here, we sought to determine the results of genetic evaluation using a variety of genomic methods in a diverse cohort of pediatric SNHL patients.
Methods: We performed genetic evaluation for children with SNHL of unknown etiology between May 2019 and July 2024 at Boston Children's Hospital. Patients received standard of care evaluation with a pediatric otologist and genetic counselor. All probands were offered genetic testing, regardless of specific auditory or phenotypic characteristics. Commercial gene panel testing of approximately 150 genes was performed for probands with adequate insurance coverage. Research-based exome sequencing (ES) was performed after nondiagnostic panel and for those who could not otherwise access testing. Short read genome sequencing (srGS) and long read genome sequencing (lrGS) were performed for a select number of undiagnosed patients. Clinical and demographic data was obtained via retrospective chart review.
Results: 637 pediatric SNHL patients underwent genetic evaluation during the study period, including 234 gene panels, 465 exomes, 56 srGS, and 19 lrGS performed. This cohort included 392 (61.5%) probands with bilateral symmetric SNHL and 245 (38.5%) with asymmetric SNHL. 73.5% (n=299) of patients obtained insurance prior authorization for gene panel testing; approval rates were 70.8% (n=120) for those with public insurance and 77.4% (n=178) for those with private insurance. Prior authorization was approved for 75.7% (n=237) of probands with bilateral symmetric SNHL and 65.6% (n=61) of probands with asymmetric SNHL. The diagnostic rate was 30.9% (n=197) overall and 42.3% (n=166) for symmetric SNHL. Diagnoses were made in 65 genes, with GJB2 (29.4%) and STRC (14.4%) being the most prevalent. 27.4% (n=54) of all diagnoses were syndromic, with an additional 4.1% (n=8) of diagnoses being potential nonsyndromic mimics. The diagnostic yield of gene panel was 41.2% (n=63); performing ES on gene-panel negative probands added 9 additional diagnoses (13.0% of all reflex ES). Overall diagnostic rate of srGS following ES was 1/56 (1.8%). LrGS following nondiagnostic srGS had a yield of 4/19 (21.1%). LrGS was able to resolve variants in segmental duplications (OTOA) and genes with highly homologous pseudogenes (STRC).
Conclusions: Gene panel testing is standard of care in evaluation of pediatric SNHL and as such was covered by most public and private insurers. While comprehensive gene panel will capture most clinically meaningful diagnoses, ES marginally increases yields. LrGS shows promise over srGS to better identify variants in difficult-to-sequence and noncoding genomic regions

Podium 16: Decoding the Aging Auditory System: Molecular Mechanisms, Functional Decline, and Therapeutic Prospects

Moderators: Kelly Harris and Ilkem Sevgili

10:30 a.m. - 12:30 p.m.

Ocean Ballroom 9 - 12

Genetic Contribution to Hearing Loss Progression in Aged Diversity Outbred Mice

Daniel Johnson*[1], Sarah Cancelarich[1], Kara Campos[1], Jacqueline Otto[1], Kevin Bugge[1], Irina Marcovich[1], Meghan Drummond[1]

[1]*Regeneron Pharmaceuticals*

Background: Complex genetic traits and diseases, including age-related hearing loss (ARHL) are difficult to study in inbred strains of mice. In this study we utilized the Diversity Outbred (DO) mice from The Jackson Labs to explore genetics of, and expression patterns contributing to, a broad range of hearing preservation observed by audiometric testing of aged DO mice. The DO mouse line is derived from random crosses of eight founder inbred strains, resulting in a highly genetically diverse population and therefore constituting a power tool for tracking genetic variants involved in ARHL.
Methods: Auditory brainstem response (ABR) and distortion product otoacoustic emissions (DPOAE) were measured in ~2-year-old DO mice (n = 20). Tail samples were collected for comprehensive SNP genotyping to reveal strain-specific genetic contributions. Mouse cochleae were isolated for RNA sequencing to identify differentially expressed genes. These sequencing data were integrated with phenotypic data to explore molecular mechanisms underlying ARHL. Additionally, we performed immunofluorescence of whole mount samples to assess hair cell survival and synaptic puncta counts.
Results: 2-year-old DO mice exhibited a wide range of hearing abilities from excellent hearing to profound hearing loss. Comparisons with original founder strains at 4-months of age showed a similar variability in hearing patterns, suggesting these differences persist in the DO age mice. Haplotypes will be evaluated for associations with the observed ARHL progression.
Conclusions: To our knowledge, this is the first study that uses the Diversity Outbred mouse line and its uniquely diverse genetics to explore genetic modifiers of age-related hearing loss. Further studies will help us understand the heterogeneous mechanisms underlying ARHL and potentially design therapies for its treatment or prevention.

Age-Related Mitochondrial Depolarization and Mitophagy Impairment in the Cochlear Biobattery

Tyreek Jenkins*[1], Jiaying Wu[1], Li Li[1], John Lemasters[1], Hainan Lang[1]

[1]*Medical University of South Carolina*

Background: Age-related hearing loss (ARHL; presbyacusis) is a global health concern affecting the communication and livelihood of many older adults. Metabolic presbyacusis, a major type of ARHL, is associated with atrophy of the stria vascularis (SV). The SV, cochlear

biobattery, is a vascularized and highly bioenergetic epithelium essential for sound transduction. Specifically, mitochondria-rich marginal cells (MCs) of the SV transport K+ into the endolymph to drive hair cell depolarization. Increasing evidence supports aberrant macrophage activity and chronic inflammation of the SV as key contributory factors of metabolic presbyacusis. However, the underlying propagating mechanisms have yet to be elucidated. Recent studies signify mitochondrial dysfunction to be a key contributor of macrophage activation and inflammation. Damaged/depolarized mitochondria undergo fission to enable mitophagy (a selective form of mitochondrial degradation), but excessive fission can impair autophagosome formation, affecting clearance of dysfunctional mitochondria, leading to oxidative stress and the release of mitochondrial damage associated molecular patterns. This may drive macrophage activation and inflammation. The present study addressed the hypothesis that mitochondria within strial MCs of aging mice are depolarized and less metabolically active with reduced mitophagy compared to young-adult mice contributing to cochlear inflammation and auditory functional decline.
Methods: Hearing sensitivity of young-adult (2-4 months), middle-aged (15-18 months), and aged (≥ 24 months) CBA/CaJ mice were assessed by measuring the auditory brainstem response (ABR). The SV was then dissected from the cochlea and loaded with tetramethyl rhodamine methyl ester (TMRM) to assess mitochondrial membrane potential via live-cell confocal imaging. In addition, SV explants were excited using two-photon imaging to evaluate NADH and FAD autofluorescence (indicators of mitochondrial metabolic activity). Mitochondrial fission was assessed using quantitative immunohistochemistry of phosphorylated dynamin-related protein 1 at serine 616. To evaluate mitophagy in strial MCs, young-adult (2-4 months) and middle-aged (10-14 months) GFP-LC3 transgenic mice were exposed to an octave-band noise (8-16 kHz) at 106 dB SPL for 2 hours to stimulate mitophagy. Cochleae were collected 1 day post noise exposure to assess mitophagy via LC3 colocalization with mitochondria. Hearing was assessed before and after noise exposure via ABR.
Results: Middle-aged CBA/CaJ mice exhibit reduced hearing sensitivity and suprathreshold function. Mitochondria within MCs are significantly depolarized with reduced metabolic activity in middle-aged animals. Furthermore, young-adult GFP-LC3 mice exposed to noise showed increased LC3 puncta per MC compared to sham exposed controls, indicating active autophagy. In contrast, middle-aged GFP-LC3 mice showed no change in LC3 puncta after noise exposure and significantly less LC3 puncta compared to young-adult mice, thus suggesting impaired mitophagic activity.
Conclusions: The understanding of age-related mitochondrial alterations in MCs of the SV will aid in the development of therapeutic agents designed to combat cochlear inflammation via targeting mechanisms aimed at preserving mitochondrial function such as mitophagy.

Single-Nucleus Profiling of Inner Ear Aging Reveals Cellular Diversity and Hair Cell Degeneration Caused by Inflammation-Induced Aberrant Rna Splicing

Mingyu Xia*[1], Jiaoyao Ma[2], Yunjie Li[2], Wenyan Li[2], Huawei Li[2]

[1]*Eye and ENT Hospital, Fudan University,* [2]*ENT Institute and ENT Hospital, State Key Laboratory of Medical Neurobiology and MOE Frontiers Center for Brain Science, Fudan University*

Background: Degeneration of the auditory and vestibular systems leads to dysfunction of two essential senses: hearing loss and decompensated balance perception. However, the cellular and molecular mechanisms governing inner ear aging and its link to dysfunctions in sensory perception remain unclear.
Methods: SA-β-gal staining, aging-related marker staining and TEM were used to analyze the the aging-related phenotypes of cochlea and utricle from 3-month-old, 12-month-old and 24-month-old C57BL6/J mice. Then, single-nucleus RNA sequencing was employed to construct a comprehensive cellular aging atlas of the mouse inner ear organs. RNAscope were utilized the spatial location of the interested cell types. To complement the snRNA-seq results, the whole inner ear tissue with the bone removed were collected for bulk RNA-seq. Age-related changes in transcriptome of HC subtypes among the two organs were analyzed and the expression of potential genes lead to hair cell degeration were validate by immunofluorescence staining. Lentiviral vector-based short hairpin RNAs targeted potential genes related to HC degenration were transfect TNF-α-induced inner ear organoid models. Bulk RNA-seq and RIP-seq were combined to explore the molecular mechanism by which Rbm25 regulates HC senescence. To identify alternative splicing targets guided by Rbm25, differentially spliced genes and binding genes by Rbm25 were analyzed.
Results: 12,355 nuclei from the cochlea and 14,398 nuclei from utricle were obtained, and 48 clusters of the two organs were identified across ages. We unveiled the heterogeneity of the mature cochlea and utricle, identified four spatially and genetically distinct utricular HC subtypes, and identified new markers for inner ear epithelial cells. Specifically, four molecularly defined and spatially specific utricular HCs hold physiologically distinct features were identified. Furthermore, we uncovered the heterogeneous and asynchronous molecular aging patterns of the two organs, including the dominant events that trigger the degeneration of two cochlear HC subtypes and four utricular HC subtypes. Theses unregulated molecular patterns during HC aging mainly including RNA splicing, protein folding, ribosome biogenesis, and those of downregulated were synapse, stereocilium, and cell adhesion. In addition, we unveiled that an inflamed microenvironment shaped by activated macrophages in the inner aged ear induced aberrant RNA splicing in HCs, which led to HC dysfunction. We found that RBM25 KD recused TNF-α-induced senescence-related transcriptome changes. Additional overlap of RBM25 spliced and binding genes revealed that RBM25 modulates inflammation-mediated HC aging through mRNA alternative splicing.
Conclusions: In this work, we constructed a cellular aging atlas of the inner ear organs, revealing insights into organ-specific and cell-specific transcriptomic features associated with inner ear aging, and uncovering the mechanisms by which the inflamed microenvironment triggers a series of complex events that lead to HC dysfunction. The underlying mechanisms of inner ear aging have implications for developing approaches to counteract age-associated hearing loss and balance disorders.

Unraveling Age-Related Cellular and Molecular Mechanisms Associated With Vestibular Hair Cells and Their Slow pace of Aging Compared to Cochlear Hair Cells

Samadhi Kulasooriya*[1], Huizhan Liu[1], Sarath Vijayakumar[1], Celia Bloom[1], Mi Zhou[1], Litao Tao[1]

[1]*Creighton University School of Medicine*

Background: The deterioration of the inner ear with aging contributes to age-related hearing loss (ARHL) and vestibular dysfunction (ARVL). Many studies have examined the age-related changes in the cochlea, while ARVL remains understudied. ARVL is the gradual loss of bilateral vestibular function accompanied by interruptions to visual and proprioceptive inputs, leading to an increased risk of imbalance, dizziness, and fatal falls. According to the National Institutes of Health, age-related falls account for 50% of all accidental deaths and it is the 6th leading cause of death in the elderly, highlighting the importance of understanding the molecular basis and development of targeted therapeutics. Despite both the cochlea and vestibule in the inner ear containing mechanosensitive hair cells (HCs) and non-sensory supporting cells (SCs) in the sensory epithelia, evidence from both humans and mice suggests that age-related functional and morphological changes in the cochlea precede those in the vestibular system. Thus, we aim to determine the key factors and underlying mechanisms driving age-related cellular, and molecular changes in the vestibule that contribute to ARVL and understand how aging processes differ between the vestibule and cochlea.
Methods: CBA/J mice aged between 2 and 24 months after birth were used for our experiments. We performed vestibular and auditory functional tests to assess age-related vestibular and cochlear functional decline. At the cellular level, we examined changes in HC count, morphology, and ultrastructure using histology, super-resolution (SR) confocal microscopy, and scanning electron microscopy (SEM). At the molecular level, we used single-cell RNA sequencing (scRNA-seq) to examine changes in the transcriptomes of vestibular HCs during aging. For comparison, we also examined changes in the transcriptomes of cochlear HCs. For scRNA-seq, vestibule and cochlea were isolated from 80 mice to obtain 5 biological replicates. Droplet-based scRNA-seq was performed using the 10x Genomics platform, and raw data were processed by Cell Ranger to obtain count matrices. Downstream quality control, analysis, and visualization were performed in R using the Seurat package. mRNA and protein expressions were validated by RNAscope and immunofluorescence respectively.
Results: Our functional analysis shows an age-related decline in both the vestibule and cochlea. Our morphology analysis indicates signs of degeneration of vestibular HCs. Our molecular analysis shows that vestibular HC aging is associated with both universal and cell-type specific aging signatures. Importantly, our comparative analysis unveiled evidence to support the differential onset of aging in the vestibule compared to the cochlea.
Conclusions: Our findings pinpoint potential molecular drivers and underlying mechanisms of vestibular aging that lead to ARVL, providing an avenue to develop targeted therapeutics. Moreover, our study will provide valuable insights into the mechanism/s involved in the disparity of the pace of aging in the two systems within the inner ear.

Pou4f3 is Critical for Stereocilia Bundle Maintenance and Hair Cell Survival in Adult Mammalian Cristae

Brad Walters*[1], Kendra Stansak[1], Tianwen Chen[1], Caroline Nall[1], Tierah Macon[1], Wu Zhou[1], Hong Zhu[1], Brandon C. Cox[2]

[1]*University of Mississippi Medical Center,* [2]*Southern Illinois University School of Medicine*

Background: The vestibular epithelia of the inner ear give rise to our senses of balance, proprioception, and motion. Age-related vestibular dysfunction represents a major health issue, affecting much of the population, and causing injurious and fatal falls. Despite the prevalence of vestibular dysfunction and its impact on mortality and morbidity, the causes of age-related vestibular decline are poorly understood. The transcription factor Pou4f3 plays a critical role in the development and innervation of inner ear hair cells, and POU4F3 mutations are known to cause hearing loss. However, significantly less is known about Pou4f3's role in vestibular function, particularly at postnatal and adult ages.
Methods: To test whether POU4F3 changes during aging, horizontal and anterior cristae from adult mice were immunolabeled with antibodies against POU4F3, SOX2, and MYO7A. Pou4f3 function was tested directly by conditional deletion from type I and type II hair cells in 8-week old male and female mice using Atoh1-CreER:Pou4f3loxP/loxP mice. Rotarod and Vestibuloocular responses (VORs) were measured six weeks after tamoxifen induction. VORs were measured in an additional cohort 6 months after tamoxifen induction. Anterior and horizontal cristae were collected and stained to visualize POU4F3 expression, stereocilia bundles (phalloidin), and hair cells (MYO7A and SOX2).
Results: At all ages investigated, a modest number of MYO7A+ vestibular hair cells lacked POU4F3 immunoreactivity. Quantification of POU4F3 immunofluorescent intensities revealed decreasing levels of POU4F3 between 2 and 19 months of age, particularly in the horizontal cristae. Type II hair cells generally had higher POU4F3 staining intensities compared to type I hair cells. VOR assessments showed that conditional deletion of Pou4f3 led to deficits in rotational responses at 6 weeks after the deletion. VOR performance largely recovered in Pou4f3 conditional knockout mice 6 months after Pou4f3 deletion, however when VORs were tested in the dark, deficits again became apparent. Phalloidin labeling revealed decreased numbers of stereocilia bundles in the horizontal and anterior cristae of conditional knockout mice. The cristae in Pou4f3 conditional knockout mice also showed loss of hair cells in the peripheral and central regions.
Conclusions: The data suggest that Pou4f3 is not persistently maintained at high levels in all hair cells throughout life as was previously thought. Furthermore, the levels of POU4F3 in vestibular hair cells may decline as a function of aging, and loss of Pou4f3 was shown to cause bundle degeneration and hair cell death which are hallmarks of vestibular aging. Thus, the data support the hypothesis that Pou4f3 may be an important molecular target for age-related vestibular dysfunction and future directions aim to see if overexpression of Pou4f3 can mitigate age-induced vestibular degeneration.

Physiological and Histological Characterization of a Macaque Model of Presbycusis

Swarat Kulkarni*[1], Amy Stahl[1], David Pitchford[1], Leslie Liberman[2], M. Charles Liberman[2], Troy A Hackett[3], Ramnarayan Ramachandran[4]

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Background: Age-related hearing loss (ARHL), also known as presbycusis, is estimated to affect nearly two-thirds of the U.S. population over 70 with numbers expected to grow as the population increases. Despite ARHL prevalence and relevance, current human studies cannot separate this pathology from lifetime noise exposure. This confounding factor limits the ability to accurately characterize ARHL and develop targeted treatments or prevention strategies. Laboratory macaques have long lifespans and live in environments with controlled noise levels that do not cause hearing loss, making them optimal presbycusis models. Here we use the macaque model to characterize presbycusis-related changes through physiological and histological measures.
Methods: We used two clinical, non-invasive physiological measures in aging macaques (Macaca mulatta, 26-35 years, n=9, 3 female): distortion product otoacoustic emissions (DPOAEs) and auditory brainstem responses (ABRs). DPOAEs were measured by playing tone pairs (L1-L2: 10 dB, f2/f1 ratio: 1.22). ABRs to broadband clicks at different presentation rates (0.001-97 kHz; 100 μs duration; 27.7-200/s), macaque-specific chirps, and frequency-specific tone bursts (0.5 – 32 kHz; 27.7/s) that span much of the audible range of macaques. We measured thresholds and response amplitudes for all ABRs and DPOAEs at all frequencies tested, and latencies for ABRs. Macaques were euthanized after physiological procedures, and cochleae were extracted, perfused, and analyzed to estimate survival of inner and outer hair cells (IHCs and OHCs) and ribbon synapses.
Results: Histology revealed little OHC loss except at the highest frequencies. IHC loss was generally very minimal, except at the highest frequencies (≥16 kHz) in few animals. Further, ribbon synapse counts differences from younger animals were minimal, except at the highest frequencies and correlated with OHC/IHC survival. Physiology revealed more pronounced changes, where DPOAE responses showed higher thresholds for older monkeys at all frequencies and lower distortion produce (DP) amplitudes in older monkeys compared to young. Broadband chirp/click ABRs show decreased amplitude and increased latency for Waves I, II, and IV relative to young macaques, with Wave I changes reflecting functional deafferentation. Analysis of responses to clicks at different rates reveals wave II response amplitudes decrease in older monkeys with age. This decline was less steep than that found in younger macaques as the presentation rate increases, indicating age-related adaptation changes between young and old monkeys. Tone-specific stimuli analysis shows reduced response amplitudes and increases in wave II and IV latencies, which were more pronounced at higher frequencies (≥4 kHz).
Conclusions: The changes in physiological response measures were more pronounced than the histological changes, suggesting that structural deficits do not fully reflect the extent of the functional hearing changes. Future behavioral, transcriptomic, and CNS histological analyses will reveal how other areas of the auditory system play a role in functional deficits.
[Work Supported by NIH R01-DC-015988 and RF1 AG 060754]

Noise Induced Hidden Hearing Loss Accelerates Alzheimer's Disease Development and Progression

Tianying Zhai*[1], Chun Liang[1], Peng Zhe[1], Yong Kong[1], Hong-Bo Zhao[1]

[1]*Yale University Medical School*

Background: Alzheimer's disease (AD) is a common progressive neurodegenerative disease. Recently, its population is increased rapidly, and the age of disease onset also becomes early. However, the underlying mechanisms remain unclear. Hearing loss is considered as a high-risk factor for AD and AD-related dementia (ADRD) development and progression. Noise can induce hearing loss, in particular, hidden hearing loss. In this study, we investigated the effect of noise on the AD development and progression. Our results suggest that hidden hearing loss can accelerate AD/ADRD development and progression.
Methods: APP/PS1 and 5XFAD AD mice were used. Mice at age of 3-4 months old were exposed to white noise (~98 dB SPL) for 2 hr and one time. ABR, DPOAE, and acoustic-evoked cortical potential (AECP) were recorded to assess hearing function and brain activity. Behavioral test of acoustic startle response (ASR) was also recorded to assess dementia or cognition decline. The auditory cortex (AC), inferior colliculus (IC), cochlear nucleus (CN), and cochleae were also collected for the bulk Poly(A) RNA Sequencing analyses to assess the genomic changes.
Results: Both APP/PS1 and 5XFAD mice show early functional changes in the auditory system. The earliest changes could be found around 2-3 months old. After noise exposure at 3-4 months old, both APP/PS1 and 5XFAD mice had temporal threshold shift (TTS) and ribbon synapses were significantly reduced as shown by hidden hearing loss. The AECP recording showed that cognition associated wave P3 in AD mice with noise exposure had significantly reduction with aging. Further analysis of AECP waveform demonstrated that the thinking and cognition associated gamma waves were changed with aging. Consistent with results of AECP recording, the behavioral test measured by ASR demonstrated an accelerated decline in noise-exposed AD mice with aging; the decline-speed was 2-3 times faster than that in no-noise-exposed AD mice. Finally, RNA-Seq analysis confirmed this noise-induced acceleration in the noise-exposed AD mice. After noise exposure, AD-associated genes had early, more significant expressions and up-regulations than the control AD mice without noise exposure.
Conclusions: Noise is a high-risk factor and can accelerate AD/ADRD development and progression.

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Structural Integrity of the Auditory-Language Brain Networks Varies With Cognitive Status and Accounts for Speech-In-Noise Deficits in Older Adults

Gavin Bidelman*[1], Jack Stirn[1], Connor Shin[1], Elaina Lewis[2], Mengyuan Zhou[3], Rose Rizzi[1], Jessica MacLean[1]

[1]*Indiana University,* [2]*Indiana University School of Medicine,* [3]*Jacobs School of Music, Indiana University*

Background: Difficulties in speech-in-noise (SIN) comprehension are ubiquitous in the aging process. There is now growing evidence that declines in hearing function might also accelerate early cognitive decline in older listeners. Older adults' poorer SIN abilities are related to abnormal neural representations and functional transmission of information within the auditory-linguistic cortices, but how senescent changes alter the structural properties of this brain circuity remains underspecified.

Methods: Here, we examined diffusion weighted imaging (DWI), behavioral speech-in-noise processing, and cognitive abilities in a large cohort (N=626) of older adults from the Human Connectome Project Aging (HCP-A). Cognitive status was assessed via the Montreal Cognitive Assessment (MOCA) to delineate older adults with and without early cognitive impairment. Anatomical tractography was mapped from DWI scans using q-sampling reconstruction and deterministic fiber tracking of bilateral arcuate fasciculus (AF) and the acoustic radiation (AR) pathways.
Results: Behaviorally, we found advancing age was globally associated with poorer SIN scores but depended critically on cognitive status; age-related declines in SIN processing were more precipitous for low- compared to high-MOCA listeners. At the neural level, DWI tractography revealed larger streamline density of the AF and AR pathways in good vs. poor SIN perceivers. Paralleling behavior, links between weakened AF/AR structural integrity and SIN were also more prominent in older adults and especially those with cognitive decline.
Conclusions: These results reveal SIN difficulties in older listeners are associated with salient changes not only in canonical language (AF) but also primary auditory-sensory (AR) pathways of the brain. Our findings underscore important links between hearing and cognitive status in the context of aging and suggest deterioration in auditory-linguistic brain structures might account for the receptive speech communication deficits that are common later in life and which are accelerated by cognitive decline.

Poster Session IV

1:30 p.m. - 3:00 p.m.

Peninsula Ballroom

T1. Synaptic Transmission at the Endbulb of Held Deteriorates in MCU Knockout Mice

Guanyu Li*[1], Ruili Xie[1]

[1]*Ohio State University*

Category: Auditory Nerve
Background: Calcium mediates important biological processes including synaptic transmission. Specifically, presynaptic action potential activates calcium influx at the synaptic terminal and that triggers the release of synaptic vesicles. Free calcium ions are quickly removed from the terminal to stop synaptic transmission. One mechanism to remove calcium is through mitochondria reuptake via mitochondria calcium uniporter (MCU). However, it remains unclear how MCU contributes to synaptic transmission, especially under high rate activity. In the anteroventral cochlear nucleus (AVCN), bushy neurons receive auditory nerve input via large endbulb of Held synapses to faithfully transfer information to the brain. Iin our research, we investigated the effect of losing MCU on synaptic transmission at the endbulb of Held using MCU knock out mice and heterozygous littermates

Methods: Whole-cell patch clamp recording was performed from bushy cells of the AVCN in acute brain slices to characterize the synaptic properties of the AN input. Synaptic transmission was activated by stimulating auditory nerve at different rates. Target neurons were filled with fluorescent dye included in the electrode solution and preserved upon the completion of the recording for post hoc identification.
Results: We found that auditory nerve stimulation reliably evoked synchronous EPSCs throughout the stimulates train in both heterozygous control and MCU knock out at the low stimulation rate of 100 Hz. At 400 Hz, however, evoked EPSCs were more depressed in MCU knock out mice with significantly increased asynchronous release after the first 20 stimuli. The asynchronously released EPSCs continued for hundreds of ms even after stimulus train ended in MCU KO mice. Auditory brainstem response (ABR) recording showed prominent hearing loss in MCU knockout mice.
Conclusions: Our results suggest the calcium regulation by MCU is crucial in maintaining reliable synaptic transmission at the endbulb of Held synapses at high-rate activities. Defects in MCU function at auditory nerve synapses may be an important mechanism underlying hearing loss

T2. Electric Threshold and ECAP Measures of Neural Health Show Varying Trends in Patients Between Cochlear Implant Surgery and Initial Activation

Jennifer Anyanwu*[1], Holden Sanders[2], Lina Reiss[2]

[1]*Philadelphia College of Osteopathic Medicine,* [2]*Oregon Health and Science University*

Category: Auditory Nerve
Background: Significant variability remains in cochlear implant outcomes. One factor that has not been investigated is post-operative recovery of the auditory nerve after cochlear implantation. Two measures of neural health include behavioral thresholds and electrically-evoked compound action potential (ECAP) thresholds. Previously, it was shown in guinea pigs that behavioral and ECAP thresholds tended to initially worsen, then improve within 2-3 weeks after cochlear implantation (Pfingst et al., 2015). However, these animals were not given chronic electric stimulation; recent data suggest that chronic stimulation may interfere with threshold recovery (Reiss et al., 2024). To date, behavioral and ECAP thresholds have not been measured in human patients between surgery and initial activation (onset of chronic stimulation). The goal of this study was to monitor neural health after cochlear implant surgery and initial activation in human patients. We hypothesized that neural health, as measured by thresholds, would show similar recovery trends after surgery but would slow or worsen after initial activation.
Methods: Five adult patients (2 Cochlear, 3 Med-El; 2 females, 3 males; age range 45-76 years) were recruited before receiving CI surgery. Behavioral thresholds were measured psychophysically using direct stimulation in a 3-alternative forced choice, 2-up, 1-down adaptive procedure. ECAP amplitude growth functions were measured using clinical software, and ECAP thresholds were estimated at a fixed amplitude value. Measurements were conducted at 1 day, 3 days, and 1, 2, 3, and 4 weeks after surgery.
Results: Behavioral thresholds either dropped (improved; n=2) or showed a drop and then increased (worsening; n=3). Average drops from day 1 to week 4 were 15.2

± 39.7 μA, 17.1 ± 53.3 μA, 6.0 ± 54.3 μA, and 2.8 ± 57.2 μA for the most apical, apical-middle, middle, and basal electrodes (n=5). ECAP thresholds were not possible to obtain for all participants, but when recorded showed a general trend of drops over time (n=2).

Conclusions: As hypothesized, behavioral and ECAP thresholds showed overall drops after implantation, though some cases showed worsening, which may be related to initial activation. The observations are consistent with those seen in guinea pig models, particularly with chronic stimulation, and may indicate disruption of neural function due to an inflammatory reaction to the implant insertion and/or chronic stimulation. Future work will examine age and health conditions as potential predictors of neural health recovery after cochlear implantation

This study was funded by a NIH NIDCD grant R56DC018387 and NIH STEMM-HEAR grant R25DC020698.

T3. Intraoperative Extracochlear Electrically-Evoked Auditory Brainstem Response for Assessment of Cochlear Nerve Function in Translabyrinthine Vestibular Schwannoma Resection

Alena Pauley*[1], Benjamin Ostrander[1], Jonathan Dilgen[2], Peter Dixon[1], Marc Schwartz[1], Rick Friedman[1], Douglas Bennion[1]

[1]*University of California, San Deigo School of Medicine,* [2]*University of California, San Deigo Hospital*

Category: Auditory Nerve

Background: Intraoperative monitoring of acoustically-evoked auditory brainstem response (ABR) waveforms is used to clinically monitor acoustic function during removal of vestibular schwannomas (VS). In some VS patients, acoustically-evoked ABR cannot reliably be used intraoperatively, particularly in patients undergoing a translabyrinthine resection. Electrically-evoked ABR (eABR) recording is an alternative for monitoring cochlear nerve integrity and may be a predictor for hearing outcomes in patients who undergo concurrent cochlear implantation following translabyrinthine tumor removal.

Methods: Patients undergoing microsurgical VS resection were enrolled. During tumor removal, a stimulating electrode was applied to the cochlear nerve to obtain eABR recordings. These were correlated with hearing outcomes collected at cochlear implant activation.

Results: In a preliminary cohort, the first four patients of the planned 20 participants with baseline functional hearing underwent translabyrinthine craniotomy for tumor removal. Two patients demonstrated intraoperative eABR response at 0.2 mA at waveform intervals that coincided with Wave III and Wave V of the auditory-evoked eABR. The other two patients had no response at this level of stimulation. Subsequent cochlear implementation found both patients with detectable intraoperative eABR had more favorable initial audiologic outcomes, including sound awareness at all electrodes and consonant-nucleus-consonant (CNC) word understanding (8 and 32%, respectively). Of the two patients without discernible intraoperative eABRs, one was stimulable along two-thirds of the array and the other had no discernable sound awareness.

Conclusions: Extracochlear eABR may uniquely allow interrogation of cochlear nerve integrity intraoperatively and may be useful as a predictive mechanism for hearing outcomes in patients undergoing cochlear implantation concurrent with microsurgical resection for VS.

T4. Uncovering Neuronal and Glial Cell Diversity in the Human Spiral Ganglion to Advance Hearing Loss Therapies

Boaz Ehiogu*[1], Emilia Luca[2], Ryosuke Yamamoto[2], Alain Dabdoub[3]

[1]*University of Toronto,* [2]*Biological Sciences, Sunnybrook Research Institute,* [3]*Sunnybrook Research Institute/University of Toronto*

Category: Auditory Nerve

Background: Sound information detected by the inner ear is transmitted to the brain by primary auditory neurons (PANs). 90-95% of PANs are type I neurons, characterized as large, bipolar neurons that encode the auditory information we perceive as sound. The remaining 5% of PANs are small, pseudounipolar type II neurons. Type II neurons are hypothesized to play a role in damage and pain signalling. PANs reside in the spiral ganglion and are ensheathed by glial cells, which regulate neuronal function and survival. The cell bodies are enveloped by Satellite glial cells, whereas the axons and dendrites are enveloped by Schwann cells. Though damage to PANs leads to degeneration and irreversible hearing loss, glial cells survive. One promising strategy for restoring hearing involves reprogramming glial cells into functional neurons. However, to advance such strategies a comprehensive profile of PANs and glial cells in humans is necessary. Therefore, to characterize the molecular signatures of human PANs and associated glial cells, we performed single-nucleus RNA and ATAC sequencing.

Methods: The spiral ganglia were dissected from human fetal inner ears at gestational week 18 (n=3). A single nuclei suspension was generated and processed following 10X Genomics' single-nucleus multiomic workflow. A standard bioinformatic analysis was conducted to resolve the underlying cellular and molecular heterogeneity.

Results: A total of 16,943 nuclei were analyzed and 8 distinct clusters were identified. Gene annotation using cell-type specific markers resolved neuronal and glial populations. Subsequent sub-clustering analysis of neurons identified three distinct clusters, indicative of cellular heterogeneity. Type I and type II neurons were defined by the average gene expression levels of PROX1 and GATA3, respectively. Putative type I neurons comprised 92% nuclei whereas putative type II neurons comprised 8%, in accordance with the known cell-type proportions. Type I neurons were further subclustered into distinct type I subtypes. Clustering analysis divided glial cells into populations of cycling and non-cycling cells. Non-cycling glial cells were defined by a gene expression signature consistent with a subtype of Schwann cells.

Conclusions: Using single-nucleus RNA and ATAC sequencing we generated a comprehensive multiomic atlas of PANs and glial cells in humans. We observed novel neuronal and glial cell types characterized by unique molecular signatures. Through characterizing the transcriptomic and epigenomic landscape of PANs and glial cells we will elucidate the gene regulatory networks governing cell-type identity. Gene regulatory networks must be repressed or activated to effectively reprogram glial cells into neurons. Thus, our study will lay the foundation for the development of reprogramming strategies capable of regenerating PANs and restoring hearing.

T5. Spike-Rate Adaptation and Facilitation in Biophysical Models of the Electrically Stimulated Human Auditory Nerve

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Category: Auditory Nerve

Background: Cochlear implants (CIs) are highly effective in restoring hearing in deaf individuals to a remarkable degree. However, speech perception varies considerably between CI recipients and the reasons are yet to be clearly identified. Biophysical models are used to predict excitation patterns of the auditory nerve. While there are sophisticated models based on voltage-gated ion channels, these models fail to reproduce temporal dynamics such as facilitation and spike-rate adaptation. These effects shape the temporal excitation of the auditory nerve, even when the synapses are bypassed during electrical stimulation. We have developed models of the human inner ear that, for the first time, predict the temporal progression of neuronal excitation in the auditory nerve.

Methods: Detailed biophysical models of auditory nerve fibers with human morphologies were extended with biophysically inspired mechanisms that induce spike-rate adaptation and facilitation. We considered two mechanisms to model adaptation: slow inactivation of sodium channels and M-type channels. Persistent sodium currents were included to tune facilitation. The models were implemented as double-cable multicompartment neurons with stochastic Hodgkin and Huxley like ion channels. 400 nerve fibers were embedded in a finite element (FE) inner ear model based on µCT scans of human temporal bones. The FE model was used to calculate the current spread along each nerve fiber via a virtually inserted electrode array in the scala tympani. Electrically evoked compound action potentials (eCAPs) were reconstructed for pulse train stimuli to validate and fit with recorded eCAP trains from CI recipients.

Results: Simulation results with only standard voltage-gated ion channels showed refractory processes and were only consistent with recorded data for the first few milliseconds. By including adaptation and facilitation mechanisms, our models could reproduce human whole-nerve responses to pulse train stimulation consistently up to 100 ms. Good agreement was achieved for the predicted reduction of eCAP amplitudes at pulse rates of 500, 900, and 1800 pulses per second (pps). Including facilitation mechanisms resulted in accurate prediction of the alternating pattern of eCAP amplitudes at pulse rates between 1000 - 2000 pps, as observed in psychophysical experiments with CI recipients.

Conclusions: Our models successfully replicated spike-rate adaptation in the human auditory nerve during electric pulse-train stimulation. By incorporating adaptation and facilitation mechanisms, our simulations accurately reproduced the temporal progression of eCAP amplitudes in human CI recipients. This opens the possibility to study effects seen in psychophysical experiments such as pitch discrimination tasks, temporal loudness integration and stimulation with modulated speech signals. Our simulation results furthermore represent an initial step toward the development of personalized coding strategies by fitting model parameters to human data. This additionally provides insights into the state of the auditory nerve in CI recipients and how it influences the temporal dynamics.

T6. Ecap Simulation of Combined Cochlear and Vestibular Stimulation Employing Realistic Human Inner Ear Anatomy

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Category: Auditory Nerve
Background: The cochlea and vestibular organs often suffer from sensory cell dysfunction or loss that require electrical stimulation. Thus, research explores stimulation through combined cochlear and vestibular implants. In-silico models of the inner ear provide a valuable input for the development of these combined implants, as they offer the possibility to test multiple scenarios in advance of in-vivo experiments and patient implantation. Based on a recently presented modeling workflow for combined inner ear simulation of the cochlea and the vestibular system, we demonstrate the capability of that workflow to simulate realistic electrically evoked compound action potentials (ECAPs) and amplitude growth functions (AGFs) with varying electrode positions and simulation waveforms.
Methods: Nerve fiber trajectories for the inner ear nerves, including the cochlear and vestibular nerves, were generated based on our previously presented modeling workflow. Electrical activity of the neurons was described by two multi-compartment nerve models, for the cochlear nerve and the vestibular nerves, respectively. Employing the finite element method, extracellular electrical fields resulting from electrode contact stimulation were computed. Various scenarios involving electrodes in both systems were simulated allowing for detailed interpretation of possible position-specific differences. Adding to that, we computed electrically evoked compound action potentials to simulate the neural responses of the fibers. Treating transmembrane currents as distributed sources, we assessed resulting electrical fields at measuring electrode positions. Simulating for a course of 12 current levels as clinically applied, we computed the resulting AGF for varying stimulation waveforms. The AGF was calculated using artifact reduction schemes as proposed by the clinical usage and for potential comparison with patient data. Multiple positions of measuring and stimulating electrodes were evaluated, relying on a realistic implant geometry.
Results: The generated ECAPs from realistic simulation scenarios show a close alignment with literature and measured data from implanted humans. They do strongly depend on the positions of both active and measuring electrodes. While the selected measuring electrode shifts the amplitude of the ECAP, the specified active electrode alters the ECAP shape, as different fiber ranges are targeted with changing potential distributions. Analyzing the AGF of different scenarios, a large influence of electrode position and stimulating waveforms is shown.
Conclusions: The presented model facilitates the exploration of the influence of combined cochlear-vestibular stimulation on both the cochlea and vestibular system. This will enhance our comprehension of how electrode placements and stimulation scenarios affect targeted stimulation within the inner ear, as well as the reciprocal influence on recorded ECAPs when stimulating these organs. Moreover, this understanding could aid new diagnostic possibilities, as the model enables the investigation of distinctive impacts of pathological conditions on ECAPs.

Furthermore, this work lays the foundation for validating our model with data from patients implanted with combined cochlear/vestibular implants in the future.

T7. Accuracy and Efficiency of a Swept Modulation Depth Stimulus for Cross-Species Neurometric Physiological Analyses

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Category: Auditory Nerve

Background: Despite advancements in hearing-aid technology, understanding speech in noise remains a significant challenge for hearing-aid users. Understanding the neural mechanisms underlying speech perception in noise, and how these mechanisms are degraded with hearing loss, is crucial for improving hearing-aid efficacy. Amplitude modulation (AM) plays an essential role in speech, and previous studies have demonstrated a relationship between modulation detection and speech intelligibility. Physiological studies have also shown that sensorineural hearing loss impacts modulation detection, particularly in noisy environments. Neurometric analyses can be used to estimate modulation detection in physiological studies by quantifying the discriminability of modulation depth based on neural coding statistics. However, estimating modulation detection through physiological recordings involves repeating measurements for each modulation depth and comparing to a reference (unmodulated tone). This process is time-consuming and poses challenges, especially in neurophysiological recordings from a single auditory-nerve (AN) fiber. While this measurement results in a unique and valuable dataset for studying the neural mechanisms underlying modulation coding, the measurement is constrained by time as the fiber may be lost before the modulation depth threshold can be determined. Similarly in human studies, long behavioral experiment durations introduce subject fatigue and reduced attention, while lower signal-to-noise ratios (SNR) in human electrophysiological measurements such as envelope following response (EFRs) and EEG require more repetitions, further extending the duration of these experiments.

Methods: We propose using a swept modulation depth stimulus, where the modulation depth changes continuously over time while keeping the root mean square of the signal constant. This stimulus offers an alternative method for studying modulation coding and determining modulation depth thresholds. Spiking data from computational models (Zilany et al., 2023 [without efferent] and Farhadi et al., 2023 [with MOC efferent]) are used to analyze phase-locking to the temporal envelope of AM stimuli. The effects of modulation frequency, direction of the sweep (upward or downward), and sound level are examined. This stimulus will be evaluated for application in physiological recordings, including AN-fiber recordings (animal models), EEG, and EFR in both human and animal studies.

Results: Preliminary results from EFRs recording in chinchilla and computational modeling confirm that a swept modulation depth stimulus can be both efficient and accurate for physiological recordings, particularly in AN-fiber single-unit recordings as time for measuring data from each fiber is limited and variable across fibers and specifically degraded in animals with hearing loss.

Conclusions: The swept modulation depth stimulus has the potential to improve amplitude-modulation detection methods in a range of physiological studies. Further research is needed to

evaluate the practical application of this stimulus in cross-species experimental setups. Additionally, the effects of efferent activity should be explored through computational modeling and physiological methods.
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T8. Effects of Lifetime Noise Exposure on Auditory Brainstem Response Morphology

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Category: Auditory Nerve
Background: This study aims to investigate the relationship between lifetime noise exposure and the morphology of the auditory brainstem response (ABR), with a particular focus on identifying subclinical hearing damage not detected by standard audiometric thresholds. Audiometry may fail to detect hearing difficulties caused by cochlear synaptopathy or auditory nerve pathology. Some studies have found a correlation between ABR wave I peak amplitude and lifetime noise exposure in humans; however, this relationship has not been consistently observed. The present study seeks to expand on current literature by utilizing a modeling technique to extract additional data from the ABR that may be associated with high levels of lifetime noise exposure, potentially enhancing the sensitivity of diagnostic measures for hearing loss.
Methods: ABRs were measured on 111 participants with audiometric thresholds ≤ 25 dB HL, and noise exposure was quantified using the Lifetime Exposure to Noise and Solvents Questionnaire (LENS-Q). A Gaussian mixture model feature extraction technique (GMM-FET) was employed to analyze the morphology of the ABR waveforms, going beyond traditional measures of amplitude and latency, to include curvature, area, and width of the waves. The study explores the relationships between model-extracted ABR wave metrics and lifetime noise exposure, accounting for the influence of sex, age, type of noise exposure (steady-state versus impulse), and tinnitus.
Results: The application of the GMM-FET provides a novel approach to analyzing ABR waveform morphology, allowing for the discovery of previously unidentifiable patterns. By examining these patterns in relation to lifetime noise exposure, this method offers a new perspective on the potential impact of noise exposure on auditory function.
Conclusions: The findings of this study highlight the potential of advanced mathematical modeling techniques, such as the GMM-FET, to reveal subtle auditory changes that traditional clinical tests and visual analysis of the ABR may miss. Given the ABR's potential ability to pinpoint site-of-lesion along the auditory pathway, these insights could further our understanding of how noise exposure affects the auditory pathway and pave the way for more refined diagnostic methods and targeted treatment options.

T9. OPEN BOARD

T10. Characterizing Medial Olivocochlear (MOC)-Mediated Enhancement and its Dependence on Auditory and MOC Stimulation Parameters

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Category: Brainstem: Structure & Function

Background: Medial olivocochlear (MOC) neurons, originating in the superior olivary complex, travel across the brainstem and out cranial nerve VIII to form synapses on outer hair cells (OHCs) along the cochlear coil. MOC activation, as measured by distortion product otoacoustic emissions (DPOAEs), leads to both suppression and enhancement of cochlear activity by presumably adjusting the OHC's role in sound amplification. MOC-mediated suppression occurs with a 100-ms onset time constant and promptly returns to baseline following stimulus termination, while MOC-mediated enhancement is much slower in its onset and offset kinetics. While MOC-mediated suppression is attributed to the release of ACh and the activation of postsynaptic α9α10 nicotinic ACh receptors (α9α10nAChRs) on OHCs, the neurotransmitter and downstream mechanisms underlying MOC-mediated enhancement remain unknown. Consequently, there are significant gaps in our understanding of how MOC-mediated enhancement is achieved and the specific mechanisms that underlie this process. To facilitate a better understanding of MOC-mediated enhancement, we conducted a systematic evaluation of MOC-mediated enhancement while varying auditory and MOC stimulation stimulus parameters.
Methods: To evaluate the effects of auditory and MOC stimulation stimulus parameters on MOC-mediated enhancement, we recorded DPOAEs in urethane/xylazine-anesthetized C57Bl/6 mice before, during, and after stimulating MOC neurons in the 4th ventricle. DPOAE measurements and stimulus generation were done with Tucker-Davis System hardware and custom software, using F1 and F2 frequencies (10 and 12 kHz, respectively) delivered to the right ear via Etymotic ER2 earphones and recorded with an ER-10B+ microphone. To study the MOC-mediated enhancement in isolation, selective α9α10 nAChRs antagonists were administered intraperitoneally to ablate MOC-mediated suppression. We systematically varied MOC shock train frequency (1 – 200 Hz), shock train duration (5 – 70 seconds), or F1 DPOAE probe levels (5 – 30 dB above threshold values).
Results: Significant MOC-mediated suppression was observed at shock frequencies between 50 - 200 Hz with the largest mean peak amplitude seen at 100-Hz. In contrast, MOC-mediated enhancement was first observed at shock train frequencies as low as 10 Hz with peak amplitudes seen at 100 – 200 Hz. At a fixed shock train frequency of 200 Hz, MOC-mediated enhancement of DPOAEs was observed with shock train durations as low as 5-seconds and appearing to reach its maximum amplitude at 20-second shock train durations. The magnitude of the isolated MOC-mediated enhancement gradually decreased as the level of the F1 DPOAE probe level increased. Finally, a second, but much slower, MOC-mediated enhancement was also observed, but it's unclear whether it utilizes similar signaling mechanisms.
Conclusions: Our study has further characterized MOC-mediated enhancement and its dependence on the frequency, duration, and intensity of MOC stimulation and/or auditory input. Observations of a second, slower form of enhancement warrant further investigation. Work is ongoing to identify the synaptic mechanisms driving MOC-mediated enhancement.

T11. Development of a Rapid Auditory Brainstem Response Threshold Estimation Algorithm for Human Audiometry: A Simulation Experiment of Suprathreshold Sampling Strategies for Accuracy and Efficiency

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Category: Brainstem: Structure & Function

Background: The auditory brainstem response (ABR) is a standard procedure for evaluating hearing sensitivity of humans and animals who are unable to undergo behavioral audiometry. During an ABR, a stimulus is played and the evoked potential is recorded. An ABR threshold, the lowest level for which a discernible ABR waveform is observed, can be mapped across stimulation frequencies. However, the considerable measurement time required for ABRs presents a barrier in both pediatric clinics and laboratories that use animal models. Previously, we developed an adaptive ABR threshold estimation algorithm that reduced testing time by a factor of 3-5. In the algorithm, interim thresholds are defined by the lowest stimulus level that elicits a minimum peak-to-peak (p2p) response, below which a peak cannot be resolved from the noise. The stimulus (frequency and level) were chosen adaptively to sample along interim estimates of the threshold, under the premise that sampling in the region of interest minimizes model uncertainty, thereby increasing threshold estimation accuracy. Here, we investigate the effect of different sampling strategies that utilize suprathreshold levels, i.e., 0, 5 and 10 dB above the interim threshold estimate. Further, we test the three sampling strategies using two p2p threshold criteria, 0.01 and 0.02 mV. In total, 6 configurations are tested.

Methods: The sampling strategies are evaluated using the Intelligent Hearing Systems SmartVS ABR simulation package, which is designed to simulate realistic ABR sessions including realistic measurement noise and artifacts. The algorithm estimates thresholds for patients with a variety of hearing profiles. For each configuration, the algorithm estimated thresholds are compared against ground truth thresholds. The performance of the algorithm is evaluated using the intraclass correlation coefficient (ICC). Further, the algorithm is evaluated by its rate of convergence towards the ground truth, variability across patients, and stability of estimate as more data is collected.

Results: All sampling strategies and p2p criteria performed well. Sampling strategies using 0, 5, and 10 dB over threshold have ICC = 0.93, 0.95, 0.94 and ICC = 0.93, 0.95, 0.97 for p2p = 0.01 mV and 0.02 mV, respectively. In both p2p cases, sampling above threshold provides more accurate results than sampling at threshold. Not only does sampling above threshold provide more accurate estimates, it also provides greater stability of threshold estimation as more data is accumulated.

Conclusions: The intuitive 'sample in the parameter space that is of most interest (i.e., at threshold)' is not the best strategy for this algorithm. Sampling strategies that emphasize near-threshold data end up training the model with primarily noise. Sampling just above threshold to achieve a higher signal to noise ratio, and extrapolating down to threshold, appears to be a better strategy.

T12. Sex Differences in the Auditory Processing of Musical Sounds as Revealed With the Frequency Following Response

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Category: Brainstem: Structure & Function
Background: Male and female young adults differ in the timing and amplitude of their Frequency Following Response (FFR) to complex sounds. These sex differences in auditory processing arise in adolescence, suggesting a hormonal mechanism that preserves the timing and amplitude of brain responses in females. Previous experiments investigating sex differences in speech-evoked FFRs from young adults have demonstrated shorter onset latencies in female responses, as well as stronger encoding of high-frequency harmonic information in the female temporal fine structure response. The current study investigates whether similar sex differences can be found in FFRs to a wide range of musical notes.
Methods: Frequency Following Responses from 45 participants (28 female) between the ages of 18-25 were collected using 36 200-ms musical note stimuli sampled from octaves 2-5 of a Rhodes Electric Piano. Each note is a complex synthesized tone with a fundamental frequency (F0) ranging from 65.41 to 493.88 Hz. All participants had normal hearing.
Results: Female participants demonstrated faster response onset timing than male participants. Females also had greater phase consistency, a measure of the consistency of frequency-specific timing in the response, than males at first harmonic frequencies. We did not observe a significant main effect of sex in response amplitudes at either fundamental frequencies or first harmonic frequencies across all notes. There was no sex difference in cross-trial consistency of response morphology or broadband signal-to-noise ratio.
Conclusions: The sex differences that we observed in musical note-evoked FFRs are closely aligned with those observed in previous investigations of sex differences in click ABRs and speech-evoked FFRs and indicate faster, more stable processing of sound in the brainstem and midbrain in females than males. These results support the idea that sex differences in auditory processing reflect more than physical sex differences (e.g. head size), and may be a product of sex hormones acting differently on the auditory system of females and males starting in adolescence. In addition, the timing consistency effect observed here further separates biological effects on auditory processing from experiential effects on auditory processing and should be considered in future research on sex, musicianship, language experience, or other experience-induced effects that prompt changes in sound encoding as measured by the FFR.

T13. Neural Circuitry Mapping of Oxytocin

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Category: Brainstem: Structure & Function
Background: Oxytocin (OT) is a neuropeptide that is partially synthesized in the paraventricular nucleus (PVN) with both endocrine and neural effects. In the endocrine system, OT is secreted into the blood stream and aids in several biological processes (i.e. reproduction). As a neurotransmitter, OT influences many social behaviors. Our work has shown that OT is present in the auditory brainstem – specifically the medial nucleus of the trapezoid body (MNTB), an

area of the brain important for sound localization. Prairie voles are often used to study social behavior due to their ability to pair bond and exhibition of biparental behavior, which are partially facilitated by OT. We hypothesize that OT in the auditory brainstem (MNTB) aids in sound localization of important conspecifics (such as opposite-sex mates) and therefore helps establish and maintain pair bonds. We predict that OT in the MNTB originates from the PVN.
Methods: To determine the connection between where OT is synthesized (the PVN) and the MNTB, we injected two types of neural tracers. First, retro dye beads were injected, in vivo, into the MNTB of anesthetized prairie voles to trace if the origin of OT in the MNTB is the PVN. After 2-3 days of incubation of the retro beads, brains were taken and stained via immunohistochemistry. Next, we will inject an OT-specific virus into the PVN and search for a connection to the MNTB.
Results: The results will be presented pending the results of the injections.
Conclusions: These objectives will aid in determining where OT in the auditory brainstem originates, providing insight into its function in the auditory brainstem.

T14. Functional Characterization of Non-Calyceal Inputs in the Medial Nucleus of the Trapezoid Body

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Category: Brainstem: Structure & Function
Background: Auditory processing requires temporal precise integration along with reliable supra-threshold output formation. Neurons of the medial nucleus of the trapezoid body (MNTB) possess these features to ensure rapid and precise feed-forward inhibition to various auditory integration centers. This temporal precise neuronal transmission is partially based on the large, somatic synapse, the calyx of Held. Under resting conditions, this highly specialized synapse engenders faithful and temporal precise one-to-one action potential (AP) transfer. However, during ongoing high-frequency activity, the temporal precision and fidelity deteriorate. Next to the large calyceal inputs, small excitatory non-calyceal (NC) inputs innervate the soma and dendrites of the MNTB neurons. Besides the excitatory nature of these NC inputs, their synaptic physiology and functionality remain largely unknown. In this study, we characterize NC inputs and elucidate how they support and modulate AP initiation generated by the calyx of Held and thereby promote fidelity and precision in auditory processing.
Methods: Whole-cell recordings from MNTB neurons in actuate brain slices were conducted. We stimulated afferent fibers at different frequencies and pulse numbers to characterize synaptic transmission and short-term plasticity of NC and calyceal inputs. External calcium concentration was elevated, to gain insights into vesicle dynamics of NC inputs. From these data, we derived the EPSC kinetics, short-term plasticity, recovery from depletion, and amount of asynchronous release. To probe the functional relevance of NC inputs to AP precision and success, we paired conductance templates of scaled calyceal and NC inputs under dynamic clamp conditions.
Results: Compared to calyceal EPSCs, the EPSCs of NC inputs are ~30x smaller and show more variability in decay time (up to 8x slower). In elevated extracellular [Ca2+] (2.5 mM) the vesicle pool could be partially depleted and recovered bi-exponentially with time constants comparable to the calyx of Held. Under physiological extracellular [Ca2+] (1.2 mM) NC inputs showed

robust frequency-dependent facilitation. During train stimulations, asynchronous release increased. After the stimulation, the decay time of the occurrence of asynchronous release depended on the stimulation frequency. AP generation, triggered by simulated calyx conductance templates, showed a frequency-dependent reduction in success rate and a deterioration of temporal precision. Simultaneous application of NC conductance templates, increased calyx evoked AP success rates and improved the temporal precision up to 50 µs. This improvement depended on the conductance size of the applied NC template.
Conclusions: Our findings demonstrate that NC inputs can promote the fidelity and temporal precision of calyx-generated APs under ongoing activity. Moreover, it indicates that MNTB neurons integrate information from different sources to modulate the timing and fidelity of their output. Thus, the NC inputs play a critical role in auditory information processing.

T15. Neuromodulation in the Descending Auditory System: Mechanisms Underlying Serotoninergic Excitation of Medial Olivocochlear Efferent Neurons

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Category: Brainstem: Structure & Function
Background: In the auditory system, the sensitivity of afferent neurons (i.e. from ear to brain) are directly modulated by efferent neurons (i.e. from brain to ear). Medial olivocochlear efferent neurons (MOCs), located in the auditory brainstem, decrease cochlear amplification by inhibiting outer hair cell (OHC) electromotility. Given the proposed role of MOCs in context dependent tasks such as selective attention, signal extraction from noise and protection from acoustic trauma, we are investigating the full complement of non-auditory input onto MOCs. Serotonin (5-hydroxytryptamine; 5-HT) has been identified as a potential neuromodulator in central auditory circuits, and existing literature suggests MOCs express multiple postsynaptic 5-HT receptor subtypes (Koyama et al, 2017; Frank et al., 2023). Serotonin has been implicated in protection from noise overexposure (Ohata et al., 2021), however, it is unclear if MOCs receive direct serotonergic synaptic input, and how such input may affect their excitability.
Methods: ChAT-IRES-Cre;tdTomato mice were used to genetically identify cholinergic MOC neurons. Anatomical investigations consisted of retrograde tracer injections into the cochlea and/or immunohistochemistry for markers of 5-HT. In-vitro patch clamping from brainstem slices was combined with exogenous application of 100µM 5-HT to characterize serotonergic responses in MOC neurons. Additional pharmacological experiments interrogating potential downstream mediators of serotonergic signaling were performed using ZD7288 to block hyperpolarization-activated cyclic nucleotide-gated (HCN) channels (50µM; mechanistically implicated in 5-HT signaling elsewhere in the auditory brainstem), apamin to block small conductance calcium-activated potassium (SK2) channels (100nM; expressed in MOC terminals at the OHC synapse), and guangxitoxin-1E to block Kv2 channels (100nM; contribute to action potential afterhyperpolarization).
Results: Histological data validated the existence of serotonergic terminals in close apposition to both retrogradely-labeled and genetically-identified MOC neurons in mouse. In patch-clamp

experiments, 5-HT increased MOC excitability, but the magnitude of the effect varied. Serotonin significantly decreased action potential (AP) threshold, and increased rate of AP firing in response to 100pA of injected current. Pharmacological experiments demonstrated that MOCs do indeed express functional HCN, SK2 and Kv2 channels. Blocking HCN channels with ZD7288 or Kv2 channels with guanxitoxin-1E did not affect AP firing rate, while blocking SK2 channels with apamin did. Preliminary data analyses suggest that 5-HT mediated increases in excitation were not affected when HCN channels were blocked by ZD7288, nor when Kv2 channels were blocked by guangxitoxin-1E. In the presence of apamin, however, 5-HT did not result in further increases in firing rate, suggesting a potential role of SK2 channels in 5-HT mediated excitability.
Conclusions: Serotonin modulates MOC neuron excitability in-vitro. Current pharmacology experiments are focused on determining the complement of channels controlling intrinsic activity in MOC neurons while simultaneously teasing out the mechanism of serotonergic action underlying the increase in MOC neuron excitability.

T16. Cochlear Amplification Modulates Synaptic Transmission at the Endbulb of Held Synapse in the Cochlear Nucleus

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Category: Brainstem: Structure & Function
Background: In the cochlea, outer hair cells push and pull the basilar membrane, dramatically amplifying its vibration, and therefore greatly expand the dynamic range of hearing. But how neurons and synapses in the central nervous system cope with this cochlear amplification and expanded dynamic range is poorly understood.
Methods: We took advantage of a mouse line (Prestin-/-) where prestin, the motor protein in outer hair cells, was genetically knocked out, therefore removing cochlear amplification completely without changing the cellular structure of the cochlea significantly. Firstly, we recorded auditory brainstem responses (ABRs) of both WT and Prestin-/- mice and evaluated their hearing performance. Secondly, we conducted patch-clamp recording in whole-mounted cochleae and investigated functions of inner and outer hair cells. Lastly, we performed patch-clamp recording in bushy cells in brainstem slices and examined the excitability of bushy cells in the cochlear nucleus and synaptic transmission in the endbulb of Held synapse.
Results: As expected, we found greatly elevated ABR thresholds in Prestin-/- mice (40 ~ 60 dB), with reduced amplitudes and increased latencies in Wave I of ABRs. In the cochlea, both outer and inner hair cells became smaller based on their whole-cell capacitance, non-linear capacitance in outer hair cells was completely removed, but exocytosis from inner hair cells remained unchanged. In the cochlear nucleus, bushy cells exhibited a slightly more depolarized resting membrane potential (3.29 mV), and an increased input resistance (67.4%), along with a smaller and shorter after hyperpolarization following spikes, all of which indicate increased excitability in Prestin-/- bushy cells. With auditory nerve stimulation, we found that Prestin-/- auditory fibers were more excitable in that the stimulation voltage required to evoke EPSCs successfully in Prestin-/- bushy cells was only 55.9 % of that for their WT counterparts. Furthermore, we found that the amplitude of evoked EPSCs was reduced by 22.2%, and their decay time constant

became shorter (0.878 vs 0.770 ms), while neither the amplitude nor the frequency of spontaneous EPSCs was significantly changed. For paired stimulation, we found that the EPSC ratio went from depression in WT bushy cells (0.769) to facilitation in their Prestin-/- counterparts (1.11). With 50 stimulations at 100 Hz, we found a smaller readily releasable pool (RRP) of synaptic vesicles (391 vs 248 vs), a quicker replenishment of RRP (54.0% vs 76.2% at 500 ms), and a reduced sustained release rate of synaptic vesicles (1881 vs 971 vs/s) in Prestin-/- bushy cells.
Conclusions: Taken together, we found that cochlear amplification causes significant and multifaceted changes in excitability of bushy cells and synaptic transmission in the endbulb of Held synapses, and these changes are likely part of mechanisms allowing central neural circuit to better serve expanded dynamic range of hearing.

T17. Inhibitory Interneurons in the Mouse Primary Auditory Cortex Drive Contrast Adaptation

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Category: Primary Auditory Cortex
Background: In natural environments, important auditory stimuli like vocalizations or predator noises are often obscured by background noise. In order to detect and identify relevant sounds, the auditory system must separate stimuli from background noise. This ability is frequently impacted in people with hearing impairments, and investigating its mechanisms is vital to developing effective treatments for hearing impairments.
One strategy for isolating signal from noise is neuronal adaptation to the statistics of the current auditory environment. In the central auditory system, neurons adapt to a reduction in sound pressure level variability (contrast) by increasing their response gain. This adaptation is especially pronounced in the primary auditory cortex (A1). Though multiple mechanisms have been proposed, the mechanisms of cortical contrast gain control are still unknown. Two genetically distinct classes of inhibitory interneurons, parvalbumin-positive (PV) and somatostatin-positive (SST) neurons, which differentially control auditory temporal adaptation and can modulate excitatory neuron gain, are potential candidates. In this study, our goal was to test the role of PVs and SSTs in auditory contrast adaptation and perception of sound in noise.

Methods: In both mice and humans, higher neuronal gain in low-contrast environments corresponds to an increased perceptual sensitivity to small changes in sound pressure level. To test whether A1 interneurons drive these contrast-dependent behavioral changes, we trained mice on a go/nogo task to detect targets presented in backgrounds of varying contrast. We presented targets at varying time-points following a contrast change in order to assess behavioral sensitivity at different adaptation timepoints.
Second, in order to assess how contrast impacts inhibitory input, we performed acute extracellular recordings in A1 of passively-listening mice, using optogenetic labeling to identify PV and SST units.

Results: As predicted by neuronal gain adaptation, behavioral sensitivity increases gradually after the transition from high-contrast to low-contrast background. However, optogenetically inactivating SSTs in A1 abolishes this change, indicating that SSTs are necessary for contrast-dependent changes in behavioral sensitivity. We are currently collecting data during PV inactivation.
Optotagging reveals that PVs and SSTs differentially encode stimulus contrast; PVs consistently encode high contrast with an increased average sustained firing rate, and respond to increases and decreases in contrast with large transient increases and decreases in firing rate, respectively. In contrast, while individual SSTs can reliably encode stimulus contrast, their responses are much more heterogeneous.

Conclusions: Our results demonstrate the involvement of A1 SST interneurons in contrast adaptation. In the future, we will directly investigate whether there is a causal relationship between PV and SST activity and neuronal contrast gain adaptation by optogenetically manipulating PVs and SSTs during acute extracellular recordings. Due to the importance of adaptation to hearing in noisy environments, out work highlights selective inhibitory mechanisms as possible targets when developing new treatments for hearing deficits.

T18. The Influence of Stimulus Duration on the Acoustic Change Complex in Normal-Hearing Adults

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Category: Primary Auditory Cortex
Background: The acoustic change complex (ACC) is a neural response elicited by a change in an ongoing sound recorded using electroencephalography (EEG). In a previous study, our group investigated ACCs in normal-hearing and hearing-impaired subjects and found a significant and robust correlation between speech perception scores expressed by speech reception threshold (SRT) and ACC outcome measures ($r^2 = 0.87$). Thus, the ACC has the potential to be used as an objective measure to assess auditory performance in patients who cannot reliably perform speech perception tests, particularly due to limitations in language proficiency and cognitive capabilities. The sound stimuli used in our previous study had a duration of 3300 ms. If shorter stimuli yield similar outcomes, the ACC recording can be shortened, making the test more appealing for clinical applications in diagnosing hearing impairment.
Methods: A cohort of 20 participants (10 women) with normal hearing were enrolled in this observational study. Auditory stimuli to evoke ACCs consisted of a 1000 Hz base frequency and a 12% frequency modulation sweep. Varying stimulus durations of this composite stimulus were presented in a randomized order: 1300 (1000+300 ms), 1800 (1500+300 ms), 2300 (2000+300 ms), 3300 (3000+300 ms), 4300 (4000+300 ms), and 5300 ms (5000+300 ms) with 300 ms referring to the duration of the frequency change. Prior to the ACC recordings, pure-tone audiometry was conducted to confirm normal hearing thresholds. The primary objective of this study was to investigate whether shorter acoustic change stimuli than 3300 ms evoke ACC responses with comparable amplitudes and latencies. Additionally, the study aimed to determine

the point at which the ACC amplitude reaches its maximum level by using the 4300 and 5300 ms stimuli. Linear mixed models were constructed to investigate the effect of stimulus duration on the ACC amplitudes and latencies.
Results: The ACC amplitude increases with stimulus duration which is caused by an increasing N1 amplitude (p LESS THAN 0.0001) while the ACC latency does not correlate with stimulus duration. Coincidentally, we found that female participants showed higher amplitudes (p = 0.0015) and shorter latencies compared to male participants (p = 0.0026).
Conclusions: Longer stimulus durations lead to higher ACC amplitudes. Based on our results, the 3300 ms stimulus duration is recommended as the optimal stimulus duration. Coincidentally, we found that women show shorter ACC latencies and higher amplitudes compared to men.

T19. Fast Transmission between Vestibular Type I Hair Cells and Their Calyceal Afferents in Mice

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Category: Hair Cells: Anatomy & Physiology
Background: Recent studies have shown that much of the transmission between the Type I hair cells (HCs) and their surrounding calyces occurs via non-quantal mechanisms, which are dependent on both pre-and post-synaptic ion channels. The non-quantal transmission exhibits both fast and slow components. In this study, the fast component of the non-quantal transmission was investigated using paired recordings from the mouse semicircular canal crista. The recordings revealed fast, bi-directional electrical coupling between the two cells. In this mode of non-quantal signaling, cleft-facing conductances in both the HC and calyx increase, enhancing their electrical coupling. This form of communication, relying on cleft resistance, is referred to as "resistive coupling."
Methods: Dual whole-cell recordings were obtained from Type I HCs and their calyces in the central and peripheral zones of the epithelia. The recording pipettes were filled with a mixed anionic solution, KF: KCl, 118:12 mM. Additionally, 50 μM Alexa Fluor 488 or 568 were added to the Type I HC and calyx. One electrode was positioned at the apical neck of the Type I HC, while the second electrode was placed at the base of the calyx, near its junction with the parent afferent. Type I HC and calyx afferent were examined in voltage clamp.
Results: Hyperpolarizing and depolarizing commands were applied to Type I HCs to examine net HC currents and their effects on the calyx, which was held at a constant potential of -100 mV. Larger depolarization resulted in increased outward HC currents. During the HC voltage command steps, calyx responses showed a proportional increase in the inward steady-state (SS) amplitude, corresponding to the outward HC current. Despite being held at a constant potential, the calyx current exhibited an instantaneous decrease at the conclusion of the HC step, followed by a return to baseline with a multi-component tail current. This instantaneous change represents a fast component of bidirectional HC-calyx coupling due to synaptic cleft resistance. To investigate if this was due to open conductances in Type I HCs, 4-AP+ (15mM) and TEA (30mM) blockers were added to the Type I HCs internal solution. Blocking Type I HCs with 4-AP+ reduced the outward current in the HC, leading to a smaller associated current in the calyx.

The addition of TEA to 4-AP+, aimed at fully blocking potassium currents in Type I HCs, led to a complete blockage of inward currents in the calyx, including the fast inward current.
Conclusions: In the mouse crista, fast transmission between the HC and calyx is driven by Type I HC potassium currents, resulting in changes in driving force across open conductances in the HC and calyx. The fast response may be involved in the vibration and movement mechanisms of the vestibular system.
Supported by NIH, NIDCD grant R21DC017577

T20. Calcium Imaging of Mechanically Evoked Hair Cell and Afferent Population Activities in Mammalian Vestibular Inner Ear

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Category: Hair Cells: Anatomy & Physiology
Background: We image Ca2+ activity in populations of utricular hair cells and afferents in order to correlate moment-to-moment activity within and between distinct physiological zones of the epithelium. The utricle provides information about head tilt and linear head motions to the brainstem and cerebellum, facilitating rapid reflexes for maintaining stable visual fields, balance, and posture. Hair cell (HC) studies have shown major differences between HC and synaptic types, while in vivo afferent studies show distinct response properties that correlate with zone in the sensory epithelium and with spike regularity. Highly irregular afferents are more adapting and innervate the central zone (striola); highly regular afferents are more tonic and innervate the peripheral extrastriola. While single-unit recordings have shown that irregular and regular afferents differ strongly in average response properties, they do not directly show moment-to-moment correlations of afferent activity. To address this information gap, we are using two-photon microscopy to correlate the Ca2+ signals of selected sets of vestibular hair cells and afferents during a common fluid-jet stimulus.
Methods: Our preparation is the excised sensory epithelium and attached afferent nerve of the mouse utricle, for which there are whole-cell recordings of hair cell and afferent responses to mechanical stimulation. We remove overlying structures to expose the mechanosensitive hair bundles, then apply a fluid jet driven with voltage waveforms for steps, sinusoids (0.5-20 Hz), or head motions as recorded from mice (courtesy KE Cullen). The evoked hair- bundle motions are viewed from above, captured in Dodt or DIC movies, and tracked with a custom algorithm. In each preparation, hair cells or afferents express the Ca2+ indicator (GCaMP 6s or 8m). Ca2+ signals (dF/F) are correlated directly or after deconvolution to generate “inferred activity”.
Results: We assume that hair cell receptor potentials (hair cells) and afferent postsynaptic potentials and spikes modulate voltage-gated Ca2+ currents that drive the Ca2+ indicator signals (Kabirova et al., this meeting). Strategic choice of regions of interest (ROIs) allows us to correlate activities within and across zones, between bundle polarities, and between hair cell types, in order to isolate the significance of each of these factors in shaping response activity. In preliminary results, correlated activity decreases with distance within a zone; activities are uncorrelated across the reversal line of bundle polarity (LPR); and within zones (on 1 side of the LPR), correlations are insensitive to small variations in bundle orientation (LESS THAN 20

degrees), consistent with the known cosine dependence of hair cell sensitivity to stimulus angle re: bundle orientation.
Conclusions: These data will provide input to a population model we are developing on how sensory information is represented across the mammalian vestibular epithelium (Lutz et al., this meeting). Funding: R01DC018304 (CKL), R01DC012347 (CKL, MK, OJL, DS, RAE), F31DC021883(OJL), CBC-AG-002 (MK), TPCN 1R90DA060338 (DS)

T21. Hair Cell Synaptic Dysfunction of Otof p.R1939Q Knock-In Mouse

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Category: Hair Cells: Anatomy & Physiology
Background: Otoferlin is a multi-C2 domain protein that is involved in synaptic vesicle fusion and vesicle pool replenishment in the inner hair cells (IHCs). Pathogenic variants in OTOF gene are often associated with human auditory neuropathy spectrum disorder (ANSD). OTOF p.R1939Q variant is the most prevalent mutant allele among Korean patients with ANSD. Here, we investigated the potential impact of this otoferlin point mutation on hair cell synapse and hearing.
Methods: A mouse knock-in model with p.R1934Q variant (Otof+/+, Otof+/pR1934Q, Otofp.R1934Q/p.R1934Q) at the orthologous mouse Otof locus was generated and excised cochlear tissues were subjected to whole cell patch clamp recordings. Despite the profound hearing deficit in Otofp.R1934Q/p.R1934Q, calcium influx into the IHCs of Otofp.R1934Q/p.R1934Q appeared intact. No significant difference in the voltage-dependence and the peak amplitude of IHC calcium current was observed among Otof+/+, Otof+/pR1934Q, and Otofp.R1934Q/p.R1934Q. In contrast, synaptic release from IHC, measured at the afferent bouton endings abutting IHC, differed according to the genotype. EPSCs of variable amplitude were detected in all 3 genotypes.
Results: Yet, the frequency of EPSC in Otofp.R1934Q/p.R1934Q was lower, the amplitude range less variable, the mean amplitude smaller, and the waveform slower.
Conclusions: In summary, the mutant otoferlin protein appeared to cause inefficient vesicular fusion without significantly affecting calcium influx into IHCs.

T22. Investigation on Inner Hair Cell Stereocilia Stimulation Mechanisms Through 3D Finite Element Model of the Mouse Organ of Corti

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Category: Hair Cells: Anatomy & Physiology

Background: The intricate cytoarchitecture of the organ of Corti (OoC) provides the structural basis for stimulation of the inner hair cell (IHC) stereocilia, which triggers the mechano-electrical transduction (MET) channels on top of the lower stereocilia rows. The stimulation mechanism of the IHC stereocilia, plays an important role in how the mechanical motion of the traveling wave and the OoC gets translated to electrical signals via MET channels. However, the mechanisms of how IHC stereocilia get stimulated by their surroundings within the OoC remains to be determined. Recent experimental data on in situ IHC individual stereocilia from the 20 kHz location indicates non-cohesive bundle motion (Wang et al., 2021) and provides a basis for further modeling studies in understanding the IHC stereocilia stimulation mechanism in response to OoC motions.
Methods: Current work investigates the role of OoC and surrounding fluid spaces in IHC stereocilia stimulation via a 3D Finite Element Model (FEM) of a slice of the mouse OoC in the middle turn. Based on recent data, the two key hypotheses that the model tests are: 1) whether the IHC stereocilia are stimulated by inner sulcus fluid due to variation in the height of the sub-tectorial space, and 2) how this stimulation mode depends on frequency. The model captures the realistic representation of the fluid space of the inner sulcus, tectorial memrane, and the cortilymph consisting of the inner tunnel of Corti, the space of Nuel, and the outer tunnel. The cellular structure of the OoC is reconstructed based on 2-photon measurements on fresh tissue (Soons et al. 2015), as well as Scanning Electron Microscopy and histology slices on fixed tissues (Davies and Forge 1987, Taylor et al. 2012). The model captures the longitudinal inclination of the outer hair cells, Deiter cells, phalangeal processes and the outer pillar cells resulting in the mosaic pattern of the reticular lamina.
Results: The model for the 20 kHz best frequency region was validated based on our recently published experimental data for passive mechanics. Importantly, the tip of the tall row of IHC stereocilia moves more than the cuticular plate of the cell in the radial direction with 2 and 3 kHz stimulations. Consistent with measurements, there are significant motions observed in the longitudinal direction along with the radial-direction motions. With the validation of the model, the relative motion of the tectorial membrane and reticular lamina, and the inner sulcus fluid motion were determined at low and high frequencies.
Conclusions: Different IHC stimulation mechanism can be observed by investigating the relative motion of different components of the OoC, and the fluid motions of the inner spiral sulcus and the sub tectorial space.

T23. Localization of Piezo2 in Vestibular and Auditory End Organs in Mice

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Category: Hair Cells: Anatomy & Physiology
Background: Piezo2 protein has been suggested to provide a novel mechanoelectrical transduction (MET) mechanism (Wu et al., 2017; Lee et al., 2024) that is complementary to the well-established MET mechanism mediated by transmembrane proteins (Tmc1, Tmc2 and Tmie)

(Pan et al., 2013; 2018; Kurima et al. 2015; Zhao et al., 2014). In a previous study, we reported that Piezo2 protein is expressed in vestibular hair cells in the cristae and maculae. Similar to auditory hair cells, Piezo2 protein is most prominent in the cuticular plate, but unlike auditory hair cells, vestibular hair cells also express Piezo2 protein in the stereocilia. In this study, we further investigated Piezo2 protein distributions in Type I and Type II vestibular hair cells in the cristae and maculae and inner and outer hair cells in the cochlea.
Methods: Offspring of Piezo2-GFP-IRES-Cre mice bred to and Ai14-TdTomato reporter mice were used in the study. Temporal bones were harvested and fixed in 4% paraformaldehyde. Following decalcification in EDTA, cristae, maculae, and cochleae were microdissected for whole mount processing. Tissues were stained with antibodies against Myosin VIIa, SOX2, TdTomato and oncomodulin (OCM). Following immunostaining, vestibular and cochlear samples were mounted on glass slides, coverslipped, and submitted for confocal imaging. High-resolution confocal z-stack images of entire cristae, maculae, and cochleae were acquired using a Zeiss LSM880 confocal microscope.
Results: TdTomato expression, representing Piezo2, was observed primarily in Type I (Myosin VIIa+ / SOX2-) vestibular hair cells in the cristae and maculae. In the utricle and saccule, TdTomato expression was greater in striolar (Myosin VIIa+ / OCM+) hair cells. In the cochlea, TdTomato was expressed in all outer hair cells and sparsely in inner hair cells.
Conclusions: Although preliminary, these results suggest that Piezo2 may play different roles in the MET of vestibular and auditory hair cells. Ongoing studies will further examine 1) Piezo1 protein distributions in cristae and maculae and 2) functional consequences of conditional knockouts of Piezo1 and Piezo2 in vestibular hair cells.
TC and JL are co-presenters; HZ and WZ are co-senior authors.

T24. Assessment of Lateral Line Efferent Innervation and Rheotaxis Behavior in chrna9 Mutant Zebrafish

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Category: Hair Cells: Anatomy & Physiology
Background: Cholinergic efferent innervation modulates hair cell response to stimuli through activation of acetylcholine receptors to hyperpolarize hair cells. While the homomeric α9 nicotinic acetylcholine receptor and its role in lateral line response to reafferent stimuli have been defined in zebrafish, the effects of α9 receptor loss-of-function mutation on efferent innervation and lateral line mediated behavior has not been evaluated. Here we assess morphology of lateral line efferent neurons and rheotaxis behavior in chrna9 loss-of-function mutants, which have a premature stop codon in the α9 receptor mRNA transcript.
Methods: chrna9 mutants, heterozygous, and wild type siblings were used in all experiments. Rheotaxis behavior experiments were conducted in 6-7 days post-fertilization (dpf) larvae using a flow stimulus regime (10s pre-stim, 20s stim, 10s post-stim, v = 13.95-20.69mm/s), and resulting swim behaviors were video recorded and analyzed. To assess efferent pre-synaptic vesicle populations, dopaminergic neuron morphology, and hair cell density in lateral line neuromasts, larvae were immunolabeled with antibodies to synaptophysin, tyrosine hydroxylase, and HCS-1. α9 receptor loss of function was validated with HCR RNA-FISH.

Results: Analysis of chrna9-/- larvae rheotaxis behavior show increased mean number of rheotaxis events in the first 10 seconds of stim that subsequently return to wild type means by the later 10 seconds. chrna9-/- efferent pre-synapses had no change in average synaptophysin immunolabel intensity or average number of puncta per neuromast. Average number of hair cells per a neuromast were also comparable. Dopaminergic neurons were detected in all larvae siblings and may present some variance in expression. chrna9-/- fish receptor loss of function was confirmed through reduced fluorescence of chrna9 mRNA transcript.
Conclusions: Chrna9 loss-of-function may have some impact on rheotaxis behavior. Immunostaining of chrna9-/- lateral line organs show comparable synaptophysin immunoreactivity and hair cell counts per neuromast as well as presence of dopaminergic efferent neurons. Additional studies aim to analyze the swimming kinematics of chrna9-/- larvae during rheotaxis behavior and to probe for other variances in associated cholinergic efferent synaptic contacts.

T25. Visualizing how Presynaptic Activity Shapes Ribbon Formation in Zebrafish

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Category: Hair Cells: Anatomy & Physiology
Background: Ribbon synapses are essential for the rapid and precise transmission of sensory stimuli of hair cells. At ribbon synapses, the voltage-gated calcium channel Cav1.3 plays a critical role in mediating this transmission. While the role of Cav1.3 at mature ribbon synapses is relatively well understood, the role these channels play during synapse formation is not well understood. Research has demonstrated that Cav1.3 activity influences presynapse or ribbon size during development, and may play a role in the maintenance of ribbon synapses (Wong et. al., 2019, Sheets et al., 2012). This work demonstrated that Cav1.3 channels are required for spontaneous calcium responses in developing hair cells. Blocking these calcium responses during development resulted in larger ribbons. Recent work using live imaging has demonstrated that two processes, ribbon precursor transport and fusion are key events that define the dynamic of ribbon formation (Hussain et. al., 2024). But whether Cav1.3-mediated activity acts of either of these processes to define ribbon size is not clear.
Methods: To study this processes we are studying ribbon formation in vivo, in larval zebrafish when larvae are 2 days old and the majority of hair cells and synapses are forming. To visualize developing ribbon precursors and ribbons were using Tg[Mb:Rib tRFP] Homo Inx line. We are using this line in combination with another transgenic line Tg[myo6b:YFP Tublin], to mark hair cells and microtubules, the tracks developing ribbons move along during developing. To visualize developing ribbons we are using Zeiss AS confocal to capture ribbon movement fusion of zebrafish at 3dpf over the course of 30 minutes.
Results: Currently we have used live imaging to visualize the number and distribution of ribbon precursors and ribbons during the time course of development in living zebrafish hair cells. For this experiment, we performed in vivo imaging of hair cells to assess kinocilium length, to approximate hair cell stage. We then acquired high resolution Airyscan imaging to count the

number and distribution of ribbons at each stage. We find there are more ribbon precursors compare to controls. Based on these results we hypothesize that Cav1.3-mediated activity is important to regulate the fusion of ribbon precursors during development. To examine ribbon fusions, we are currently acquiring Airyscan timelapses (every 45sec for 30 min) to monitor ribbon movement and fusion during development.
Conclusions: Our work will highlight how activity shapes the formation of ribbons in hair cells. Understanding ribbon formation is important because ribbons can detach from the active zone and change in size and location after damage. Thus, understanding how ribbons are established at the active can help us to develop strategies to repair synapses after damage and ultimately restore sensory system function.

T26. Stereocilia Elongation is Regulated by Formin-Dependent Organization of Ankle Links

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Category: Hair Cells: Anatomy & Physiology
Background: Stereocilia are actin-based, mechanosensitive protrusions at the apical surface of auditory sensory hair cells that detect sound. The actin filaments within stereocilia are parallel and unbranched, with barbed ends located at stereocilia tips, where actin is incorporated during stereocilia elongation. Based on the unbranched nature of the actin network within stereocilia, we speculated that formin proteins contribute to the elongation of stereocilia actin filaments. Several formins are thought to be expressed in auditory hair cells based on mRNA sequencing, with FMN1 and DAAM1 being the most abundant.
Methods: To explore this hypothesis, we examined the effects of the formin inhibitor SMIFH2 on inner hair cells from mouse cochlear explants. We assessed stereocilia size as well as the localization of formins and key effectors using high-resolution microscopy.
Results: Unexpectedly, SMIFH2 treatment caused stereocilia to elongate, suggesting that inhibiting formins somehow promoted actin assembly at stereocilia tips. We localized DAAM1 and FMN1 to the base of developing stereocilia, where their immunostaining was coincident with ankle-link components, including MYO7A. Ankle links are transient adhesion complexes between adjacent stereocilia that are required for normal stereocilia development. Treatment with SMIFH2 disrupted the localization of FMN1, DAAM1, and MYO7A, causing these proteins to be lost from the ankle link region and instead localize along the stereocilia shaft and at the stereocilia tip. Since formins are unlikely to stimulate actin assembly in the presence of SMIFH2, we asked whether MYO7A could itself promote stereocilia elongation. EGFP-MYO7A had little effect on stereocilia dimensions; however, expressing a previously identified Myo7a mutant associated with hearing loss did phenocopy SMIFH2 treatment.
Conclusions: Together, our results indicate that the formin proteins FMN1 and DAAM1 are required to organize ankle-link adhesions between stereocilia. Additionally, the loss of this organization promotes stereocilia elongation, which is mediated by MYO7A. These findings suggest an unexpected role for ankle link adhesions in the regulation of actin assembly at the tips of stereocilia.

T27. The Role of MYO7A Isoforms in Tuning Hair Cell Function

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Category: Hair Cells: Anatomy & Physiology

Background: In auditory hair cells, tip-link tension is essential for the sensitivity of the mechano-electrical transduction (MET) process. Our previous study provided evidence that the unconventional Myosin VIIa (MYO7A) is essential for tensioning the tip-link MET complex. We further discovered that MYO7A isoforms with unique N-terminal extensions are differentially expressed in inner and outer hair cells (IHCs and OHCs), correlating with reported differences in tip-link tension. The goal of the present study was to explore the hypothesis that the differential expression of functionally distinct MYO7A isoforms directly affects hair cell physiology such as tip-link tension and resting open probability, and hearing sensitivities across hair cells at different frequencies.

Methods: MAS-ISOseq long-read sequencing by PACBIO was performed on cochlea cDNA to identify new transcript isoforms. Isoform-specific MYO7A deletion or HA affinity tagged mouse lines were generated. MET currents were recorded in response to fluid jet stimulations, and hair bundle motion was monitored by a high-speed camera. SEM and immunofluorescence microscopy were used to investigate hair bundle morphology. ABRs and DPOAEs were measured to test hearing. Cryo-EM was conducted to resolve the structure of mouse MYO7A isoforms.

Results: MAS-ISOseq identified two major isoforms of Myo7a in the auditory system: a widely studied canonical isoform (MYO7A-C) and a previously unreported novel isoform (MYO7A-N). Analysis of isoform-specific HA KI mice revealed that IHCs predominantly express MYO7A-C, and a significantly lower level of MYO7A-N. In OHCs, MYO7A-C and MYO7A-N showed an opposing gradient along the tonotopic axis. Simultaneous deletion of both MYO7A-C and MYO7A-N abolishes MYO7A immunoreactivity in all hair cells, accompanied by disorganized hair bundles and profound hearing loss. Therefore, we conclude that MYO7A-C and MYO7A-N are the major isoforms in hair cells. We previously reported that IHCs in mice lacking MYO7A-C (Myo7a-ΔC mice) showed a significant reduction in resting open probability in IHCs, consistent with the proposed role of MYO7A in generating tip-link tension. We now show that deletion of MYO7A-N (Myo7a-ΔN mice) results in progressive hearing loss and OHC loss. ATPase hydrolysis assays demonstrated that MYO7A-N exhibits significantly lower ATPase activity than MYO7A-C, consistent with a proposed role in maintaining a higher tip-link tension in OHCs. Lastly, we determined the molecular structures of the two MYO7A isoforms by cryo-EM, with specific focus on structural difference of the ATP binding pocket and actin interface in MYO7A-C and -N. Lastly, we employed AlphaFold3 to map known MYO7A deafness mutations to the structure.

Conclusions: In summary, our studies reveal the isoform diversity of MYO7A in the cochlea and highlight their essential roles in tensioning the MET complex. The differences in motor activity between the two isoforms are consistent with their proposed role in fine-tuning the tip-

link tensions, with potential importance for establishing the remarkable frequency range of mammalian hearing.

T28. Evaluating the Loss of Esrrg on the Cochlear Ribbon Synapses

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Category: Hair Cells: Anatomy & Physiology
Background: Estrogen-related receptor gamma (Esrrg) encodes a nuclear receptor that acts as a transcription factor in the absence of a ligand and bears high sequence similarity to Esrrα and Esrrβ. It is expressed in highly metabolically active tissues such as the heart, kidney and brain, modulating transcriptional pathways of mitochondrial, synaptic and metabolic function. From genetic studies, Esrrg has been implicated as a causative gene for congenital hearing loss and associated with age-related hearing loss in women. In mice, Esrrg is expressed at E10.5 in the developing otic vesicle and the vestibulo-cochlear ganglia at E16.5. However, little is known about the exact role of this gene in the cochlea. Recently, we showed that conditional knockout (cKO) of Esrrg in mouse inner ear leads to an early onset hearing loss and a phenotype suggestive of an auditory neuropathy.
Methods: Here, we performed immunostaining of the cochlea in Esrrg-mutant mice (Esrrg tm1d/tm1d/Sox10-Cre) and littermate controls at P12, around the time of hearing onset. Cochlear samples were fixed in 4% PFA, decalcified in 4.13% EDTA, pH7.4 and processed for whole mount immunostaining with anti-Myosin7a (hair cell marker), and the hair cell synapse markers, anti-CtBP2 (presynaptic ribbons) and anti-GluA2 (postsynaptic receptor). Confocal z-stacks were collected at 0.25µm z-plane intervals in the mid-apical (8 kHz) and mid-basal (24 kHz) cochlear coils on a Zeiss LSM 700 microscope using a PlanApo 63x (1.40 NA) oil-immersion objective. Quantitative analysis of the ribbon synapse was performed on maximum intensity projections of the Z stacks using Fiji software. For analysis of structural organisation in 3D, Imaris software was used. We performed whole-cell patch-clamp recordings from adult inner hair cells (IHC) at P42-P84 to measure calcium- dependent exocytosis. Recordings were performed using 1.3 mM extracellular calcium and at body temperature.
Results: Analysis of the IHC from co-labelling with anti-CtBP2 and anti-GluA2 suggests that there is a reduction in the amount of postsynaptic GluA2-positive AMPA densities in Esrrg-mutant mice with a decrease in the number of colocalised synapses. We are currently validating these results and investigating changes in synaptic distribution across the two distinct frequencies. Functional analysis revealed that adult IHCs show reduced exocytosis, although with normal calcium current.
Conclusions: Further characterisation is ongoing to help define the role of this gene in IHCs.

T29. Spatiotemporal Models to Investigate Population-Level Activity in the Vestibular Inner Ear

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Category: Hair Cells: Anatomy & Physiology

Background: The vestibular inner ear detects head motion through displacement of mechanosensitive stereocilia bundles on hair cells (HCs). Primary vestibular afferents encode these bundle deflections with distinct firing patterns: irregular and regular. Both afferent classes encode head motion via spike rates, but irregular afferents provide more information via precise spike timing. Computational studies thus far have modeled HCs and neurons at the single-unit level to explain how various biophysical properties may influence neural coding but it is unclear which properties are the most salient. Furthermore, the vestibular epithelium is spatially organized into zones related to afferent subtype: irregular and regular afferents emerge respectively from central (striola) and peripheral (extrastriola) zones. Our model inner ear organ, the utricle, detects linear acceleration in multiple ~horizontal directions because the hair bundles vary systematically within zones in their orientation (preferred stimulus direction), and reverse polarity (stimulus orientation preference) sharply at the striola/lateral extrastriola boundary. Although we know key differences in the mean activity of HCs and afferents in the striola and extrastriola and across the line of polarity reversal (LPR), we lack information about how HC and afferent activities represent head motions moment-by-moment on the vestibular epithelium. Here we address this question with a computational model of population activity by hair cells and afferents of the mammalian utricle.

Methods: HCs and afferents are simulated with Hodgkin-Huxley style equations and incorporate physiological data from our work in excised utricles plus the in vivo literature on vestibular afferents. The utricular epithelium is simulated as a three-layer feedforward model in which each layer represents a two-dimensional plane of, respectively, 1) HCs, 2) synaptic terminals and 3) spike initiation zones. To incorporate spatial features of the epithelium, the first layer contains HC orientation vectors. Feedforward integration between layers represents dendritic arbor size and types of synaptic inputs to afferents. We will systematically change parameters (e.g. dendritic arbor size or proportion of hair cell subtype) between the two zones and compare spiking statistics. Pairwise correlations will be calculated with respect to spatial distance between neuronal receptive fields and relative to the striola-extrastriola boundary.

Results: Preliminary findings using two-photon calcium imaging data suggest that HC activity is spatially dependent such that nearby HCs are more correlated. Furthermore, these correlations are robust for bundle polarity variations up to 20 degrees.

Conclusions: Ongoing work in our laboratory will allow us to compare our simulated responses to population activity in HCs and synaptic terminals evoked by bundle motions and collected with two-photon calcium imaging and to deconvolve the relatively slow Ca2+ signals with receptor and postsynaptic potentials recorded with high temporal precision (Luong et al., Kabirova et al. this meeting).

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T30. Do Changes in Resting Mechanotransduction Current Impact the Cytoskeleton Actin Composition Within Stereocilia and Cuticular Plate of Auditory Hair Cells?

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Category: Hair Cells: Anatomy & Physiology

Background: Stereocilia are actin-based organelles on inner ear sensory cells. Stereocilia from cochlear hair cells are deflected by sound-induced vibrations causing the opening of mechano-transduction (MET) channels at their tips. These channels regulate the resting influx of calcium into the cell, which impacts the morphology of stereocilia (Velez-Ortega et al., eLife, 2017). The stereocilia cytoskeleton has both β- and γ-actin isoforms. In vitro these isoforms show different polymerization and depolymerization rates in the presence of calcium (Bergeron et al., J Biol Chem, 2010). It is unknown whether changes to the resting calcium influx affect the ratio of β- to γ-actin in the stereocilia. Therefore, we evaluated the effects of MET channel blockage on the composition of the stereocilia and cuticular plate in mouse auditory hair cells.

Methods: Organ of Corti explants were isolated from C57Bl/6 mice at early postnatal days and cultured in control conditions or in the presence of tubocurarine, a MET channel blocker, for several hours. Samples were then fixed and immunostained against β- and γ-actin using isoform-specific antibodies and imaged using a Leica SP8 confocal microscope with high-resolution objectives (Leica 100X with 1.44 NA, and Olympus 100X with 1.7 NA).

Results: At a partial (~80%) block of MET channels, we observed significant changes in the ratio of β- to γ-actin in the cuticular plate of inner and outer hair cells (IHC and OHC, respectively), however, we did not notice significant MET-driven changes to the stereocilia cytoskeleton. Interestingly, in control conditions, preliminary results using an objective with higher numerical aperture (1.7 NA) show differences in the ratio of β- to γ-actin between non-transducing (tallest) and transducing (shorter) rows of stereocilia in OHC bundles. We are currently evaluating changes to the actin composition of the stereocilia cytoskeleton using saturating concentrations of the MET channel blocker.

Conclusions: Our results indicate that changes to the resting MET current not only impact the morphology of stereocilia but also lead to remodeling of the actin cytoskeleton within the cuticular plate. However, the physiological impact of this remodeling in the cuticular plate remains to be studied. Our preliminary results also suggest key differences in the cytoskeleton composition of transducing vs. non-transducing stereocilia in OHC, that could play a role in the resistance to MET-dependent remodeling in the tallest row of the bundle.

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T31. Auxiliary Subunit LRRC52 Regulates Bk Channel Function and Localization in Outer Hair Cells

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Category: Hair Cells: Anatomy & Physiology
Background: Cochlear hair cells transduce mechanical energy into receptor potentials with amplitude and time course shaped by activation of Ca2+- and voltage-dependent K+ channels (BK channels). BK channels in inner hair cells (IHCs) are unusual in that, even in the absence of Ca2+, they are activated at more negative potentials than any other known BK channel. We showed in IHCs that a specific regulatory gamma subunit, LRRC52, is a key determinant of the BK channel negative activation range as well as their clustering and localization (Lingle et al., 2019, PNAS 116:18397-18403). In outer hair cells (OHCs), the receptor potential mediates the sensitivity and frequency tuning of cochlear responses to sound through control of OHC electromotility. Here, we investigated the role of LRRC52 in mouse OHCs, where BK channels contribute to efferent inhibition of OHCs during stimulation of cholinergic medial olivocochlear (MOC) neurons.
Methods: Explants of the organ of Corti from C57/BL6 mice of 19-23 days of age were used for patch clamp recordings of BK channel currents. Confocal immunofluorescence images of BK channel localization were obtained from explants at 4-8 weeks of age. ABR, DPOAE, and VsEPs measurements were made in WT, LRRC52HET, and LRRC52KO animals. Noise exposures (100 dB SPL, 8-16 kHz, 2 hours) were done at 8 weeks of age in awake mice, followed by functional assessments at 1 day and 2 weeks post exposure. MOC-mediated suppression of DPOAEs during MOC stimulation was recorded in each strain before/after iberiotoxin (IBTX).
Results: Patch-clamp electrophysiology: The BK current present in control mid-apical coil OHCs was absent from LRRC52KO cells over the voltage-range tested up to +200mV. MOC inhibitory postsynaptic currents were mediated by both small conductance Ca2+-activated K+ channels (SK channels) and BK channels in control OHCs but only by SK channels in LRRC52KO cells.
Molecular anatomy: In OHCs from WT mice, BK channels were localized on the basolateral membrane in clusters apposed to presynaptic terminals of MOC neurons. In LRRC52KO mice, the BK channels were mislocated, dispersed throughout the basolateral membrane. Synapsin labelling of MOC terminals appeared unaltered in LRRC52KO mice.

Cochlear/vestibular function: ABR, DPOAE, and VsEP response metrics (thresholds, amplitudes, and latencies) appear unaffected in LRRC52KO mice.

Noise exposure: Both WT and LRRC52KO mice exhibited a temporary threshold shift at 1 day post exposure and no permanent threshold shift at 2 weeks post exposure.

MOC-mediated DPOAE suppression: MOC-mediated suppression of DPOAEs was progressively reduced in LRRC52HET and LRRC52KO mice as compared to WT animals. Perilymphatic IBTX reduced MOC-mediated suppression of DPOAEs, with little-to-no effect in LRRC52KO mice and a reduced contribution in LRRC52HET mice as compared to WT.

Conclusions: These results demonstrate that LRRC52 subunits contribute to BK channel voltage activation, localization, and efferent inhibition in cochlear OHCs.

T32. Molecular Identity of a Gating Spring

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Category: Hair Cells: Anatomy & Physiology

Background: Mechano-electrical transduction (MET) channels convert mechanical stimuli into electro/chemical cell signals. An elastic element, coined "gating spring", conveys forces to the channel gate to pull it open. This behaviour conveys a nonlinear gating compliance to the mechanics of hair bundles and the Drosophila hearing organ (Johnston's organ; JO), yet the molecular identities of the underlying machinery are still unclear. In Drosophila, this nonlinear compliance represents the gating spring activation of at least two different types of MET channels, one of which is lost selectively in mutant flies lacking the TRP channel NompC. NOMPC is a bona fide MET channel possessing an ankyrin repeats domain (ARD, 29 ankyrins) at its N-terminus, which assumes a spring like helical structure and is connected by a small linker to the remainder of the channel.

Methods: We tested different NOMPC constructs in adult fly mutants (lacking native NOMPC) as well as in S2 cells (not natively expressing NOMPC). These constructs were: i) wild-type NOMPC with GFP tag, ii) 2xARD-NOMPC (ARD duplication), iii) 2xLinker-NOMPC (Linker duplication).
We employed a Laser Doppler vibrometer to measure antennal displacements, while simultaneously recordings compound action potentials from JO neuron axons near the antennal nerve.

Electrostatic stimulation was used to move the arista. We used an adapted gating spring model including two types of MET channels to describe the gating compliance seen in the displacement response of the arista.

The same NOMPC constructs were transfected in Drosophila S2-cells. Spontaneous and mechanically evoked currents were recorded in outside-out membrane patches. We also employed the cysteine crosslinker agent 1,6-Hexanediyl-Bismethan-ethiosulfonate (MTS6).

Results: Duplicating the ARD does neither affect the gating compliance in adult flies nor the spontaneous activity or mechanical sensitivity of NOMPC in S2 cells.
Duplicating the linker, however, does affect the NOMPC gating compliance in adult flies and affect spontaneous activity and mechanical sensitivity in S2 cells. The NOMPC associated gating spring stiffness was about halved in adult flies and the mechanical sensitivity in S2 cells shifted by a factor of two towards larger stimuli. This correlates with the assumption of the linker functioning like a Hookean spring.

We also added cysteine pairs to one of the two linkers in the 2xLinker-NOMPC construct and treatment with MTS6 resulted in the restoration of mechanical sensitivity.

Conclusions: We found that duplicating the NOMPC ARD has virtually no effect on NOMPC gating in vitro, NOMPC MET function in vivo, or the effective gating spring stiffness that can be deduced from the gating compliance. By contrast duplicating the linker, connecting the ARD and the presumed channel gate, halved NOMPC pressure/displacement sensitivity in vitro/in vivo as well as the effective stiffness of the gating springs. Taken together, we think to have molecularly identified the gating spring component in NOMPC.

T33. Stiffness Changes and Force Production of Outer Hair Cells During Mechanical Stimulation

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Category: Hair Cells: Anatomy & Physiology
Background: Outer hair cells (OHCs) are essential for mammalian ear for providing amplifying mechanical feedback to the vibration in the cochlea. These cells have two motile mechanisms: piezoelectric lateral wall and the hair bundle. The present study predict how the interplay of these mechanisms determines their response to external mechanical stimulation.
Methods: A theoretical model for OHCs, which incorporates piezoelectricity of the lateral membrane, structural elasticity and mechanosensitive hair bundle, was examined to predict the responses of OHCs to mechanical stimulation.
Results: [1] For high frequency stimulation, the mechanosensitivity of the hair bundle dominates amplifying feedback of the cell body. The mechanosensitivity of the lateral wall contributes to the stiffness of the cell body.
[2] For mechanical stimulation at low frequencies, piezoelectricity of the lateral wall determines the amplifying force. Hair bundle conductance makes a positive contribution to the stiffness of the OHC.

[3] If the membrane potential is held constant, the cell body shows biphasic reduction of stiffness (an analog of gating compliance) with increasing external force, associated with the conformational transition of the motile molecule. However, the stiffness of the cell body increases if the membrane potential is allowed to change during mechanical stimulation.

Conclusions: OHCs show rather complex behavior due to the interplay between two kinds of mechanosensitive elements in those cells. They can lead to pure piezoelectric resonance at low frequencies and semi-piezoelectric resonance involving the hair bundle at high frequencies. The stiffness of the cell body increases with mechanical stimulation, in contrast to stiffness reduction while the membrane voltage is kept constant.

T34. Lack of MAP2 Cause Sensorineural Hearing Loss and Vestibular Dysfunction

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Category: Hair Cells: Anatomy & Physiology
Background: MAP2 is one of the major neural microtubule-associated proteins (MAPs) and is evolutionally conserved from C. elegans to mammals. This suggests its importance in neuronal function. However, functional elucidation of MAP2 is mostly limited in vitro, and the importance of MAP2 for the sensory neural system remains unclear.

To elucidate the function of MAP2 in vivo, we observed the behavior of MAP2 knockout (MAP2 KO) mice. It normally developed into an adult without any apparent abnormal bodily movement but showed a reduced surprise response or avoidance to loud noise. MAP2 is expressed not only in neurons but also in cochlear hair cells. Therefore, we hypothesized that the loss of MAP2 causes hearing loss and vestibular disorder.

Methods: Auditory function and sensory cell morphology were assessed by Auditory brainstem response (ABR), immunofluorescence, and Scanning Electron Microscopy. Phenotypic changes in protein expression was evaluated for myosin VIIa, MAP2, and F-actin through immunohistochemistry.
Vestibular functions were tested by Rotarod Test, Beam Test and Open Field Test.

Results: ABRs to sound stimuli at 2, 4, 8, 16, 32, 50, 60, 64, 68, and 72 kHz were measured. Response thresholds of MAP2 KO mice were higher than those of wild type (WT) mice at 4 kHz -32 kHz, with a maximum increase of 40 dB at 16 kHz. ABR above 50 kHz was not observed in MAP2 KO mice even with the loudest stimulus (90 dB SPL). In addition, there was an increase in response latency and a decrease in response amplitude, which are characteristic of sensorineural hearing loss.
Immunostaining showed that MAP2 was expressed in the cochlear outer hair cells (OHC), inner hair cells (IHC), and cochlear neurons. The amount of MAP2 expression was higher in the OHC than IHC. The number of OHCs of base turn of the cochlear decreased in MAP2 KO mice, while the number of IHCs did not change in any turn. In addition, electron microscopy scanning revealed that the loss of the MAP2 gene did not affect the length of OHC stereocilia but increased their misalignment compared to WT mice. By the rotor rod test, MAP2 KO mice had a decreased time until fall compared to WT mice. By the beam test, MAP2 KO mice took more time to cross the beam and lost their footing more often than WT mice.

Conclusions: These results suggest that MAP2 deficiency causes hearing loss and equilibrium dysfunction and that MAP2 contributes to the maintenance of hearing sensitivity to sound, especially in the high-frequency range, and to balance. The possible role of MAP2 in regulating the stiffness of microtubules and actin in hair cells will be discussed.

T35. Does Stereocilia Separation-To-Height Ratio Accurately Define the Geometric Gain?

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Category: Hair Cells: Anatomy & Physiology
Background: Geometric gain is a critical parameter in cochlear hair bundle (HB) models that influences the bundle's mechanical and electrical response to sound. It is often approximated as the ratio of the horizontal spacing between stereocilia pivots to the average stereociliary height. This approximation is used to estimate how mechanical deflections of the HB affect gating spring tension, which modulates ion channel activity. However, relying on this simplified ratio

does not accurately capture the true mechanics of the system, leading to potential inaccuracies in predictions of HB sensitivity, stiffness, and related quantities from the models.
Methods: We analyzed geometric gain at five cochlear locations in adult mice (30 kHz, 12 kHz, 8 kHz, 2 kHz, and 750 Hz), comparing two definitions: the approximated "stick geometric gain," derived from two infinitesimally thin stereocilia and the "true geometric gain," based on the complete morphology of stereocilia using our fully nonlinear two-row isolated HB model without adaptation. We use morphological and mechanical data from published experiments. To assess the effects of these geometric gains on bundle sensitivity, operating range, and stiffness, we implemented the model developed by Tinevez et al. (Biophys. J., 93(11), 4053-4067 (2007)), referred to as the TJM model, without adaptation, as it employs the stick approximation. We then compared its predictions with our two-row model that inherently utilizes the true geometric gain.
Results: The stick geometric gains were calculated as 0.25, 0.17, 0.15, 0.11, and 0.09 across decreasing characteristic frequencies, while the corresponding true geometric gains were consistently lower at 0.09, 0.08, 0.06, 0.05, and 0.04. This reveals a substantial two- to three-fold overestimation of geometric gain by the stick model that neglects bundle morphology. As a result, the TJM model predicts larger changes in gating spring tension for the same set of model parameters defined in the two-row model, leading to overestimated bundle sensitivity and narrower activation curves. Stiffness predictions at ~0.4 open probability were similarly larger, with errors ranging from 75% to 125%.
Conclusions: The overestimation of bundle sensitivity and stiffness, coupled with an underestimated operating range, compromises the accuracy of HB dynamic models. The commonly used geometric gain approximation, based on a simplified stick model, fails to capture the complexity of HB morphology, particularly the length and inclination of the tip link. These structural factors significantly influence gating spring tension and sensitivity, highlighting the need for a precise definition of the geometric gain and models that accurately represent the true geometry of HBs. As the next step, we plan to explore how changes in the geometric gain affect response prediction when adaptation effects are included in the system.

T36. The Effect of TMC1 Deafness Mutations on Cochlear Hair Cell Loss

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Category: Hair Cells: Anatomy & Physiology
Background: Transmembrane channel-like protein 1 (TMC1) is an essential component of the hair cell mechanoelectrical transduction (MET) complex in inner ear, which allows us to perceive sound and balance. TMC1 has been identified as the pore-forming submit of the MET complex and to be involved in hair cell membrane homeostasis. Auditory hair cells from mice carrying TMC1 deafness-causing mutations show signs of degeneration at different postnatal development stages, therefore, causing hearing loss. However, the molecular mechanisms of TMC1 mutations in hair cell degeneration remain unclear. Here we investigate the impact of different mutations in TMC1 on hair cell degeneration and dysregulation of membrane homeostasis.
Methods: We performed morphological and quantitative analysis of the spatial and temporal cochlear hair cell degeneration in mice harboring different TMC1 mutations. Hair cell loss was

quantified via myosin7a and phalloidin counterstaining. Dysregulation of hair cell membrane homeostasis was characterized by phosphatidylserine (PS) externalization and visualized with annexin V staining.
Results: In mice carrying two copies of dominant TMC1 pore region mutation D569N, we observed hair cell degeneration in all hair cells at postnatal day 15 (P15) with increased hair cell degeneration rate from apex to base. Homozygous mice carrying the recessive mutation D528N, which is also located at the ion conduction pore of TMC1, start to show signs of outer hair cell (OHC) degeneration at P30 and worsen from base to apex, while inner hair cell (IHC) loss occurs at P15 and progresses from apex to base. Interestingly, hair cells from mice carrying the TMC1 W554L mutation located outside the pore region, maintain normal morphology through early adulthood with only signs of mild OHC degeneration at the basal region at P30. Constitutive PS externalization was observed during hair cell degeneration in TMC1 pore mutant mice while mice carrying the TMC1 W554L mutation showed reduced PS externalization.
Conclusions: Our data shows hair cell degeneration is facilitated by TMC1 mutations that alter the MET channel properties and dysregulate hair cell membrane homeostasis. Our results suggest that TMC1 could play an essential role in hair cell survival by maintaining plasma membrane asymmetry, a novel insight that could have significant implications for our understanding of hearing loss and potential therapeutic interventions.

T37. Paralemmin-3 – an Essential Constituent of the Submembrane Cytoskeleton of Auditory Hair Cells

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Category: Hair Cells: Anatomy & Physiology
Background: In the mammalian inner ear, cochlear inner hair cells (IHCs) enable accurate and faithful synaptic sound encoding, while outer hair cells (OHCs) perform frequency-specific sound amplification and fine-tuning through their intrinsic voltage-dependent somatic electromotility. This latter process is facilitated by the unique trilaminate structure of the OHC lateral wall, which consists of the transmembrane motor protein Prestin found within the plasma membrane, the subcortical actin- and spectrin-based cytoskeleton, and the cytoplasmic subsurface cisternae. This complex system is essential for both, mechanical rigidity and stability as well as cell expansion and contraction during electromotility. Whereas the ultrastructure of the lateral wall is well described, its molecular composition is incompletely understood. Here, we identified paralemmin-3 (Palm3) as a novel protein specifically localized to the lateral walls of

auditory hair cells that may play a crucial role in connecting the plasma membrane to the underlying cytoskeleton.
Methods: A comprehensive characterization of the functional and morphological consequences of Palm3 deficiency was conducted in Palm3-KO mice. First, auditory brainstem response (ABR) and distortion product otoacoustic emissions (DPOAE) recordings were conducted on 3- and 10-weeks-old WT littermate and Palm3-KO mice. Second, tonotopic hair cell survival and the overall involvement of Palm3 in the structural integrity and maintenance of auditory hair cells were investigated using immunohistochemistry, light sheet fluorescence-, and confocal microscopy. Third, by employing electron tomography on high-pressure frozen and freeze-substituted organs of Corti, the structural integrity of the lateral wall of WT and Palm3-KO OHCs was examined on ultrastructural level. Finally, intracochlear delivery of adeno-associated viruses (AAV) encoding eYFP-tagged wild-type Palm3 was carried out to try to revert the pathophysiological phenotype of Palm3-KO.
Results: Palm3-KO mice exhibit early-onset and progressive hearing impairment resulting from diminished cochlear amplification. Subsequent multiscale morphological analyses of acutely dissected cochleae revealed structural collapse of OHCs, leading to progressive and extensive OHC loss along the tonotopic axis. Furthermore, Palm3-KO OHCs exhibit disrupted distribution and attenuated expression of several membrane-associated proteins – including Prestin and α2-Spectrin – suggesting a role of Palm3 in plasma membrane scaffolding. In line with this hypothesis, electron tomography of OHC lateral walls revealed significantly fewer and structurally perturbed cisternae structures in Palm3-KO compared to WT littermates. Finally, AAV-mediated rescue of Palm3 partly restored hearing function, enhanced OHC survival and restored OHC cell shape as well as Prestin and α2-Spectrin expression.
Conclusions: Palm3 is a novel protein of the lateral plasma membrane of auditory hair cells that is essential for adequate membrane scaffolding and OHC survival.

T38. Acbd7 is Essential for Maintaining Auditory and Vestibular Functions Associated With Hair Cell Synaptic Transmission

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Category: Hair Cells: Anatomy & Physiology
Background: Identifying key genes that govern hair cell (HC) function is the foundation for exploring the working principle of inner ear HCs and crafting therapeutic strategies for auditory and vestibular disorders. By employing single-cell RNA sequencing on cochlear organoids, we have identified Acbd7, a member of the acyl-CoA binding protein (ACBP) family, as a novel marker for both auditory and vestibular HCs. The ACBP family of genes plays an indispensable role in the metabolism of long-chain fatty acids and in the regulation of neurogenesis and neuronal function. However, the specific contribution of Acbd7 to inner ear development and function has not yet been fully elucidated.
Methods: We conducted single-cell transcriptome sequencing of cochlear organoids and clarified the expression profile of the HC-specific Acbd7 gene through in situ hybridization. Utilizing Acbd7 knockout (KO) and HC conditional KO (CKO) mouse, we evaluated auditory function via auditory brainstem response (ABR) and vestibular function through off-vertical axis

rotation (OVAR) and angular vestibulo-ocular reflex (aVOR) tests. The effects of Acbd7 deficiency on HC count, nerve fibers, and synapse were analyzed using immunofluorescence and electron microscopy. Patch-clamp electrophysiology was applied to evaluate synaptic vesicle release and ion channel function in HCs. Finally, we adopted transcriptome sequencing to explore the mechanisms by which Acbd7 affects HCs function, providing an integrated understanding of its regulatory networks.
Results: We have discovered Acbd7 as a novel marker for hair cells, with a dynamic expression profile throughout the auditory and vestibular systems. Acbd7 KO and CKO mice presented with progressive hearing loss with a reduction in the mean peak amplitude of ABR wave I and an extension in latency. Assessments of vestibular function in Acbd7 KO mice using off-vertical axis rotation (OVAR) and angular vestibulo-ocular reflex (aVOR) tests revealed utricle and horizontal crista ampullaris abnormalities. A reduction in hair cell ribbon synapses was observed in both cochlear and vestibular HCs of Acbd7 KO mice. Whole-cell patch-clamp recordings demonstrated that Acbd7 deficiency results in diminished Ca2+ currents and a decrease in membrane capacitance. Moreover, transcriptome sequencing in Acbd7 KO mice revealed a downregulation of genes associated with Ca2+ signaling and calmodulin binding, highlighting the essential role of Acbd7 in the sustenance of hair cell synaptic function.
Conclusions: n this work, we identified Acbd7 as a novel HC marker essential for maintaining auditory and vestibular functions through its role in regulating synaptic function, particularly by modulating Ca2+ activity and calmodulin binding-related genes. Our findings provide a theoretical basis for investigating synaptic-related auditory and vestibular pathologies.

T39. BAIAP2L2 Modulates Actin Protrusion Shape via Espin-1-Dependent Actin Regulation

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Category: Hair Cells: Anatomy & Physiology
Background: Stereocilia are actin-rich mechanosensitive organelles in auditory and vestibular hair cells. They require precise regulation of their length and morphology for proper hearing and balance. The mechanoelectrical transduction (MET) complex is localized at the tips of the second row and lower mechanotransducing prolate stereocilia. It is widely accepted that MET and actin regulation are reciprocally regulated, underscoring the importance of structure-function relationships at stereocilia tips. Key proteins at the stereocilia tips, such as Espin-1, EPS8, Myo3A, and the membrane curvature regulator BAIAP2L2, are critical for stereocilia regulation. While the retention of BAIAP2L2 at the stereocilia tip is calcium-dependent, Espin-1 operates independently of calcium. Both proteins influence MET activity and stereocilia morphology, and the V169A mutation in the I-Bar domain of BAIAP2L2 has been linked to progressive hearing loss.
Methods: However, the mechanisms by which Espin-1 and BAIAP2L2 precisely regulate stereocilia length and shape, and whether they work in a coordinated manner, remain largely unknown. To explore potential mechanisms and common regulatory pathways, we conducted

immunolocalization studies and tested their interactions using the COS-7 heterologous expression assay and multiple deletion and mutated constructs of the proteins.
Results: Our data show that BAIAP2L2 and Espin-1 co-localize at the stereocilia tips, with BAIAP2L2 positioned near the membrane and Espin-1 located closer to the actin filament bundle. Their distributions exhibit substantial overlap, suggesting possible interactions. Combinatorial co-transfection assays show that the Espin-1 and BAIAP2l2 interact via SH3 and PR1 domains creating the possibility of multiple interactions with EPS8 and other tip proteins including components of the MET complex such as PCDH15 and CIB2. Further, co-transfection of BAIAP2L2 and Espin-1 with and without EPS8 in COS-7 cells induces the formation of phase-separated condensates, originating at filopodia tips and migrating to the cell body. We show by immunogold EM that these condensates have the characteristic dense and amorphous appearance. This supports our hypothesis that BAIAP2L2 and Espin-1 not only interact directly via SH3 and PR1 domains but also the interaction results in phase separation. Additionally, we demonstrate that the BAIAP2L2 V169A mutation, which is associated with hearing loss, reduces the formation of these condensates but does not prevent interactions of BAIAP2L2 with EPS8 and Espin-1.
Conclusions: These results taken together suggest that BAIAP2L2 interactions with Espin-1 via phase separation condensates may be crucial for maintaining normal MET function by coordinating the regulation of membrane curvature and stereocilia length.

T40. Comprehensive Profiling and Structural Analysis of Kinocilia in Adult Cochlear and Vestibular Hair Cell

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Category: Hair Cells: Anatomy & Physiology
Background: The kinocilium is an essential component of sensory hair cells, playing a critical role in hair bundle development and the establishment of planar cell polarity within the sensory epithelium. In vestibular hair cells, kinocilia persist throughout an animal's lifespan, while in the organ of Corti, they begin to disappear by postnatal day 8 (p8). Despite its conserved features with other cilia and flagella, the specific nature, and properties of the kinocilium remain not well understood. We are investigating the architecture of vestibular hair cell kinocilia and conducting molecular profiling from isolated adult hair cells to uncover the unexplored distinct structural and functional characteristics of kinocilia in the inner ear.
Methods: We employed single-cell RNA sequencing to profile the gene expression of 1,522 cochlear and vestibular hair cells from 10-week-old CBA/J adult mice. We then mapped the overlap between ciliary proteins identified in public databases and our transcriptomic data across four hair cell subtypes. Additionally, we compared these findings with previously published neonatal mouse transcriptomic data to further explore the overlap with ciliary proteins. In parallel, immunostaining and high-resolution confocal microscopy were used to localize key motility-related proteins within the kinocilia of both neonatal and adult cochlear and vestibular hair cells. Conventional electron microscopy (EM) provided detailed insights into the ultrastructural complexity of kinocilia.

Results: Our single-cell transcriptomic analysis uncovered numerous genes related to cilia and basal bodies involved in motility and sensory perception. Gene enrichment revealed that vestibular hair cells strongly express motile cilia components, akin to those in sperm, the trachea, and unicellular organisms, along with primary cilia genes. We identified over 100 genes associated with motile cilia, including those essential for forming and maintaining the 96-nm repeats in the ciliary axoneme. Using immunofluorescence antibody labeling, we confirmed the localization of Foxj1, a key regulator of motile cilia, and identified the 96-nm repeat "ruler" proteins CCDC39/CCDC40, along with IFT-B complex components CLUAP-1 and IFT172. Transmission and scanning electron microscopy revealed greater complexity and regional variations along the kinocilium, including a cone-like structure at the tip and a microtubule arrangement that differs from previous EM studies.
Conclusions: This study provides the first comprehensive profiling of kinociliary genes in cochlear and vestibular hair cells. Our analysis uncovered distinct spatial and temporal expression patterns, with certain ciliary genes showing region-specific elevation, suggesting specialized functions in both motile and primary cilia. We offer new insights into the molecular and structural organization of motile cilia in hair cells, revealing that the kinocilium may have structural variations not fully captured in previous studies. These findings indicate a more complex ciliary architecture, with potential implications for mechanosensitivity in vestibular hair cells.

T41. Myosin Xva Isoforms Participate in the Mechanotransduction-Dependent Remodeling of the Actin Cytoskeleton in Auditory Stereocilia

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Category: Hair Cells: Anatomy & Physiology
Background: Modified microvilli in the inner ear, known as stereocilia, detect sound-induced deflections through the opening of mechano-electrical transduction (MET) channels at their tips. At rest, a small MET channel current results in a constant calcium influx. This entry of calcium ions at rest regulates the morphology of the stereocilia cytoskeleton (Velez-Ortega, et al. Elife, 2017). However, the molecular mechanisms involved in this activity-driven cytoskeleton plasticity are largely unknown. The unconventional myosin XVA (MYO15A) is a key protein in the regulation of stereocilia dimensions during hair bundle development, as well as in the maintenance of mature stereocilia. Here, we evaluated the role of MYO15A in the MET-dependent remodeling of the stereocilia cytoskeleton in auditory hair cells using mice deficient in one or multiple isoforms of MYO15A.
Methods: Organ of Corti explants were isolated from mice that lack either all isoforms of MYO15A (Myo15sh2/sh2) or only the long isoform (Myo15ΔN/ΔN) along with their heterozygous and wild-type control littermates at postnatal days 4 or 7. The explants were incubated in the presence of MET channel blockers (benzamil or tubocurarine) or their vehicle controls. The efficiency of the blockage was assessed via the uptake of the FM1-43 dye. Samples were fixed and prepared for electron microscopy (EM) to evaluate morphological changes in stereocilia via scanning EM (SEM) or focused ion beam SEM (FIB-SEM) using a FEI Helios

NanoLab Dual Beam. Other samples were fixed, immunostained against the row identity proteins GNAI3, EPS8, or ESPNL, and imaged with a Leica SP8 confocal microscope.
Results: No MET-dependent remodeling was observed in hair bundles lacking all MYO15A isoforms, while the absence of the long isoform of MYO15A led to exaggerated remodeling of the shorter transducing rows. Interestingly, stereocilia in the non-transducing tallest row – which do not exhibit morphological changes after MET channel blockage in wild-type hair cells – did exhibit MET-dependent remodeling in the absence of one or both alleles of the long isoform of MYO15A. In addition, we found impaired trafficking of the row identity proteins GNAI3 (tallest row) and ESPNL (shorter rows) in mice lacking the long isoform of MYO15A.
Conclusions: Our study demonstrates that MYO15A isoforms are necessary for MET-dependent remodeling of the stereocilia actin cytoskeleton. Additionally, the long isoform of MYO15A likely delivers molecular machinery that increases the cytoskeleton stability of stereocilia, ensuring that the MET-dependent actin remodeling occurs in a controlled manner. Surprisingly, the absence of the long isoform of MYO15A also affects the row identity of developing stereocilia which could explain the observed MET-dependent remodeling of the actin cytoskeleton within stereocilia from the tallest row.

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T42. Elastic Links Are Required for High Positive Correlations Between Inner-Hair-Cell Stereocilia

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Category: Hair Cells: Anatomy & Physiology
Background: Inner-hair-cell hair bundles (IHBs) are the sensory organelles required for mammalian hearing. IHBs convert sound-induced forces within the inner ear into receptor currents, a key step in the transmission of sound signals to the brain. The transduction of forces into receptor currents is limited by thermal-scale fluctuations in the stereocilium deflections, which cause fluctuations in the receptor current. These fluctuations compete with the sound signal driving firing of the auditory neurons, increasing the threshold of hearing.
An IHB comprises tens of stereocilia, filaments emanating from the hair-cell surface, which are deflected by only a few nanometers in response to piconewton sound-induced forces. Stereocilium deflections cause ion-channel opening and closing, producing receptor currents. If the fluctuating deflections of the tallest stereocilia are highly positively correlated, the ion channels tend to open and close simultaneously, decreasing fluctuations in the receptor current. If, on the other hand, the fluctuating stereocilium deflections are weakly or negatively correlated, the ion channels tend to open and close at different times, increasing fluctuations in the receptor current.

Stereocilium fluctuations can be positively or negatively correlated, depending on the mechanical properties of the IHB. We propose that elastic links between the tallest stereocilia

promote positive correlations between stereocilium deflections, decreasing the threshold of hearing.

Methods: We use a mathematical model to determine how the IHB's mechanical properties regulate stereocilium fluctuations. In the model IHB, the stereocilia are attached to the hair-cell apex and coupled to each other by elastic and damping links. The model parameters are based on published experimental observations.
Results: We find that stereocilium deflection fluctuations are larger at the edge of the IHB than at the center. Correlations between stereocilium deflections decrease with the distance between the stereocilia and with increasing numbers of stereocilia within the IHB. Eliminating the elastic links between the tallest stereocilia makes the stereocilium deflections negatively correlated, whereas increasing the elastic link strength increases positive correlations between stereocilium deflections.
Conclusions: Elastic links between the tallest stereocilia in the IHB ensure positive correlations between stereocilium deflections, decreasing the threshold of hearing. We show the importance of quantifying fluctuations in the IHB and determining how the mechanical properties of the IHB limit these fluctuations.

T43. Viscoelasticity Accounts for Fast Adaptation in Outer-Hair-Cell Bundles

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Category: Hair Cells: Anatomy & Physiology
Background: Outer-hair-cell hair bundles (OHBs) are required for normal hearing. They transduce sound-induced forces into the receptor currents that drive cochlear amplification. In response to step stimuli, OHB receptor currents adapt – they decrease after transiently increasing. Adaptation is thought to maintain an OHB's sensitivity to sound stimuli when it is deflected by step stimuli. However, the mechanisms underlying OHB adaptation are under debate. We show here that viscoelastic elements can account for the fastest components of adaptation.
OHB comprise stereocilia, filamentous rods protruding from the apical surface of the outer hair cell. Neighboring stereocilium pairs of different height are linked by gating springs, which are attached to mechanoelectrical transduction (MET) channels in the shorter stereocilia. Sound-induced stimulus forces deflect the stereocilia, elongating and shortening the gating springs, opening and closing the MET channels.

Adaptation in OHBs has slow (GREATER THAN 10 ms) and fast (LESS THAN 1 ms) timescales. There is evidence that slow adaptation depends on the influx of calcium though the MET channels, but that fast adaptation does not depend on calcium influx. Experiments using very rapid stimuli show that fast adaptation has two components with timescales of 0.1 ms and 0.7 ms on average. We propose a mechanism for fast adaption that accounts for the fast-adaptation timescales.

Methods: To evaluate the feasibility of our proposal, we develop a new mathematical model of the OHB. The model OHB comprises pivoting stereocilia, gating springs, the kinetics of MET-

channel gating, and two viscoelastic adaptation elements in series with the MET channels. The morphology of the model OHB is based on published experimental observations. To determine mechanical parameters, we fit the model OHB to 11 published experimental observations, which constrains 11 mechanical parameters in the model.
Results: The model OHB reproduces 11 independent experimental observations, including the experimentally measured fast adaptation timescales, the fraction of adaptation associated with each fast adaptation timescale, and the extent of fast adaptation. Additionally, the model OHB predicts that the tip link is viscoelastic and contributes to fast adaptation. In response to oscillatory stimuli, the model OHB also shows that fast adaptation causes hysteresis in the displacement-current activation curve.
Conclusions: We find that viscoelastic elements in series with the MET channels can account quantitatively for fast adaptation in OHBs. Strikingly, we find that viscoelastic tip links contribute to fast adaptation. Our findings lend support to the hypothesis that fast adaptation in OHBs does not require calcium influx through the MET channels.

T44. The shaker-1 Mutation Highlights the Role of Myosin Viia in Maintaining of the Morphological Integrity of the Shortest Rows of Stereocilia in Cochlear Hair Cells

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Category: Hair Cells: Anatomy & Physiology
Background: The transduction of acoustic information into electrical signals depends on the mechanically induced displacement of stereociliary bundles projecting from the apical surface of the sensory hair cells. Hair bundle deflection opens mechano-electrical transducer (MET) channels located at the tips of the shorter rows of adjacent stereocilia. The gating of the MET channels requires force supplied by the tensioning of tip links during sound-induced bundle displacement. The motor protein MYO7A, an unconventional myosin responsible for syndromic (Usher 1B) or non-syndromic recessive deafness in humans when mutated, has long been associated with tip-link tensioning, but conclusive evidence is still lacking. In this study, we investigated the role of MYO7A in mature hair cells using conditional knockout mice.
Methods: The role of MYO7A in hair cells was investigated using the shaker-1 mouse, which has a missense mutation in the shaker-1 gene (Myo7aSh1) which interferes with the motor protein function. Patch-clamp electrophysiology was used to record the MET current, which was elicited by displacing the hair bundles of the hair cells with a piezo-driven fluid jet. The morphology of the stereociliary bundles and their molecular composition was investigated using immunofluorescence microscopy and scanning electron microscopy. Hearing function was assessed using auditory brainstem responses. Dual AAV-Myo7a was used to rescue the function in shaker-1 mice.
Results: We found that hair cells from shaker-1 mice progressively lose the MET current, despite the MET channels showing normal resting Po and sensitivity to intracellular Ca2+. These

late biophysical changes occurred while the 2nd and 3rd rows of stereocilia in the hair bundles start regressing, eventually leading to hearing loss by about P30. Noise exposure exacerbates the disruptions of the stereociliary bundles and the progression of hearing loss. We also show that the delivery of Myo7a via dual-AVV into the cochlea of Myo7aSh1/Sh1 mice leads to a partial recovery of the hearing function in adult mice.
Conclusions: We found that MYO7A is required for maintaining the functional integrity of the stereociliary hair bundles, and that gene-based rescue partially restores the function of MYO7A in cochlear hair cells.

T45. Evoked Calcium Signals in Intact Vestibular Epithelium and Their Relationship to Electrical Changes in Hair Cells and Afferent Neurons

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Category: Hair Cells: Anatomy & Physiology
Background: During head motions, each vestibular hair cell generates receptor potentials that reflect the direction, size and frequency content of the motion. Current knowledge of how mammalian vestibular epithelia respond to motion is largely based on data collected serially from individual hair cells or primary afferents, as well as recordings of summated potentials that reflect output of the vestibular inner ear. But such recordings don't provide us with information on how hair cell subpopulations of vestibular epithelia and their afferents are representing head motion moment-by-moment.
Methods: Progress with genetically encoded calcium indicators allowed us to produce mouse lines with GCaMP8m expression in specific cell types: hair cells and afferent, allowing us to record simultaneously the responses of multiple cells to stimuli on both hair cell and afferent stages in the intact epithelium of the mouse utricle (Luong et al., this meeting).
Results: We are exposing mechanosensitive hair bundles by removing otoconia and stimulating them with a fluid jet. Hair bundle deflection leads to entry of cations, including Ca2+, through transduction channels and the resulting receptor potential activates voltage-gated CaV1.3 channels in the basolateral membrane. Stimulus-evoked transmission to afferent synaptic terminals gives rise to postsynaptic Ca2+ signals likely due to voltage activation of CaV1.2 channels near the spike initiation zone. But we lack information on calcium dynamics in vestibular hair cells and afferents. Here we are investigating the relationship between current/voltage changes and Ca2+ responses in individual hair cells and afferent neurons.
Conclusions: Direct comparisons of single-cell Ca2+ and electrical signals will guide our interpretation of population Ca2+ signals and modeling of how different populations within the vestibular epithelium and nerve represent head motions (Lutz et al., this meeting).

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T46. Technical Details on Single-Molecule Microscopy of MYO7A Trafficking in Live Hair Cell Stereocilia

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Category: Hair Cells: Anatomy & Physiology

Background: Stereocilia are a bundle of cylindrical F-actin protrusions that develop on the apical surface of hair cells and function as biological mechanosensors for sound and acceleration. Although wild-type motor activities of unconventional myosins are essential for developing functional stereocilia, it is not fully understood how each myosin molecule localizes itself and its cargo using the energy from ATP hydrolysis. Here, we introduce the technical details of our workflow for single-molecule microscopy in live hair cell stereocilia to approach this open question with recent updates demonstrating our current analyses of MYO7A's trafficking in stereocilia.

Methods: Explant cultures of utricles or saccules (hereafter "vestibules") were prepared from mouse neonates at postnatal day 2–5 and transfected using a Helios® gene gun to express HaloTag-fused proteins. EGFP or EGFP-fused protein was co-expressed as a transfection marker. HaloTag-fused protein molecules were fluorescently labeled using JFX554-ligands and illuminated by a 561-nm light sheet at 0.2 kW/cm2 using a dual-inverted selective plane illumination microscope (diSPIM). HaloTag-fused human β-actin (HaloTag-actin) was used to characterize fluorescent puncta. Trafficking of MYO7A was analyzed using HaloTag-fused full-length MYO7A, MYO7A-HMM (a head + neck fragment) and MYO7A-R/K (a mutant disabling the tail-mediated motor autoinhibition). The p.F36V mutant of FK506-binding protein 12 (FKBP) was fused to the C-terminus of MYO7A-HMM for conditional homodimerization using an FK506 derivative, AP20187. Conditional heterodimerization between FKBP and the FKBP-rapamycin binding domain (FRB) by a Rapalog, AP21987, was used to tether MYO7A-HMM to the plasma membrane or the F-actin core.

Results: In vestibular hair cells expressing HaloTag-fused proteins, the density of fluorescent puncta decreases as the concentration of JFX554 ligands decreases. HaloTag-actin molecules are distinguished from each other with 0.01–0.03 nM of JFX554 ligands. Fluorescent intensities of HaloTag-actin puncta show a quantum distribution consistent with single-molecule detection. Single molecules of HaloTag-fused MYO7A and its fragments are visible with a slightly higher concentration of JFX554 ligands, 0.3–0.6 nM, suggesting their low expression level. Imaging of MYO7A in stereocilia is established using HaloTag-MYO7A-HMM-FKBP conditionally dimerized by 200 nM AP20187. Kymograms generated from time-lapse acquisition every 1 sec are useful for visualizing processive movements of MYO7A-HMM, which occur at 101 ± 53 nm/s (n = 42; mean ± SD) in stereocilia. Processive movements are also detected for a constitutively active mutant, MYO7A-R/K, but not for full-length MYO7A suggesting that MYO7A can dimerize (or oligomerize) in stereocilia when the motor domain is exposed. MYO7A-HMM does not show processive movements when tethered to the plasma membrane or F-actin, both of which are possible with MYO7A's interacting partners.

Conclusions: An experimental workflow to visualize single protein molecules in live hair cells was established and utilized to analyze MYO7A's trafficking in stereocilia. We are improving this workflow to analyze various protein-protein interactions necessary for developing functional stereocilia.

T47. MYO15A Rescues Elongation of Developmentally-Stunted Stereocilia in Adult Hair Cells

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Category: Hair Cells: Anatomy & Physiology

Background: Actin-based cytoskeletal projections called stereocilia underlie hair cell mechanosensory function. Genetic mutations that stunt stereocilia growth result in profound deafness in both mouse models and human populations. Examples include genes encoding the Elongation Complex (EC) proteins, which are required for stereocilia elongation and row-specific patterning of the stereocilia bundle. Gene therapy approaches in mouse mutants lacking functional EC proteins WHRN or EPS8 showed promising outcomes at early postnatal stages. However, AAV-based Whrn or Eps8 gene replacement in adult mice no longer rescued stereocilia growth or patterning, suggesting that interventions may be limited to a strict developmental window. Gene replacement has not yet been piloted for remaining EC members that provoke a similar stereocilia phenotype when mutated: MYO15A, GPSM2, and GNAI.

Methods: As Myo15a transcript size poses a challenge for viral packaging, we created a Cre-inducible mouse model that expresses the developmental Myo15a isoform 2 fused to HaloTag (ROSA26 DIO-HaloTag-Myo15a). To achieve timed, hair-cell-specific Cre expression, we used either embryonically-expressed Atoh1-Cre or Gfi1-CreERT induced with tamoxifen in 6-week-old adults. To supply GPSM2-GNAI, the polarization module of the EC, we generated an AAV-PHP.B viral vector expressing HA-Gpsm2-P2A-Gnai3. We used the motor-deficient Myo15a shaker-2 allele and a Gpsm2 null allele to assess possible rescue of stunted stereocilia growth phenotypes with confocal microscopy. Stereocilia height and number were measured using Imaris software, and row organization was assessed by immunolabelling row-1- or row-2-specific proteins.

Results: Early Atoh1-Cre-driven induction of HaloTag-Myo15a in shaker-2 mice rescued both elongation and row specificity, resulting in apparently normal stereocilia bundles. In Myo15a+ controls, early HaloTag-Myo15a overexpression led to stereocilia overelongation following a tonotopic gradient where shorter stereocilia at the cochlear base overgrew dramatically compared to the natively longer stereocilia toward the apex. Mature induction of HaloTag-Myo15a did not cause obvious overelongation of phenotypically normal Myo15a+ bundles, but remarkably resumed elongation of stunted shaker-2 stereocilia at 6 weeks of age. Stereocilia tip localization of other EC proteins was restored in adult shaker-2 stereocilia expressing HaloTag-Myo15a. However, elongated shaker-2 stereocilia lacked a row-specific height gradient and had uniform enrichment of row-1 protein markers. In ongoing work, we are co-expressing AAV-delivered GPSM2-GNAI with HaloTag-Myo15a to test whether stereocilia bundle polarization and row height gradation can be belatedly achieved in adult mutant hair cells. We confirmed that HA-GPSM2 localizes to row-1 stereocilia tips when delivered at both mature and postnatal stages,

and further, that AAV-HA-Gpsm2-P2A-Gnai3 delivery rescues abnormal stereocilia bundle morphology in Gpsm2 null mice at early postnatal stages.
Conclusions: Late expression of Myo15a is uniquely able to promote growth in stereocilia that failed to elongate during development. This demonstrates that stereocilia growth is not temporally restricted, which provides encouragement for adult interventions aimed at repairing developmentally stunted or damaged stereocilia.

T48. AAK1 Regulates Membrane Homeostasis of Cochlear Hair Cells

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Category: Hair Cells: Anatomy & Physiology
Background: We recently reported a common variant rs1396793 in the 5'UTR of adaptor-associated kinase 1 (AAK1), to be associated with a reduced risk of noise-induced hearing loss (NIHL). Aak1 regulates clathrin-mediated endocytosis (CME) through phosphorylating the u2 subunit of adaptor protein-2 complex (Ap2). Aak1 expresses highly in cochlear hair cells and spiral ganglion neurons. Active CME has been previously found around the hair cell cuticular plates, while the physiological function of CME in cochlear hair cells was only recently explored in terms of its role in susceptibility to ototoxic agents. We here expand from our previous finding that upregulated Aak1 protects hearing from noise exposure and found that the downstream activities of Aak1 is to maintain hair cell apical membrane homeostasis and structural integrity.
Methods: Aak1-flox and Ap2m1-flox mice were crossed with Gfi1-cre or Atoh1-cre mice to generate Aak1 or Ap2m1 hair-cell conditional knockout mice (Aak1-cKO or Ap2m1-cKO). Hearing functions were measured by ABR and DPOAE. Morphology of hair cell stereocilia and cuticular plate were assessed by scanning and transmission electron microscopy. Imaging of Annexin V labelling, localization of phosphorylated-Ap2m1 and Rab5a were performed by confocal microscopy.
Results: In general, the ABR and DPOAE thresholds of Aak1-cKO mice were normal on P30 but elevated progressively thereafter. By P90, Aak1-cKO mice were profoundly deaf with OHC death in a base-to-apex severity gradient.
Our first finding was that Aak1 in hair cells is involved with phosphorylation of adaptor protein Ap2m1, which is crucial for cargo recognition in endocytosis. Phosphorylated Ap2m1 signals were found around the cuticular plates of inner and outer hair cells, and in the basal part of IHCs of the WT mice, while completely absent in the hair cells of Aak1-cko mice.

Second, Aak1-cKO mice maintained normal stereocilia and kinocilia morphology until P7. On P9, the OHCs developed blebbing of the cuticular plate and degeneration of hair bundle. Cytoplasmic channels around the cuticular plate gradually enlarged, compromising the cuticular plate integrity. Stereocilia of Aak1-cKO OHCs were intensely labelled by Annexin V, indicating phosphatidylserine externalization.

Third, to validate that Ap2m1 is downstream to Aak1, Ap2m1-cKO mice were tested and showed similar but more severe phenotype to Aak1-cKO mice. Large blebbing were found in the cuticular plates of OHCs at P9 and eventually developed into massive loss of OHCs by P21.

Fourth, Rab5a, an early endosome marker was found accumulated around the OHC cuticular plate of both Aak1-cKO and Ap2m1-cKO mice, suggesting obstruction of membrane trafficking.

Conclusions: Our results showed Aak1 is essential for the maintenance of OHC stereocilia and cuticular plate by regulating membrane homeostasis in the OHC apical pole. Further investigations include confirmation of Ap2m1 and CME as downstream of Aak1, and the role of CME in the pathogenesis of NIHL and ARHL.

T49. The role of PCDH15-CD2 Phosphorylation in Hair Bundle Morphogenesis and Function

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Category: Hair Cells: Anatomy & Physiology
Background: Hearing depends on the precise formation of V-shaped stereociliary hair bundles on cochlear auditory hair cells. Key to hair bundle formation and function are extracellular filaments including tip-links, lateral links, ankle links, and kinociliary links. Protocadherin 15 (PCDH15) is a crucial component of these links and mutations in PCDH15 cause Usher syndrome. Of the three PCDH15 isoforms, the CD2 isoform is essential for kinociliary and tip links. Its absence leads to kinocilium detachment, hair bundle orientation defects, impaired mechanotransduction (MET), and profound deafness. However, CD2's binding partners and interaction mechanisms remain unclear.
Our lab found that a Wnt/G-protein pathway involving kinases like AKT, PAK, and GSK3b regulates kinocilium positioning and hair bundle formation. We hypothesized these kinases phosphorylate the CD2 C-terminal tail to regulate its binding with PDZ domain proteins, affecting hair bundle development. Using immunoprecipitation and tandem mass spectrometry (MS/MS), we identified three phosphorylation sites on the CD2 C-terminal tail near its PDZ-binding motif (PBM).

Methods: To investigate the role of phosphorylation sites (S1771, S1776, T1781) in PBM-PDZ interactions, we generated CD2 expression constructs carrying phospho-deficient or phospho-mimetic mutations via site-directed mutagenesis. We performed affinity pull-down assays to examine interactions between mutant CD2 and the PDZ protein Whirlin, a known CD2 binding partner.
To determine the role of CD2 phosphorylation in vivo, we generated three CD2 knock-in mouse models carrying corresponding phospho-mutations using CRISPR-Cas9. Hair bundle morphology and kinocilium positioning were analyzed using fluorescence microscopy, and CD2 localization was evaluated using super-resolution fluorescence microscopy. To assess

mechanotransduction and hearing, we performed FM1-43 dye uptake assays at P6 and auditory brainstem response (ABR) measurements in adult mice.

Results: In vitro binding assays showed that phospho-deficient mutations decreased CD2's binding affinity to Whirlin, with the mutant eliminating all phosphorylation sites (CD2-4A) showing the most significant impairment. Conversely, the phospho-mimetic S1776D increased CD2's binding affinity to Whirlin. These results suggest that CD2 phosphorylation regulates its interaction with Whirlin and possibly other PDZ-containing partners.
The three CD2 knock-in mice exhibited mild defects in kinocilium positioning but did not show kinocilium detachment or hair bundle structural defects at P0, suggesting CD2 phosphorylation plays a minor role in hair bundle development. Interestingly, CD2 immunolocalization via super-resolution microscopy revealed reduced staining intensity in CD2-4A hair cells, suggesting phosphorylation affects CD2's targeting to the hair bundle. Moreover, CD2-4A mice showed a modest reduction in FM dye uptake and significantly higher auditory brainstem response (ABR) thresholds at 22kHz and 32kHz. Experiments to measure MET current and ultrastructural analysis of mutant hair bundles are ongoing. This work is supported by a Fellowship Grant from the AOS.

Conclusions: Our biochemical experiments in vitro and genetic analysis of CD2 knock-in mice in vivo indicate a critical role of CD2 phosphorylation in hearing function.

T50. Novel Heterozygous USH1C Mutation Impacts Hair Cell Mechanotransduction and Causes Progressive Hearing Loss

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Category: Hair Cells: Anatomy & Physiology
Background: The USH1C gene encodes harmonin, a crucial scaffolding protein for maintaining normal mechanosensory function in hair cells. Pathogenic USH1C mutations led to hereditary syndromic or non-syndromic hearing loss (NSHL) following an autosomal recessive pattern. However, autosomal dominant (AD) forms of USH1C-related hearing loss are rare, and their underlying pathogenic mechanisms remain largely unclear.
Methods: We performed exome sequencing on family members with a AD-NSHL pedigree and identified gene variant USH1C c.701C GREATER THAN T; p.Pro234Leu. A USH1C c.701C GREATER THAN T knock-in mouse model was then generated using CRISPR/Cas9. The hearing phenotype of the KI mouse was evaluated by ABR and the histological and physiological characteristics of cochleae hair cells were examined using super-resolution immunofluorescence staining, SEM and patch-clamp recording. The involvement of aberrant hair cell development and mechanotransduction activity was evaluated using biochemical and biophysical experiments.
Results: We identified a novel heterozygous missense variant (c.701C GREATER THAN T; p.Pro234Leu) of USH1C gene in a Han Chinese family. The affected individuals with symmetric hearing impairment, as evidenced by increased detection of pure-tone audiometry thresholds in either the right or left ear. The Ush1C knock-in mice exhibited histological and physiological abnormalities in the cochleae, in particular progressively elevated hearing thresholds, increased

susceptibility to noise, fusion or loss of hair cell stereocilia, and reduced hair cell mechanoelectrical transduction (MET) activity. Mechanistically, the biochemical and biophysical experiments showed that the mutation disrupts the interaction between the PDZ2 domain of harmonin and cadherin 23, a tip link component, leading to improper MET machinery assembly.
Conclusions: This study identified a novel mutation (p.P234L) in the USH1C gene that contributes to autosomal dominant NSHL, representing a novel inheritance pattern for USH1C-related hearing loss. The findings expand our understanding of the genetic basis of this condition and can aid in the diagnosis of genetic hearing loss. Moreover, the study provides insight into the pathogenic mechanisms that underlie autosomal dominant NSHL, which could aid the development of targeted therapies for hearing impairment. Additionally, this research offers new insights into the assembly of the hair cell MET apparatus, which could contribute to a broader understanding of the molecular basis of hearing loss and guide future research efforts in this field.

T51. Hair Cell Apoptosis and Scramblase Activity in Tmc1 Mutations

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Category: Hair Cells: Anatomy & Physiology
Background: Sound transduction in cochlear hair cells arises by activation of mechano-electrical transducer (MET) channels for which transmembrane channel-like protein isoform 1 (TMC1) is the pore forming subunit. We made mice harboring mutations in TMC1 that culminate in hair cell apoptosis and deafness by postnatal day (P) 28. However, mechano-transduction is normal at P6, when TMC1 is fully expressed. The goal is to understand when and how apoptosis is triggered.
Methods: We studied three mutations, Tmc1 p.D569N, p.M412K and p.T416K. MET channels in all mutants were gated normally, albeit with diminished Ca2+ permeability at P6. T416K was the least affected and D569N showed reduced TMC1 expression. Three methods were used to assay apoptosis: (i) Calcein-AM, entering cells and becoming fluorescent on demethylation, a reaction absent in apoptotic cells; (ii) Mitochondrial dysfunction assayed with Mitotracker or Mitolight; (iii) Scramblase activity, phosphatidyl serine externalization, revealed by Annexin V labeling. Scramblase activity was previously observed after treatment with aminoglycoside antibiotics like dihydrostreptomycin (DHS; Goodyear et al. 2008) or in Tmc1 mutants (Ballesteros and Swartz 2022). Cochleas from P3-P7 mice were fixed in paraformaldehyde and viewed in a Nikon A1 confocal. ABRs were measured from P14 on.
Results: We confirmed that DHS triggered apoptosis in outer hair cells from both wild type and Tmc1 mutant mice. After exposure to 0.1 mM DHS, Annexin V label was still observed in Tmc1 knockout mice, but only if Tmc2 was present. Thus, either TMC1 or TMC2 can support scramblase activity. Annexin V label was accompanied by reduced labeling by Calcein-AM or Mitotracker, endorsing it as an indicator of apoptosis. All three methods that demonstrate apoptosis were seen in P6 Tmc1 mutants, with the strength of label increasing from T416K to M412K to D569N, proportional to the degree of reduction in channel Ca2+ permeability. Reduced labeling with Mitotracker was directly correlated with reduced label of Calcein-AM.

Mitochondrial targeting was confirmed with the uncoupling agent FCCP, and Mitolight assay to monitor mitochondrial membrane potential. ABR measurements showed both Tmc1 p.D569N and Tmc1 p.M412K mice, but not Tmc1 p.T416K, were deaf by P15.
Conclusions: Our results demonstrate hair cells in Tmc1 mutants have already embarked on an apoptotic pathway at P6 despite MET channel activation appearing normal. The pathway may partly involve changes in cytoplasmic Ca2+, as the severity of Tmc1 mutations was proportional to the reduction in Ca2+ permeability of the mutant channel. Scramblase activity, probably activated by changes in Ca2+ and mediated by TMC1, is seen only in apoptotic hair cells and is not a modulator of normal MET channel function.

T52. Identifying Key Molecules Involved in the Biogenesis, Transport, and Recycling of Synaptic Vesicles at Ribbon Synapses

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Category: Hair Cells: Anatomy & Physiology
Background: Sensory hair cells utilize specialized ribbon synapses to reliably transmit sensory information to the brain. Ribbon synapses have high rates of spontaneous vesicle release and function without fatigue. To sustain this level of release, a continuous supply of synaptic vesicles must be trafficked to the presynapse. Recently we showed that Kif1a-mediated transport delivers new synaptic vesicles along microtubules to the hair cell presynapse. Loss of Kif1a resulted in a depletion of synaptic vesicles at the synapse, along with impaired neurotransmission. However, many other factors are needed to ensure that synaptic vesicles are made, transported and recycled appropriately. We use the zebrafish model to uncover and study these factors.
Methods: To study synaptic vesicle distributions in hair cells, we use the zebrafish lateral-line system and use immunohistochemistry against synaptic vesicle markers such as Vglut3, Rab3a, and CSP. Additionally, to visualize synaptic vesicle populations in vivo, we use the vital dye Lysotracker. We are using RNA-FISH to investigate expression of candidate genes. Then, to assess gene function we are using CRISPR-Cas9 to create mutations in candidate genes involved in synaptic vesicle packaging, transport, and recycling in zebrafish. Further, we will assess ribbon synapse function in our mutants using in vivo calcium imaging and electrophysiology, to measure evoked calcium responses and vesicle release.
Results: Currently we are using CRISPR-Cas9 to test the following candidate genes: Ap3, Rab3a/Madd, and Dynamin2, that we hypothesize are critical for synaptic vesicle biogenesis, transport, and endocytosis, respectively. Preliminarily, we have found that first-generation ap3m2 CRISPR mutants show less Lysotracker label throughout the cell when compared to wild-type zebrafish hair cells. This indicates that Ap3m2 may be important to produce new vesicles. We are currently testing our other candidate genes using a similar approach.
Conclusions: Characterizing these mutants will allow us to shed light on the process of vesicle packaging, movement, and recycling in hair cells. This work will deepen our understanding of the multiple pathways that converge to supply synaptic vesicles at ribbon synapses.

T53. The Cochlear Hook Region Detects Harmonics Beyond the Canonical Hearing Range in Guinea Pigs

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Category: Inner Ear: Cochlear Mechanics

Background: Ultrasound, or sound at frequencies exceeding the conventional range of human hearing, is not only audible to mice, microbats, and dolphins, but also creates an auditory sensation when delivered through bone conduction in humans. Although ultrasound is utilized for brain activation and in hearing aids, the physiological mechanism of ultrasonic hearing remains unknown. In this study, we hypothesized that the hook region, the basal edge of the cochlear turn, plays a key role in ultrasound perception. The purpose of this study is to investigate the vibratory response of the sensory epithelium to ultrasound stimuli.
Methods: To apply ultrasonic stimulation, a piezo actuator with a stainless steel rod was attached to middle ear ossicles of an anesthetized guinea pig. Auditory brainstem response (ABR) and cochlear microphonic potential (CM) were recorded. Using a made-to-order optical coherence tomography (OCT), we imaged the epithelium in the hook region and measured vibratory response induced by the ultrasonic stimulation.
Results: In guinea pigs, we found that ultrasound above the hearing range delivered through ossicles of the middle ear evokes an ABR and a mechano-electrical transduction current through hair cells, as shown by the CM. The CM synchronizes with ultrasound, and like the response to audible sounds is actively and nonlinearly amplified. In vivo optical nano-vibration analysis revealed that the sensory epithelium in the hook region, the basal extreme of the cochlear turns, resonates in response both to ultrasound within the hearing range and to harmonics beyond the hearing range.
Conclusions: Electrophysiological and optical measurements in guinea pigs revealed that the hair cells in the cochlear hook region electrically and mechanically resonate both with ultrasound within the hearing range and with harmonics beyond the hearing range. Our data indicate that the original detectable frequency range of the cochlea is not equivalent to the hearing range determined by air-conducted sound stimuli via tympanic membrane. The upper limit of the detectable frequency range is much higher than that of the hearing range.

T54. Intracochlear Responses Following Acoustic Trauma

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Category: Inner Ear: Cochlear Mechanics

Background: The ear is a remarkable organ that adapts to a very wide range of sound levels and frequencies. However, the delicate structures that transform incoming acoustical energy into neural signals for the brain are susceptible to acoustic trauma. Damaged ears are no longer able to perform these crucial tasks, resulting in hearing impairment. What is not well known is how damage changes the cochlea's overall mechanical response to sound. It is known that individual

cells/structures within the cochlea do not operate in isolation; their bidirectional motion coupling within and along the entire cochlear partition (CP) is essential to create the vibrations that are transduced by hair cells and thus shape the acoustic image of the outside world.
Knowledge of cochlear mechanics in “normal ears” is quickly advancing using optical coherence tomography (OCT). More and more evidence show that the complex motion of the CP is the key to maintain cochlear function. By comparing CP responses obtained before and after acoustic trauma from the same CP cross-section, our data provide new insights into the mechanisms underlying acoustic trauma induced hearing loss.

Methods: Most of the methods have been detailed previously (Meenderink et al., 2022). We utilized OCT (Thorlabs Telesto III TEL321C1) to precisely characterize sound-induced motions of the CP at the 2nd turn of the gerbil cochlea. Acoustic trauma was induced by exposing the ear to high-intensity tones (100 dB SPL) for incrementally increasing durations. Cochlear damage was assessed using two-tone induced distortion product otoacoustic emissions (DPOAEs). Variations of the CP motions were illustrated by the reconstructed sound-induced 2D vibrometry maps (both amplitude and phase) at specific frequency from multiple A-lines with a great spatial resolution of 10 μm. Then, we use classical sensitivity curves obtained from the outer hair cells (OHC) and other surrounding regions to illustrate the tuning properties.
Results: DPOAE reduction was highly consistent across experiments, allowing us to categorize damage into three levels: low, mid, and high degrees. As damage increased, the DPOAE reduction expanded from localized to more basal regions. Intracochlear 2D vibrometry maps revealed a systematic, level-dependent effect on response magnitude, with 30 dB responses being most affected, while 70 dB responses showed the least change. The reduction in the outer hair cell (OHC) region spread to adjacent structures, though the relative motion between the OHCs and surrounding structures changed little. Cochlear gain (the sensitivity difference between low and high-intensity responses) decreased as damage increased, accompanied by a loss of frequency selectivity. Additionally, phase accumulation shifted towards lower frequencies with increasing damage.
Conclusions: Our findings provide intracochlear evidence on the effects of acoustic trauma, explaining various sensory loss in frequency selectivity and sensitivity.

T55. The Contribution of Tapering to Cochlear Tonotopy

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Category: Inner Ear: Cochlear Mechanics
Background: A classic expression for the cochlear characteristic frequency is $\fc\propto\sqrt{k/m}$ with m mass and k stifness. In this expression k represents basilar membrane (BM) stiffness; a general expression for the mass is $m=m_o+m_f$, where m_o is organ of Corti mass, and m_f represent inertial load of the fluid in the scalae.
In some modeling frameworks m_o is dominant ($m\approx m_o$) while in others $m_o\ll m_f$ so that $m\approx m_f$. Regardless of this controversy, m is often considered to be roughly constant along the cochlea (e.g., in classic box models) requiring $k\propto \fc^2$. However, experimental estimates of BM stifness have consistenly shown that $k\sim \fc$. We

review this issue, by focusing on the role of the tapered geometry of the cochlear duct for the cochlear tonotopy.

Methods: We use 2D finite-difference models to represent the passive mechanics at the base of the cochlea; the physical partition stiffness is determined to ensure the correct tonotopy and BM delays. To test the role of the geometry of the cochlear duct, we compare constant-height box models with models including realistic tapering of the duct. To further gain insight, we compare results of models where $m\approx m_f$ ($m_o=0$), with models where $m\approx m_o$.
Results: In models with realistic tapering of the duct $k\sim \fc$ is necessary to ensure the correct tonotopy and cochlear delays at the base of the cochlea, in accordance with published estimates of BM stiffness and previous modeling work. This happens regardless of whether the organ of Corti of mass is considered significant or not. When assuming $m\approx m_0$, the model requires that $m_o\propto \fc^{-1}$, consistent with recent {\em in vivo} estimates of the organ of Corti area in the gerbil base. In box models of constant height $k\sim \fc^2$ is necessary to ensures the correct tonotopy and cochlear delays at the base of the cochlea, regardless of what term dominates m.
Conclusions: The most striking observation is that tapered models where $m_o=0$ require $k\sim \fc$, implying that $m_f\propto \fc^{-1}$. While this is an excellent mathematical explanation, a simple yet insightful explanation for how cochlear tonotopy comes about does not rely on the concept of resonance. When $m_o=0$, the partition mechanics has a stiffness and a viscous dominated region; the transition between these two regions happens at the frequency $f_t\propto k$ assuming constant damping. Approaching f_t, the BM response receives a short-wave hydrodynamic boost, producing a modestly peaked response. Above f_t, dissipative forces become dominant, attenuating the traveling wave. According to this reasoning, the peak BM response occurs just below f_t, so that $\fc\approx f_t\propto k$. A physically analogous situation happens when $m\approx m_o$, with the difference that the partition inertia enhances short-wave hydrodynamics producing a steeper and more realistic roll-off past CF.

T56. Integrating Mouse Cochlear Proteomics and Single-Cell Transcriptomics to Develop a Comprehensive Database of Inner Ear Tissue Expression

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Category: Inner Ear: Cochlear Mechanics
Background: Proteomic analysis of sensory organs such as the cochlea is essential for understanding the molecular mechanisms of hearing, studying the pathophysiology of hearing disorders, identification of tissue specific protein biomarkers, and enhancing the design of sensory prosthetics. There is, however, limited proteomic data on tissue specific expression in the inner ear. We therefore sought to review publicly available proteome datasets and correlate them with published adult cochlear single cell RNA-Seq datasets to create cochlear cell type-specific protein expression database

Methods: Published single cell RNAseq data was analyzed utilizing an in-house bioinformatic pipeline and compared to a published publicly available cochlear proteomic datasets. The retrieved proteomic datasets were cross-referenced against cochlear single cell transcriptome datasets to identify cell- and tissue-specific protein expression in the cochlea. Immunohistochemistry and/or in situ RNA hybridization methods were used to validate some of the identified cochlear tissue specific protein markers
Results: Combining analyses of transcriptome and proteome datasets offers the opportunity to generate a multidimensional resource that correlates cochlear RNA expression to protein expression. As a result, we identify key proteins that could serve as tissue specific markers and corelating the protein expression to their corresponding mRNA transcripts gave insights into their functional relationships and regulatory mechanisms. The study also expands our knowledge on the distribution and expression of proteins across different cochlear cell types, including hair cells, supporting cells, stria vascularis, and neurons
Conclusions: The results of our study provide preliminary information on the proteome-transcriptome correlation in the cochlea, suggesting a framework for future comprehensive inner ear cellular proteome resources for the hearing and balance research community. Comprehensive cochlear tissue specific proteomic analysis will serve as a pivotal tool to explore the molecular basis of hearing disorders, and aid in finding potential biomarkers and therapeutic targets.

T57. Time-Course of MHCII Expression in Rat Spiral Ganglion After Hair Cell Loss

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Category: Inner Ear: Damage and Protection of Neurons & Synapses
Background: Spiral ganglion neurons (SGNs) receive auditory information from cochlear hair cells, the auditory receptor cells, and transmit it to the CNS. SGNs gradually degenerate after hair cell death, potentially adversely affecting the efficacy of cochlear implants. The reason for post-deafening SGN degeneration remains unclear. Gene expression profiling shows dramatic upregulation of immune response-related genes, including MHCII, a molecule involved in antigen presentation to lymphocytes and adaptive immune response, in the spiral ganglion following deafening. Preliminary data showed the MHCII knockout prevents SGN degeneration, indicating a causal role of MHCII-mediated antigen presentation and adaptive immune response in SGN loss. We investigated if the temporal expression of MHCII matches SGN degeneration and if MHCII-mediated antigen presentation occurs before SGN degeneration. We show by immunolabeling that the MHCII expression increases after deafening, before SGN loss.
Methods: Male and female Sprague-Dawley rats were injected with kanamycin in the second postnatal week to kill hair cells and euthanized at postnatal days 21, 32, 39, 45 and 70. Sections were immunolabeled with the following antibodies: Tuj1 to label SGNs, anti-MHCII antibodies to identify MHCII-expressing potential antigen-presenting cells, and anti-IBA1 to label macrophages. Image analysis was performed using IMARIS software. The outline of the Rosenthal canal for each turn was manually traced to measure the cross-sectional area and to calculate macrophage, MHCII+APC which were counted in every fourth near-midmodiolar section.

Results: In deafened rats at postnatal day 21, the number of MHCII+ macrophages is not significantly different from hearing rats. At P32, P39, P45 and P70, deafened rats showed increased MHCII+ macrophages and percentage of MHCII-expressing macrophages, compared with hearing controls.
Conclusions: Previous findings from RNA sequencing showed the upregulation of MHCII genes after SGN death at P32 and P60. Consistent with that, immunohistology shows an increase in MHCII+ macrophages after P32, P39, P45 and P70. The significant SGN degeneration starts at P39 so our results show that post-deafening upregulation of MHCII occurs before SGN degeneration. These results implicate the temporal match between the upregulation of MHCII expression and SGN degeneration. Together with the protection of neurons in MHCII-KO mice after deafening in a previous study, the MHCII gene is shown to play an important role in SGN loss after deafening.

T58. SGN-Specific Integrin Alpha 8 Knockout Mice Displayed Altered Synaptic Count

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Category: Inner Ear: Damage and Protection of Neurons & Synapses
Background: The synaptic connection between inner hair cells (IHCs) and spiral ganglion neurons (SGNs) is crucial for fast and synchronized neurotransmitter release, enabling the precise relay of sound signals from the cochlea to the central auditory system. Integrin alpha 8 (Itga8), a heterodimeric, bidirectional cell adhesion receptor, has emerged as a key player in cochlear function, particularly in regulating cilia biogenesis within sensory cells. In this study, we utilize a conditional knockout model driven by Celf4iCreER mice to target SGNs, achieving consistent and precise recombination efficiency specifically. The loss of Itga8 significantly affects hair cell synapses, demonstrating Itga8's critical role in cochlear development and function. Our findings establish Itga8 as an essential regulator of synaptic integrity, and its disruption during development leads to profound abnormalities in synaptic counts, positioning Itga8 as a vital component in the pathophysiology of hearing disorders.
Methods: For this study, we used genetically engineered mouse models from Jackson Laboratory (Strain #: 034391) to knock out Itga8 specifically in SGNs using the Celf4 promoter. Cre-recombination was induced with tamoxifen at a dosage of 9 mg/40 g, administered in two doses of 4.5 mg each on embryonic days 14 and 15 (E14 and E15) within the expression window of Itga8 (E16-P6). Samples were collected at various developmental stages from embryonic to adulthood.
Using immunohistochemistry techniques, detailed morphological assessments were conducted throughout the three cochlear regions (apical, mid-turn, and base).

Results: Using our cell type-specific Cre-derived mouse line targeting SGNs, we achieved high recombination efficiency of Celf4-positive cells expressing tdTomato in the SGNs of Celf4-CreER^EGFP+/-; Rosa-Tdtomato+/+ mice. Notably, we observed tonotopic variation in Cre recombinase efficiency across different cochlear regions, including the apical, medial, and basal turns. Immunohistochemistry was performed using hair cell markers, SGN markers, and synaptic markers. Our analysis revealed significant alterations in synaptic counts in the Itga8 conditional

knockout between IHCs and outer hair cells (OHCs) and among the regions as well, further indicating region-specific synaptic changes within the cochlea.
Conclusions: In conclusion, our findings demonstrate that the specific deletion of Itga8 gene from the SGNs leads to significant changes in synaptic count, highlighting the essential role of Itga8 in maintaining synaptic integrity and function.

T59. Cochlear Health in Ci Users With DFNA9

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Category: Inner Ear: Damage and Protection of Neurons & Synapses
Background: DeaFNess Autosomal 9 (DFNA9) is an autosomal dominant hereditary disorder caused by pathogenic variants in the COCH gene. These mutations induce the formation of aggregates that are toxic to the fibrocytes in the extracellular matrix, ultimately leading to degeneration of spiral ganglion neurons (SGNs). An important biomarker for evaluating the health and functioning of the SGNs, is the electrically evoked compound action potential (eCAP).
The aim of this study was is to evaluate the health of the SGNs in CI users with DFNA9 by studying their eCAP responses and impedances.

Methods: Fifteen carriers of the p.Pro51Ser variant in the COCH gene who received a cochlear implant (CI) (DFNA9 group) and 15 matched control CI subjects without DFNA9 were included. All subjects used a MED-EL Synchrony implant. Impedances and eCAP threshold, amplitude and slope of the eCAP amplitude growth function (AGF) were compared between both groups. Matching of the two groups was based on sex, age at implantation, duration of deafness, and type of implant.
Results: Analyses of electrode impedances between DFNA9 and non-DFNA9 patients, show a significant interaction between time and group in the middle and basal electrodes, indicating that electrode impedances were similar in the early phase after implantation between the two groups, but increased significantly more for the DFNA9 group up to one year after implantation. Secondly, the results show that the success rate (present or absent) to record eCAP responses is lower in the DNFA9 group: eCAPs were detectable in 75.5% of the intraoperative measurements (145/192) in comparison to 96.9% (186/192) in the group without DFNA9. ECAP absence in the DFNA9 group was observed across the entire electrode array, but more pronounced in the basal region (channels 11 and 12). Additionally, comparing the parameters of the AGF, the maximum eCAP amplitude was consistently smaller and the AGF slope consistently shallower for the DFNA9 group compared to the control group throughout the electrode array. Finally, the eCAP thresholds in patients with DFNA9 were higher compared to those in the control patients for all cochlear locations.
Conclusions: The results of this study reveal that both impedances and advanced eCAP measures consistently suggest a reduced excitability and neuronal health in our DFNA9 population. The increased impedances are likely to result from intrascalar aggregate and fibrosis formation seen in DNFA9, whereas the DNFA9 eCAP responses point towards the accelerated neural degeneration.

T60. Volume Electron Microscopic Mapping of Mitochondria in the Inner Ear

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Category: Inner Ear: Damage and Protection of Neurons & Synapses
Background: In the mammalian cochlea, afferent synaptic connections between the cochlear inner hair cell (IHC) and spiral ganglion neurons (SGNs) exhibit great diversity in their structure and function, which likely enables audition over a wide dynamic range. To maintain a proper synapse gradient, both pre- and post-synaptic mitochondrial networks may undergo function-morphological adaptations owing to differential local energy demands and ion homeostasis.
Methods: Utilizing volume electron microscopy and machine-learning-assisted image analysis, we quantitatively mapped the organization and morphologies of mitochondria in both IHCs and SGNs.
Results: Specifically, in various mouse modes of ribbon loss, we set out to study the spatiotemporal changes in the distribution of ribbon-associated mitochondria at the IHC basolateral pole, as well as terminal enrichment and heterogeneity of postsynaptic SGN mitochondria.
Conclusions: The anticipated results may reveal mechanistic insights in pre- and postsynaptic contributions to the IHC deafferentation - one of the major causes for coding deficits of the cochlea.

T61. The Effect of Nitric Oxide on Peripheral and Central Auditory System

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Category: Inner Ear: Damage and Protection of Neurons & Synapses
Background: There are overlapping symptoms between vestibular migraine and Meniere's Disease. We first aimed to investigate the possibility of endolymphatic hydrops in migraine. Furthermore, we included a CGRP (Calcitonin-gene-related peptide) receptor blocker as the treatment group to study its effects on the cochleovestibular system.
Methods: There were 30 male Wistar rats aged 2-5 months. Group 1 was the control. Group 2 was the migraine with diluted NTG injection at a dose of 10 mg/kg IP every other day for nine days. Group 3 was the treatment group with Olcegepant in a volume of 1 mg/kg IP one hour and 15 minutes after the NTG injections. The hearing thresholds were determined by click and tone-burst ABR at 6, 12, 16, 20, and 32 kHz. DPOAE was performed at 6, 8, 10, and 12 kHz. ECOG was performed, and SP/AP ratios were calculated. The orofacial formalin test, Open field test, tail hang reflex, and head circling were evaluated. Tail blood samples for TNF-alpha, IL-6, and intracardiac blood samples for CGRP were gathered on day nine. The trigeminal ganglion and organ of corti, saccule, stria vascularis were dissected after the perfusion. Half of the animal's

cochleas were paraffin-embedded for staining. The other half of the cochleas were immunostained after the whole mount dissection.
Results: The migraine group was more immobile (p=0.001). DPOAE results were extracted; emissions at 6, 8, 10, and 12 kHz were higher on day nine compared to day one in the migraine group (p=0.0006, p=0.002, p=0.009, p=0.014). There was a substantial decrease in all the above frequencies in the treatment group (p=0.002, p=0.028, p=0.018, p=0.006). There was also a significant difference at 6 kHz tone-burst ABR threshold change between the control and the migraine and the treatment groups (respectively p=0.038, p=0.018). In addition, at 12 and 20 kHz, there was a significant difference between the control and the migraine groups (p=0.030, p=0.018). Tone burst ABR thresholds were lower on day nine than on day one in the migraine group. SP/AP ratios were compared within the groups; the treatment group had a lower ratio on day nine (p=0.049).
TNF-Alpha was significantly high in the migraine group (p=0.048).

The PSM (proportion of scala media) was calculated through the H and E staining of the cochlea sections, and there was no significant difference between the groups. The CGRP count was higher in the treatment group and significantly different from the migraine group (p=0.02) and the control (p=0.006). Also, the glutamate R2 was considerably higher in the treatment group than the migraine group (p=0.012).

Conclusions: NO has a beneficial effect on the central and peripheral auditory systems. Inhibiting NO lowers the SP/AP ratio. In the efferent system, there is a signaling pathway for a reciprocal relationship of glutamate with CGRP.

T62. Personalized Porous Gelatin Methacryloyl Sustained-Release Nicotinamide Protects Against Noise-Induced Hearing Loss

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Category: Inner Ear: Damage and Protection of Neurons & Synapses
Background: There are no Food and Drug Administration-approved drugs for treating noise-induced hearing loss (NIHL), reflecting the absence of clear specific therapeutic targets and effective delivery strategies. Enhancing mitochondrial function remains a promising potential treatment for NIHL. NAM, a NAD+-booster able to enhance mitochondrial function, is a potential therapeutic molecule, while a sustained drug-delivery system is also required to provide long-term hearing protection for NIHL.
Methods: C57BL/6 mice aged 4 weeks with NAM local supplementation were exposed to an 8-16kHz octave-band noise at 104 dB SPL for 2 hrs. Personalized PGMA@NAM was produced by gelatin methacrylation, crosslinking and freeze drying in 3D reconstruction model of the mice cochleae. Drug diffusion efficiency of PGMA@NAM was observed in vitro and in vivo. Gelatin sponge (GS) or personalized NAM-encapsulated porous gelatin methacryloyl (PGMA@NAM)

were placed onto the round window membrane 2 days or 5 days before noise exposure. ABR test and immunofluorescence were conducted to mice at 3, 7 and 14 days post noise. Cochleae of the mice were collected 1 day post noise for NAD+ level, mtDNA copy number, mitochondrial function measurement and TEM observation. The number of mitochondria in HCs and SGNs were counted at 3 days post noise.
Results: NAD+ level reduced in cochlea after noise and noise exposure results in mitochondrial dysfunction in auditory neuron rather than hair cells. Supplementation of NAM maintain mitochondria homeostasis in cochlea and protect auditory neuron from neuroexcitatory toxic injury in vitro and in vivo. GS@NAM delivery onto round window membrane sustained NAD+ level, ROS level as well as OXPHOS gene expression levels. NAM treatment increased the number of mitochondria in the cochleae within the noise condition, especially in the IHCs and SGNs. Auditory Brainstem Response (ABR) threshold shifts significantly decreased by NAM treatment in comparison to the opposite ears with nearly 30 dB difference at the frequencies of 16 and 32 kHz after noise trauma, with synapse preservation in the IHCs. Distinct fluorescence signal in the injected ear of the PGMA group did not decrease throughout the 12-day period. When PGMA@NAM was placed on the RWM of mice 5 days before noise exposure, PGMA@NAM treatment significantly ameliorated hearing loss and synapse degeneration compared with contralateral and PGMA groups at 3 and 14 dpn.
Conclusions: NAD+ decreases in cochlear after noise trauma, and nicotinamide supplementation boosts NAD+ and prevents neuroexcitatory in cochlear by round window adminstration. Personalized porous gelatin methacryloyl (PGMA) achieves long term drug release in cochlear, and nicotinamide encapulated PGMA enhances mitochonria and synapse function, and protects noise induced hearing loss in mice.

T63. Microplastics and Inner Ear Function

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Category: Inner Ear: Damage and Protection of Neurons & Synapses
Background: Inner ear dysfunction was widespread across all age groups. While the hazardous effects of microplastics (MPs) were increasingly reported, it remains uncertain if MPs induce these problems. In this study, we investigated whether MP polyethylene affect inner ear function in a murine model.
Methods: To detect hearing loss and balance defect after polyethylene (PE) exposure, we evaluated hearing threshold levels, assessed cerebral glucose metabolism, conducted transcriptome analysis, and performed behavioral studies. C57BL/6J mice (5-week-old) were grouped into control (n = 10) and PE-fed groups (n = 10). Mice were orally administered 100 ppm/100 μL of PE every day for 4 months.
Results: We identified the accumulation of PE in the cochlea and vestibular region. The fragmented PE in inner ear was 3.00 ± 0.38 μm in size; the administered PE concentration was 1.14 ± 1.06 mg/g. Fourier transform infrared spectrometry confirmed that the properties of the recovered MP were identical with those of PE fed to the mice. Transcriptomic analysis showed up-regulation of PER1, NR4A3 and CEBPB at the PE exposed inner ear tissue and it was

confirmed using qRT-PCR, western blotting, and immunofluorescence staining. We observed abnormalities in balance related behavior assessment in the PE group. Exposure to PE increased the hearing thresholds and decreased glucose metabolism in the bilateral lateral entorhinal cortex, right primary auditory cortex, and right secondary auditory cortex.
Conclusions: We can conclude that PE exposure induced inner ear dysfunction such as hearing loss and balance disorder.

T64. JAK2: A Key Modulator of Aminoglycoside-Induced Hearing Loss

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Category: Inner Ear: Damage and Protection of Neurons & Synapses
Background: Aminoglycosides are a class of antibiotics widely used to treat severe bacterial infections. While highly effective, they can have detrimental effects on the inner ear, leading to irreversible hearing loss. The exact mechanisms underlying aminoglycoside ototoxicity are complex and not fully understood, but several pathways have been implicated, including oxidative stress, mitochondrial dysfunction, and inflammation. Janus kinase 2 (JAK2) is a nonreceptor tyrosine kinase involved in signal transduction for various cytokines and growth factors. Dysregulation of JAK2 signaling has been implicated in a number of diseases, including hematological disorders and autoimmune diseases. Here, Utilizing genetically modified mice we investigated the role of JAK2 signalling in hearing development and response to aminoglycoside ototoxicity.
Methods: Animal Models:
We generated genetically modified mice using a PAX2-Cre driver to express Cre recombinase in the inner ear. These mice were crossed with mice carrying either a loxP-flanked JAK2 exon 1 (Knockout) or loxP-flanked exon 14 and a loxP511-flanked mutated JAK2 exon 14 with a valine to phenylalanine mutation (V617F) (constitutive activation) This resulted in two lines:

JAK2 LoxP: Cre-mediated recombination removed the endogenous JAK2 exon 1, leading to a complete knockout of JAK2 in the inner ear.

Constitutive JAK2 Activation: Cre-mediated recombination inverted the mutated exon 14, resulting in a constitutively active form of JAK2.

Hearing Tests:

Hearing thresholds were assessed in both JAK2 knockout and constitutive JAK2 activation mice at 4 weeks of age and at 3 weeks after completion of the kanamycin treatment protocol by auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE) testing.

Aminoglycoside Treatment:

JAK2 knockout mice were administered kanamycin via sub-cutaneous injection at a dose of 600 mg/kg twice daily for 14 days. Control mice received saline injections.

Tissue Collection and Analysis:

At the end of the study tissues were processed for histological analysis to assess hair cell morphology and synaptic density.

Results: We generated a mouse model with a constitutive knockout of JAK2 in the inner ear. These mice did not exhibit any spontaneous hearing loss, suggesting that JAK2 is not essential for normal inner ear function. However, when exposed to aminoglycosides, these JAK2 knockout mice demonstrated significantly increased sensitivity to ototoxicity compared to wild-type controls. Conversely, mice with constitutively activated JAK2 in the inner ear developed hearing loss even without aminoglycoside exposure.
Conclusions: Our findings suggest that JAK2 signaling plays a critical role in modulating susceptibility to aminoglycoside-induced ototoxicity. While JAK2 deletion does not directly cause hearing loss, it enhances the vulnerability of the inner ear to aminoglycoside damage. Conversely, constitutive JAK2 activation can lead to spontaneous hearing loss, indicating a complex interplay between JAK2 signaling and inner ear health. These results highlight the potential therapeutic implications of targeting JAK2 signaling for the prevention or treatment of aminoglycoside-induced ototoxicity.

T65. Cochlear Nitrative Stress and Associated Signaling in Noise-Induced Hearing Loss

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Category: Inner Ear: Damage and Protection of Neurons & Synapses
Background: Noise-induced hearing loss (NIHL) is a significant public health issue worldwide. Nitrative stress is emerging as an important factor in NIHL, but the underlying mechanisms are not fully understood. This study investigates cochlear nitrative stress, identifies nitrated cochlear proteins, and elucidates associated signaling mechanisms in noise-induced auditory dysfunction in mice.
Methods: Young adult CBA/J mice were exposed to 90 dB broadband noise 2 h/day for two weeks. Hearing threshold shift was evaluated by recording Auditory Brainstem Responses (ABR). Outer hair cell (OHC) activity was measured by recording Distortion Product Otoacoustic Emissions (DPOAE), and hair cell loss was assessed by immunohistochemistry. Cochlear synaptic dysfunction was evaluated by measuring ABR wave-I amplitude and latency and the functional synapses in the cochlea were estimated by immunostaining with anti-GluR2 and anti-CtBP2. The levels of the nitrosative stress marker 3-nitrotyrosine in the mice cochleae were measured by immunostaining with anti-nitrotyrosine. The nitrated cochlear proteins were immunoprecipitated with anti-nitrotyrosine and analyzed by mass spectrometry, and associated signaling pathways were identified using bioinformatics analysis.
Results: Noise exposure increased the hearing thresholds by 10-20 dB (p LESS THAN 0.05; n=6), affected the activity of OHCs (16, 24, and 32 kHz; p LESS THAN 0.05), and induced hair cell loss in the basal turn of the cochlea (p LESS THAN 0.05). Noise exposure decreased wave-I amplitude (Click and 32 kHz; p LESS THAN 0.05) and increased wave-I latency (24, 32 kHz; p LESS THAN 0.05), suggesting noise-induced cochlear synaptopathy. In agreement, an

examination of the pairing of pre- and post-synaptic markers indicated that noise exposure decreased the number of paired synapses (p LESS THAN 0.05; n=3) in the basal turn of the cochlea. Moreover, noise exposure significantly increased the nitrotyrosine levels in the OHCs and spiral ganglion cells (p LESS THAN 0.05), suggesting noise-induced cochlear nitrative stress. Proteomics analysis revealed that noise exposure induced the nitration of 744 cochlear proteins (n=4) while bioinformatics analysis of associated KEGG pathways indicated that the citrate cycle, oxidative phosphorylation, ribosome, and synaptic vesicle cycle were among the most highly enriched cellular processes.
Conclusions: This study indicates that noise exposure induces cochlear nitrative stress, resulting in the nitration of several cochlear proteins. More importantly, many of the nitrated cochlear proteins are associated with critical signaling pathways that regulate auditory function. Together, these findings provide new insights into the role of nitrative stress in NIHL.

T66. The Transfected Efficiency of Different AAVs in the Mouse Cochlea

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Category: Gene Therapy
Background: The virus AAV-based gene therapy currently is the predominant method used in genetic interventions for many diseases, including hearing losses. Different AAV subtypes have different cell- and tissue-specific targeting and transfection efficiency. However, the knowledge about the efficiency and cell specificity of AAVs' transfection in the cochlea is limited; the reported results also appeared confused and contradictory, which has hampered to apply the efficient gene therapies in the auditory system. In this study, we have systematically examined the transfected efficiencies of most commonly used AAVs in the cochlea to provide important and also required information for efficient performance of gene therapy in the hearing system.
Methods: Commonly used AAV1, AAVie, and AAV-Anc80L65 with different promotors were tested. The AAV with eGFP or mNeonGreen were micro-injected into the right cochlea via the posterior semicircular canal (PSCC) route. After 7, 14, and 30 days, the cochlea samples were collected, and the transfection was examined by confocal microscopy. The transfected efficiency was also measured by digital PCR (dPCR). In some mice, acoustic startle response (ASR), ABR, and DPOAE were also recorded to assess animal hearing function and hearing behavioral changes.
Results: First, we found that the transfected efficiency of AAV was largely determined by promotors. For the same AAV subtype, the transfected efficiency could be various largely with using different promotors, from almost 100% to near 0%. Second, the specificity of cell-targeting of transfection was also largely dependent on the used promotors. The difference of transfection efficiency among different AAV subtypes was visible, but less than that with different promotors. Third, the transfected efficiency was also dependent on the injection time. The transfected efficiencies and targeted cell types in newborn pups and adult mice were different. Finally, there was the cross-talking between the right (injection) ear and the left ear. The AAV transfection could be detectable in the left ear, in particularly, as the injection in pups.

Conclusions: The AAV transfection efficiency and specificity of cell-targeting in the cochlea are dependent not only on AAV subtypes but also on promotors.
Supported by NIH R01 DC017025 and DC019687 to HBZ

T67. Differential Hearing Restoration in the DFNB9 Mouse Model through Aav Gene Therapy with Human and Mouse Cdna

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Category: Gene Therapy
Background: Hearing loss is the most common sensory deficit in humans and a major concern for Public Health issues. The clinical prevalence of congenital deafness is approximately 1 in 700 newborns, 80% of these cases are attributed to a genetic cause. Deafness caused by mutations in the otoferlin gene, known as DFNB9, accounts for 2 to 8% of genetic deafness cases. Our team developed and characterized a DFNB9 murine model, demonstrating that otoferlin is crucial for the final steps of synaptic exocytosis, ensuring ultrafast vesicular neurotransmitter release at inner hair cell (IHC) ribbon synapses. This finding led to the conclusion that DFNB9 deafness is a genetically linked auditory synaptopathy. Notably, the team developed a therapeutic vector that provides the first proof of concept that viral gene therapy can effectively treat hearing impairment in a preclinical murine model of human DFNB9 deafness. The human version of this therapeutic vector is currently in phase II clinical trials for DFNB9 patients. One of the main unresolved questions in both fundamental and clinical research is whether the preclinical mouse model treated with DFNB9 gene therapy achieves similar hearing recovery and sound signal processing regardless of whether the administered vector delivers human or mouse cDNA.
Methods: To address this issue, we administered either human or mouse cDNA at two different time points, before (P2) and after hearing onset (P15) using a dual-AAV approach. After that, hearing function was evaluated using auditory brainstem responses (ABR), startle reflex, and pre-pulse inhibition (PPI). Following auditory screening, the expression and localization of otoferlin in the cochlea were verified with immunohistochemistry. Moreover, IHC membrane capacitance measurements were performed to evaluate the exocytosis function in the primary synapse of treated mice.
Results: Our results using murine cDNA validated previous results obtained with gene replacement therapy for DFNB9: ABR thresholds were restored and the startle reflex and PPI were fairly rescued. Conversely, we found that treatment with human cDNA was not sufficient to elicit the same degree of auditory recovery, ABR thresholds were higher, wave I amplitudes were lower, and latencies were increased. Similarly, we observed an impaired startle reflex and absence of PPI when using the human cDNA. IHC membrane capacitance measurements demonstrated that Ca2+-dependent synaptic exocytosis was only partially recovered after

treatment with human OTOF. In contrast, as expected, Ca2+-dependent synaptic exocytosis was restored after treatment with murine Otof.
Conclusions: These results suggest there is an intrinsic functional deficit of human Otoferlin protein when expressed in the murine cellular environment, as Ca2+-dependent synaptic exocytosis was only partially restored with the human cDNA. This study has significant implications for future gene therapy approaches and highlights the need to explore and optimize the compatibility of therapeutic proteins across species, especially in translational research aimed at human applications.

T68. Developing RNA Editing Therapy for DFNA5 Hearing Disorder in hPSC-Derived Inner Ear Organoids

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Category: Gene Therapy
Background: Mutations in DFNA5 (gasdermin E, GSDME) gene cause dominant delayed onset progressive hearing loss in humans. All DFNA5 mutations result in exon 8 skipping; cochlear implants and hearing aids are the only treatment options. The mouse model of DFNA5 does not exhibit hearing loss phenotype, making it particularly challenging to study DFNA5 hearing loss or develop therapeutic interventions. In this context, we created Inner Ear Organoids (IEOs) using human pluripotent stem cells carrying a DFNA5 mutation and developed RNA editing strategy to investigate the disease pathology and testing potential therapies.
Methods: 1. We build a DFNA5 hiPSC line (DFNA5delTTC/+), by introducing a 3-nucleotide deletion (TTC deletion) within intron 7 of normal hiPSCs, a mutation reported in multiple DFNA5 families.
2. IEOs were generated by sequential modulation of signaling pathways, including BMP, FGF, and WNT, to derive hair cells from the iPSCs carrying the DFNA5 mutation.

3. An RNA editing strategy was developed to disrupt the DFNA5delTTC transcript with exon 8 skipping by screening of CasRx/gRNA systems. gRNAs were designed to target the junction site between exon 7 and exon 9 to precisely target the DFNA5delTTC transcript. Expression vectors for hfCasRx/gRNAs with GFP were assessed for signal reduction to identify the most effective gRNA for knockdown efficacy.

4. The most effective gRNA was packaged into AAV2-hfCasRx/AAV2-gRNA-2 vectors and delivered to DFNA5delTTC/+-IEOs to study the treatment effect.

Results: 1. The DFNA5delTTC/+-IEOs displayed normal development of otic vesicles with the production of hair cells, supporting cells, and neurons up to day 40. Past day 40, significant loss of hair cells and supporting cells were detected, with a complete loss of both cell types by day 60. TUNEL assay detected significant cell death starting at day 40.
2. RNAseq study revealed the activation of cell death pathways in the DFNA5delTTC/+-IEOs compared to controls.

3. AAV-hfCasRx/AAV2-gRNA-2 delivered to the reporter cell line reduced DFNA5delTTC-GFP signal significantly, a demonstration of efficient RNA editing to abolish the mutant transcripts.

4. Constitutive expression of hfCasRx/gRNA-2 in DFNA5delTTC cell lines was built and exhibited efficient knockdown of DFNA5delTTC mRNA.

5. AAV2-hfCasRx/AAV2-gRNA-2 delivery into the DFNA5delTTC/+-IEOs rescued hair cells and supporting cells, demonstrating the treatment effect of DFNA5delTTC/+ inner ear cell types by RNA editing.

Conclusions: Conclusions: The human DFNA5delTTC/+-IEOs reveal that DFNA5 mutations primarily affect cell survival without significantly impeding the development of inner ear cell types, consistent with relatively normal hearing in young DFNA5 patients who subsequently develop progressive hearing loss. We developed an efficient RNA editing strategy to effectively abolish the mutant DFNA5 transcripts and rescue the inner ear cells. Our work lays the foundation to develop an RNA editing therapy to rescue DFNA5 hearing loss in humans based on the understanding of the mechanism and intervention work in vitro.

T69. Refining Spiral Ganglion Glial Cell Targeting With AAVie-K558R Serotype

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Category: Gene Therapy

Background: Natural hearing restoration remains one of the major challenges in addressing irreversible sensorineural hearing loss due to the lack of spontaneous auditory nerve regeneration in mammals while many interventions such as hearing aids or cochlear implantation can rehabilitate hearing, those who experience auditory neuropathy spectrum disorder (ANSD) phenotypes may not benefit from such interventions due to hypoplastic or aplastic cochlear nerves. However, those with ANSD are anticipated to have near-normal hair cell function. Thus by regenerating spiral ganglion neurons (SGNs), restoration of natural hearing for ANSD patients may be possible. Various reports have demonstrated successful neuroregeneration via viral-mediated direct cell reprogramming of glial cells. One of the vectors of choice is adeno-associated virus (AAV), which reliably delivers gene products to target cells and has been shown to restore hearing in many genetic mouse models. The tropic profile of AAV capsids is extensively studied with regard to the supporting cells (SC) and hair cells (HC) of the sensory epithelium but not in the spiral ganglion. A recent novel AAV serotype AAVie-K558R has been shown to transfect all SC subtypes, and it is known that SC and glial cells exhibit similarities. Our project now aims to ascertain the transfection profile of AAVie-K558R with a glial-specific promoter in the spiral ganglion, and to evaluate whether the delivered reporter genes can be restricted to the inner ear glial cells.

Methods: A single injection of AAVie-K558R-CAG-eGFP (AAVie-CAG) or AAVie-K558R-GFAP-mScarlet (AAVie-GFAP) suspension was delivered to the wild-type (C57/CBA) mice at P1-2 via the posterior semicircular canal (PSCC). Cochleae were harvested at 7 and 30 days

post-injection; auditory brainstem responses (ABRs) were collected before cochlear harvesting at 30 POD. The uninjected contralateral ear served as an internal control. Immunohistochemistry was performed with Sox2, Sox10 and TuJ1 staining. Cell-type specific transfection efficacy was quantified with cryosection imaging.
Results: Wildtype mice injected with AAVie-CAG demonstrated non-specific but robust eGFP expression in the inner ear at POD7. eGFP expression co-localized with TuJ1+, Sox2+, Sox10+ and Sox2+/Sox10+ cells in Rosenthal's Canal (RC). Co-localization of eGFP with Sox10+ cells was also observed in the osseous spiral lamina (OSL). AAVie-GFAP demonstrated more specific expression in the inner ear. Reporter gene mScarlet signal co-localized to Sox2+, Sox10+ and Sox2+/Sox10+ cells in the RC. Sparse co-localization of mScarlet was observed in the OSL.
Conclusions: Our preliminary results demonstrate how AAVie-CAG can transfect inner ear Sox10+ and Sox2+ cells in neonatal mice; with a glia-specific promoter, AAVie-GFAP transfected only Sox10+ and Sox2+ cells in the RC and OSL of neonatal mice. Further investigation of potential time-dependent change of expression and ABR testing are necessary to verify whether AAVie-CAG and AAVie-GFAP injections remain non-ototoxic. Additionally, testing the same vectors in adult mice would be warranted in further characterizing AAVie tropism.

T70. Dual Vector Gene Therapy Strategy Rescues Hearing in Recessive and Dominant Types of Hearing Loss

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Category: Gene Therapy
Background: Transmembrane channel-like 1 (TMC1) is an ion channel present in hair cells of the cochlea and the vestibular system. It is an important part of the mechanosensory transduction complex, which is involved in the conversion of mechanical information into electrical signals. Because of this, deficiencies in TMC1 cause dominant DFNA36 and recessive DFNB7/11 hearing loss.
Methods: In this work, we aimed to develop a gene therapy strategy that can target both dominant and recessive mutations that cause hearing loss. We use two humanized mouse models: heterozygous Tmc1D569N/+ which carry a dominant mutation in TMC1 and homozygous Tmc1N193I/N193I which carry recessive mutations. Neonatal mice were injected with a 1:1 mix containing two viral vectors: AAV9-PHP.B-spCas9 and AAV9-PHP.B-gRNA-Tmc1. The Tmc1 coding sequence carried synonymous mutations in the PAM sequence. This strategy allowed the endogenous Tmc1 gene to be disrupted, and an exogenous Tmc1 gene to be expressed. To assess hearing restoration after dual vector injection, we tested auditory brainstem responses (ABRs) and distortion products of otoacoustic emissions (DPOAEs). Electrophysiological recordings from inner hair cells were performed to assess sensory transduction currents. We evaluated hair cell survival by immunostaining with anti-Myo7a.
Results: We found that Tmc1D569N/+ mice had progressive hearing loss, as well as a reduction in transduction currents when compared to control mice (Tmc1D569N/+ Imax = 108.5 ± 13.4 pA, control Imax = -206.4 ± 31 pA). Furthermore, these mice show a significant tonotopic loss

of inner and outer hair cells as early as 4 weeks of age (apex, Tmc1D569N/+ = 10.0 ± 0.5 cells, WT = 12.2 ± 0.3 cells, pvalue = 0.03, one way ANOVA). Importantly, mice injected with AAV9-PHP.B-hTMC1 and AAV9-PHP.B-spCas9 recovered hearing thresholds, at 4, 8 and 12 weeks post-injection. Finally, Tmc1N193I/N193I mice, which are completely deaf by 4 weeks of age, were also treated with the dual gene therapy approach. Our results show that they also exhibited recovery of hearing thresholds, in some cases similar to those of WT mice. Preliminary results indicated dual vector injection reduced hair cell loss in both mutant lines.
Conclusions: These results demonstrate a successful gene therapy strategy that could target numerous Tmc1 gene deficiencies with the use of just one injection combining two viral vectors.

T71. Adenine Base Editor-Based Gene Therapy to Improve Mitochondrial Function in Hereditary Deafness Associated With Mitochondrial DNA Replication

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Category: Gene Therapy
Background: Mitochondrial diseases, characterized by dysfunction in the respiratory chain, often present with a wide range of symptoms due to multisystemic involvement. These conditions can result from mutations in mitochondrial DNA (mtDNA) or nuclear genes, with mtDNA encoding essential genes for mitochondrial function and replication. Each cell contains thousands of mtDNA copies, organized into nucleoids crucial for maintaining mitochondrial function.
Mutations in nuclear genes, such as those affecting mitochondrial single-stranded DNA-binding protein 1 (SSBP1), can disrupt mtDNA replication and maintenance. This leads to mtDNA depletion and deletions, causing a spectrum of mitochondrial diseases, including optic atrophy, sensorineural deafness, and myopathy. Despite advances in understanding these diseases, effective treatments remain elusive.

Gene-editing technologies, particularly CRISPR and base editors, offer new avenues for treatment. Traditional CRISPR methods can introduce unintended genetic changes, but base editors, which create single-strand breaks instead of double-strand breaks, allow for precise genetic modifications. Base editors have shown promise in clinical trials for various genetic disorders, and they may provide a viable approach for correcting mutations associated with mitochondrial diseases.

Methods: To correct patient-derived cells harboring a heterozygous missense mutation (c.272G GREATER THAN A:p.Arg91Gln), we tested two ABE variants.
Results: The NG-ABE8e variant exhibited the highest editing efficacy and demonstrated optimal recovery of mitochondrial function. This can be attributed to the fact that most bystander editing results in silent mutations and that the off-target sites are not located within the open reading frame. The NG-ABE8eWQ had a more specific editing window and therefore displayed a diminished editing efficacy, but still showed substantial functional recovery. Importantly,

although the mutations introduced into the NG-ABE8eWQ reduced overall editing capacity, it also led to notably fewer off-target effects.
Conclusions: We identified a novel SSBP1 mutation and its causal relationship to mitochondrial disease. We also demonstrated gene editing could significantly enhance mtDNA replication fidelity and mtDNA copy number by correcting the mutation using two different base editors. Although base editing-based therapeutics have strong potential for mitochondrial diseases, substantial challenges persist before they can be clinically applied. Efficacy and specificity can be of particular concern in mitochondria diseases with multisystemic involvement. Nevertheless, several of the phenotypes-associated SSBP1 mutations appear to be tissue-specific, primarily including optic atrophy and sensorineural deafness. The eye and ear sensory organs are strong candidates for gene therapies and genome editing due to their unique properties such as a small, enclosed compartment, immune privilege, and accessibility via established injection approach. Therefore, we believe that base editing-based gene therapies may be a feasible strategy for correcting SSBP1 mutations. In a clinical landscape where most patients with mitochondrial diseases lack a definitive treatment, the ability to restore mitochondrial function through base editing may be an attractive option for physicians and patients.

T72. Hearing Restoration by in Vivo Base Editing in a Humanized MPZL2 Mouse Model of DFNB111 Deafness

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Category: Gene Therapy
Background: Gene replacement therapy using AAV.ie-Mpzl2 cDNA delivery restored the hearing loss and inner ear structural integrity. However, MPZL2 protein is expressed across multiple cell types within the inner ear and the current absence of AAV serotypes and specific promoters that can precisely target these cells, gene replacement therapy may result in both overexpression of the MPZL2 protein in the targeted cells and ectopic expression in non-targeted cells, potentially leading to cytotoxic effects. Furthermore, it is widely acknowledged that AAV-mediated gene therapies often fail to sustain their therapeutic effects over extended periods. Base editors (BEs) possess therapeutic potential for correcting point mutations without generating DNA double-strand breaks, and potentially provide a one-time treatment for hereditary deafness caused by recessive mutations.
Methods: We explored the clinical significance of adenine base editor (ABE)-mediated gene therapy targeting the MPZL2 founder mutation (c.220C GREATER THAN T) in East Asian, the primary cause of DFNB111. We tested various combinations of ABE variants, four types of Cas9 variants with different PAM, and sgRNAs to correct the c.220C GREATER THAN T mutation. To test in vivo editing, we generated a humanized MPZL2 c.220C GREATER THAN T knock-in (KI) mouse model by inserting a human cDNA containing the pathogenic variant into the mouse Mpzl2 locus.

Results: The humanized MPZL2 c.220C GREATER THAN T KI mouse model exhibited moderate and progressive hearing loss, mirroring human DFNB111, and demonstrated structural deficits in the inner ear of the KI mice. We optimized the BE system to rectify the c.220C GREATER THAN T founder mutation and opted ABE8eWQ-SpRY-sgRNA system with editing frequency of 60% in human KI clonal cells. Administration of dual AAV.ie system packaging the ABE8eWQ-SpRY-sgRNA into the inner ear successfully restored the auditory function for the long-term and rescued inner ear structural integrity. Furthermore, the ABE8eWQ-SpRY-sgRNA system elicited neither bystander editing nor off-targets in in vivo.
Conclusions: The established humanized mouse model and the successful correction of the MPZL2 founder mutation using a single PAM-flexible ABE represent a significant step toward treating most cases of DFNB111.

T73. AAV2.7m8 Transduces Inner Hair Cells and Supporting Cells in Adult Non-Human Primate Inner Ears

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Category: Gene Therapy
Background: AAV2.7m8 has been previously shown to transduce the mouse cochlear inner and outer hair cells efficiently. In addition, it has also been shown to be capable of transducing supporting cells effectively. However, in order to assess the applicability of AAV2.7m8 for clinical use, it must be tested in larger animals with inner ears that are more similar to humans in terms of anatomy and physiology. In this study, we examine AAV2.7m8 transduction in adult non-human primate (NHP) inner ears.
Methods: Four adult (average age 20 years) rhesus macaques were used in this study. Two animals underwent AAV2.7m8-CMV-eGFP injection (3.0 X 1011 GC) via the horizontal semicircular canal (HSC), and the other two animals underwent AAV2.7m8-CMV-eGFP injection via the round window (RW). ABR was used to assess auditory function one week before gene delivery and two months after gene delivery. Immunohistochemistry was used to assess the transduction efficiency two months after viral injection.
Results: AAV2.7m8-CMV-eGFP is capable of transducing the inner hair cells and supporting cells in the adult NHP inner ears when delivered via the HSC approach. However, the transduction rate is much lower when AAV2.7m8-CMV-eGFP is delivered via the RW approach. ABR thresholds remain stable before and after inner ear gene delivery.
Conclusions: Our preliminary results showed that AAV2.7m8-CMV-eGFP is capable of transducing the adult NHP inner hair cells and supporting cells. The viral transduction is much more efficient when gene delivery was performed through the HSC compared to the RW.

T74. Evaluation of CRISPR-Based Allele-Specific Editing on Patient Dermal Fibroblasts for a Novel DFNA9-Causing Missense Variant in the LCCL Domain of Cochlin

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Category: Gene Therapy

Background: Hereditary hearing loss (HL) is one of the most common inherited sensory disabilities in the world. The onset of HL in patients with non-syndromic autosomal dominant hereditary HL (DFNA) tends to be slower than patients with non-syndromic autosomal recessive hereditary HL (DFNB). Therefore, the later onset of DFNA allows for a longer therapeutic window for clinical intervention. Here, we report the progress in our clinical study, where a novel recessive variant in the COCH gene is identified to co-segregate with HL in a DFNA9 family. We also examined the in vitro pathophysiology of this novel mutation and assessed the feasibility of CRISPR-based genome editing targeting this novel COCH variant.

Methods: DFNA patients were recruited for clinical and genetic evaluations at the National Institute on Deafness and other Communication Disorders (NIDCD), the National Institutes of Health (NIH) (Study title: Natural History of Autosomal Dominant Hearing Loss, ClinicalTrials.gov ID: NCT04501081). Skin biopsy specimens were obtained from study subjects for generation of DFNA primary dermal fibroblast cell lines. Once the causative variant of each DFNA study subject was identified, guide RNAs (gRNAs) were designed to induce non-homologous end joining (NHEJ) of the mutant allele. Lentiviruses containing various Cas9 nucleases and gRNAs were used to induce genome editing in DFNA fibroblast cell lines. Sanger sequencing was performed to verify the genotype and presence of editing in the COCH gene. Next generation sequencing was performed to assess genome editing efficiency.

Results: We have identified a novel variant in COCH (c.269T GREATER THAN A) that causes a missense substitution (p.Val90Glu) in the LCCL (Limulus factor C, Cochlin, and Lgl1) domain of cochlin, encoded by COCH, segregating with DFNA9 . To study the pathophysiology of the p.Val90Glu missense substitution of cochlin, we used in vitro cellular systems and super-resolution microscopy to examine its effects on protein trafficking. We found that this variant impaired cochlin trafficking from the endoplasmic reticulum in COS7 cells. Additionally, using the primary fibroblast cell line obtained from the DFNA9 patient, we have identified a gRNA that preferentially induced genome editing of the mutant allele.

Conclusions: We identified a novel recessive variant (c.269T GREATER THAN A causing p.Val90Glu) in COCH associated with DFNA9 . The pathophysiology of this genetic variant was evaluated, and CRISPR-based genome editing tools were optimized in vitro for preferentially targeting the COCH mutant allele.

T75. Applied Statistical Tool to Fabricate Nanocarrier for Targeted Drug Delivery to Inner Ear

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Category: Inner Ear: Drug Delivery

Background: The primary goal of our investigation is to fabricate an effective biomaterial-based drug delivery system that can locally deliver the drugs at the inner ear site to protect hair cells in the cochlea. We used a systematic approach called quality-by-design to develop nanocarriers (NCs) for drug-induced ototoxicity (DIO). DIO occurred due to treatment regimens such as aminoglycosides, platinum drugs that diminishes sensory hair cells, which are responsible for taking hearing signals to the brain. We studied antioxidant therapeutic drugs against the cisplatin toxicity.

Methods: Two PPS-mPEG-based tailor-made polymeric NCs have been designed using biodegradable and biocompatible polymers using the response surface design, where the response included particle size, PDI, EE, and DL to successfully assemble the polymeric nanocarrier. The approach was based on mathematical and statistical analysis to design NCs with desired size, polydispersity index (PDI), entrapment efficiency (EE) and drug loading (DL). The exhaustive characterization, storage ability, cryoprotectant choices, validation, and in vitro assessments of NCs on HEI-OC1 has also been part of this investigation. The particle size and PDI were determined by DLS, NTA, TEM and EE/DL were calculated using HPLC. The analysis of variance (ANOVA), lack of fit, actual predicted plot, residual predicted plot, and studentized residuals for each response were obtained from least squares fit model to evaluate whether the model fit well. The scatter index (SI%) of each response was determined using the root mean square error (RMSE) and mean response ($\bar{X}$). Statistical analysis of data was carried out through ANOVA (one-/two-way) and the tests were validated using Šidák's multiple comparison post-hoc test, where p LESS THAN 0.05 was considered as significantly different.

Results: Both NCs were successfully prepared and characterized. Actual vs. predicted plots of responses were studied after performing 16 experiments and running the standard least square model with emphasis on effective screening on response surface design. The counter profiler, prediction profiler, lack of fit analysis, residual vs predicted plots, studentized residual fit plots of each response were studied for NCs optimization. The ranges for particle size are about 100-200 nm, 55 - 80% EE, and 8 – 10% DL and PDI values (≤0.3) showed uniform size distribution based on varieties of antioxidant model drugs analyzed by DLS, NTA, TEM and HPLC. HEI-OC1 investigations were successful.

Conclusions: The synthesized NCs were shown similar to the predicted values of the prediction profiler and confirmed the validity of the obtained predicted factor values. The good fit of the model for each response was confirmed by ANOVA (P LESS THAN 0.05), Lack of Fit (P GREATER THAN 0.05), Actual Vs. Predicted plots, Scatter Index (SIn≤25%), Residual Vs. Actual response plots and Studentized fit plots. Collectively, the predictive mathematical experimental tool, statistical analysis, and cell- models against cisplatin toxicity showed a successful novel formulation to alleviate DIO.

T76. Local Administration of a Ph-Adjusted Sodium Thiosulfate-Containing Gel for Protection of Cisplatin Ototoxicity

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Category: Inner Ear: Drug Delivery

Background: The antineoplastic drug cisplatin is one of the most important drugs that damage the inner ear and cause hearing loss. Despite the reduced use of high-dose treatments, cisplatin ototoxicity remains a clinical problem, particularly in the pediatric oncology population. Various otoprotective strategies have been investigated to mitigate cisplatin-induced hearing damage. Pharmacological interventions studied in both in vitro and in vivo laboratory settings have identified numerous substances that can reduce cisplatin's ototoxic effects, though few have been taken to clinical trials. Important considerations in developing effective strategies for inner ear protection include that cisplatin readily undergoes non-enzymatic ligand-exchange reactions with nucleophiles, and its reactivity is pH-dependent. We and others have previously shown that sodium thiosulfate (STS) in a gel formulation can reduce ototoxicity when injected into the middle ear before systemic cisplatin administration. The present study aimed to investigate the influence of pH in combination with STS on otoprotection

Methods: Four groups of normal-hearing albino guinea pigs (n=32) were given a single transtympanic injection in the left ear (0.10–0.15 ml) of a hyaluronan-based gel with STS (0.1 M) and pH 6.5 (n=8) or pH 8.0 (n=8) or with placebo (sodium chloride; NaCl) and pH 6.5 (n=8) or pH 8.0 (n=8). Thirty min later, the animals were given a single intravenous injection of cisplatin (8 mg/kg b.w.). The protective potential of the gels on cisplatin-induced ototoxicity was assessed by comparing the percent loss of IHCs and OHCs in the injected and non-injected ears four days later.

Results: The OHC loss was most severe in the basal part of the cochlea, decreased with increasing distance from the round window, and was largest in the first row and smallest in the third row. The IHC loss was very low. Irrespective of pH, middle ear administration of a hyaluronan-based gel containing STS reduced the ototoxicity of cisplatin while NaCl did not. There was a tendency toward a minor difference in OHC loss in the basal turn between animals receiving a transtympanic injection of STS-gel at pH 8 and pH 6.5, with a slight advantage for the animals receiving the gel with the higher pH.

Conclusions: Cisplatin is a widely used, older cytostatic drug that harms the inner ear, making it essential to continue developing otoprotective strategies. When considering pharmacological otoprotection, key factors include the method of otoprotector delivery, the timing of administration relative to cisplatin, and the selection of otoprotective agents. In this experimental study, otoprotection was achieved through intratympanic administration of a hyaluronan-based STS gel. Further studies are warranted to investigate whether higher pH may offer greater otoprotection.

T77. Programmable NIR Responsive Nanocomposite Enables Noninvasive Intratympanic Delivery of Dexamethasone to Reverse Cisplatin Induced Hearing Loss

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Category: Inner Ear: Drug Delivery

Background: Local drug delivery to the inner ear has significant otological clinical promise for cisplatin-induced hearing loss (CIHL) therapy due to local higher concentration and less side effect than systemic administration. However, the multiple detoured barriers-round window membrane (RWM) and the uncontrolled drug release can still decrease the therapeutic effect through the local drug delivery.

Methods: Here, a novel near-infrared (NIR) responsive nanocomposite--gold nanorod@dexamethasone-mesoporous silica-saponin (AuNR@DEX-MS-saponin, NPs/DEX) as a local drug delivery system of the inner ear through intratympanic injection were exploited to control drug release spatiotemporally, and enhance the permeability of the RWM. The morphology characterizations of the NPs/DEX were assessed through the shape, size, aspect ratio, hydrodynamic size, zeta potential, PDI, and FTIR spectra. The photothermal effectiveness of NPs/DEX were evaluated after the NIR irradiation by a thermal imaging camera. Besides, the drug control release profile of the synthesized nanocomposites was evaluated with a UV–Vis spectrometer. Moreover, the biocompatibility of the nanocomposites and oto-protection against cisplatin induced HEI-OC1 cell damage were tested in vitro through CCK8 kit. Finally, the protective effect of the NPs/DEX in vivo was investigated in CIHL guinea pig model through the assessment of the ABR threshold, the evaluation of the hair cell loss, and synaptic ribbons damage in cochlea.

Results: The homogenous fabricated nanocomposite, a silica-coated gold nanorod enclosed saponin and loaded with super low DEX, had a very negative ζ-potential, which can increase the permeability of the RWM. And the synthesized nanocarrier exhibited triggered release of DEX upon NIR-light radiation. Moreover, the synthesized nano vector exhibited biocompatibility and significant protective effect in vitro regardless of NIR radiation against cisplatin induced HEI-OC1 cell damage. Particularly, the Cis + NPs/DEX + DEX + Laser group almost completely restored the hearing of guinea pigs exposed to NIR radiation at all frequencies. The loss rate of OHCs in the Cis + NPs/DEX + DEX + Laser group was significantly reduced compared with the Cis + Dex group (1st, P LESS THAN 0.05; 3rd, P LESS THAN 0.05; 4th, P LESS THAN 0.001) and Cis group (2nd, P LESS THAN 0.01; 3rd, P LESS THAN 0.05; 4th, P LESS THAN 0.01), respectively. Besides, the synaptic ribbons number per IHC in Cis + NPs/DEX + DEX + Laser group was higher than Cis group at all turns (1st, P LESS THAN 0.0001; 2nd, P LESS THAN 0.001; 3rd, P LESS THAN 0.05; 4th, P LESS THAN 0.001).

Conclusions: The fabricated NIR-based nanocomposite loaded with super low DEX act as a permeation enhancer and a remote-control drug release platform in recovering CIHL, attenuating hair cell loss and alleviating synaptic ribbon damage in response to NIR laser irradiation. And thus, our findings provide insight into the NIR-responsive local delivery system for inner ear illness.

T78. Generating and Evaluating a Novel Bioactive Coating for Cochlear Implant Electrodes

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Category: Inner Ear: Drug Delivery
Background: Cochlear implants have revolutionised the treatment of profound hearing loss, restoring sound perception to individuals worldwide. However, the surgical implantation of electrodes into the cochlea can induce biological responses, such as cell death, inflammation, and fibrotic tissue growth. Over time, these responses reduce auditory clarity and can lead to implant failure. To mitigate these outcomes, we have developed a novel bioactive coating for cochlear implant electrodes that releases an ASK1 inhibitor. Notably, ASK1 inhibition reduces cell death, inflammation, and fibrosis in several disease models, and has been deemed safe through to Phase 3 clinical trials.
Methods: To develop this coating, we loaded and tested the elution properties of several polymer and hydrogel combinations, including Polyhydroxyethylmethacrylate (pHEMA), Polycaprolactone (PCL) and polyethylenevinyl acetate (PEVA).
Results: PCL and PEVA both exhibited drug burst effects, followed by sustained drug release. In contrast, pHEMA coatings prevented meaningful small molecule release. Our ongoing studies now aim to evaluate the coating's efficacy for reducing cell death, inflammation, and fibrosis in vivo, as well as durability and flexibility parameters. We are also performing molecular studies to provide a clear protective mechanism alongside robust safety and efficacy data.
Conclusions: These data are expected to accelerate product translation while contributing to the burgeoning field of protective coatings for cochlear implants.

T79. Identification of Genes That Are Involved in Development of Cochlear Supporting Cells

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Category: Development: Cellular/Systems
Background: The sensory epithelium of the cochlea, the organ of Corti, is a highly derived and rigorously patterned structure that acts to convert auditory stimuli into electrical impulses. This structure consists of a highly diverse cellular mosaic that includes two different types of mechanosensory hair cells, and at least six different types of associated supporting cells. While there has been considerable research conducted on the development of hair cells, the formation of supporting cells, which are equally necessary for normal hearing, has not been sufficiently examined. Therefore, this project aims to improve our understanding of supporting cell development by characterizing the formation of pillar cells, Deiters' cells and Hensen's cells during the early post-natal period.
Methods: Cochlear duct cells were dissociated at postnatal day (P) 1, P3, P5, and P7 to obtain individual cells for analysis using single-cell RNA sequencing techniques. Bioinformatic

approaches, including Slingshot and Trade-seq, were employed to identify potential transcription factors that may modulate the development of different supporting cell types. To validate some of the cell-type-specific genes identified in the data set, we employed immunofluorescent labelling and RNA scope to visualize the localization of these transcripts within supporting cell types in cross-sections from postnatal cochleae.
Results: The results of our single-cell RNA sequencing analysis indicate that Hensen's cells exhibit a distinctive gene expression profile during the initial postnatal days and segregate into two distinct types of Hensen's cells from P3 onwards. Deiters' and Pillar's cells display a shared transcriptional expression pattern, which presents a challenge in the identification of these cell types. Furthermore, preliminary analysis of the single cell-RNA seq data identified four potential gene candidates (Zeb2, Rab3c, Ednrb, Agr3) that may be crucial for the development of cochlear supporting cells.
Conclusions: This study presents a single-cell RNA-seq atlas for the development of supporting cells at different early postnatal time points. The validation of these results indicates that these data will serve as a valuable resource for the examination of multiple developmental events during the formation of the mammalian cochlea, including the formation of the tunnel of Corti and the onset of hearing.

T80. ETV Transcription Factors are Necessary for Organ of Corti Development and Pillar Cell Differentiation

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Category: Development: Cellular/Systems
Background: The organ of Corti (oC) is composed of hair cells and supporting cells that are required for translating sound stimuli into electrical signals. Malformation of the structure of the oC can lead to hearing loss, underscoring the importance of both hair cells and supporting cells for normal auditory function. Despite this, the genetic mechanisms governing the development of these cells remain largely unknown. We aim to use single cell RNA sequencing to identify novel genes that are necessary for proper oC development.
Methods: Cochlear duct cells were isolated on each embryonic (E) day between E11 and E16 and single cells were collected using the 10X Genomics Chromium Controller. Data were then processed and merged using the Seurat package, and reciprocal PCA (RPCA) for integration. For pseudotime analysis, Slingshot and Tradeseq were utilized. To simultaneously reconstruct gene regulatory networks and identify key transcription factors involved in cell development, SCENIC analysis was performed.

Expression of candidate genes in specific cell types was determined using immunohistochemistry or in situ hybridization.

Mice with different combinations of deletions of Etv1, Etv4, and Etv5 were generated using Etv1-flox, Etv5-flox , and Etv4-deletion alleles along with Emx2-cre to drive recombination. Changes in cochlear development and cell type specification were determined using immunolabeling at different development time points.

Results: After elimination of low quality and non-epithelial cells through sequence analysis, we isolated prosensory and sensory cells for further analysis. The resulting data set included clusters representing inner hair cells, outer hair cells, inner pillar cells, lateral supporting cells, pharyngeal cells, Cdkn1b-negative immature sensory progenitors, Fgf20-positive medial sensory progenitor cells, and Prox1-positive lateral sensory progenitor cells.
Pseudotime analysis revealed that lateral sensory progenitor cells differentiate from Fgf20-positive/dkn1b-negative medial sensory progenitor cells . Furthermore, SCENIC analysis suggested that the transcription factors ETV4 and ETV5 are active in developing inner pillar cells.

ETV4 and ETV5 belong to the PEA3 subfamily of ETS transcription factors, which also includes ETV1. These genes are often co-expressed and have been shown to functionally compensate for one another. Single molecule in situ hybridization (RNAscope) indicated co-expression of Etv1, Etv4, and Etv5 in the lateral side compartment of the sensory epithelium at E14. At E16 and later ages, expression of all three transcripts was restricted to pillar cells.

To determine the roles of Etv signaling in cochlear development, Etv1/Etv4/Etv5 triple-knockout mice were generated. At embryonic day 18 (E18), triple-knockout mice exhibited an absence of NPY/NGFR-positive cells, indicating that Etv-deficient mice lack pillar cells.

Conclusions: Our study provides key insights into the pathways of cellular differentiation within the oC. Notably, we discovered that lateral sensory progenitor cells arise from Fgf20-positive medial progenitor cells. Furthermore, the results demonstrate a crucial role for Etv genes in pillar cell development.

T81. The Transcription Factor ZMYM4 is Expressed in Otic Neuronal Precursors and Spiral Ganglion Neurons

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Category: Development: Cellular/Systems
Background: The transcription factor Zinc finger MYM-containing type 4 (ZMYM4) has recently been shown in Xenopus laevis to be expressed within the preplacodal ectoderm (PPE) and the neural crest cells (NCCs) during early embryogenesis. Further, loss of ZMYM4 results in a down regulation of NCC and PPE markers with a concomitant expansion of the neural ectoderm. In contrast, overexpression had little to no effect on placode or NCC development. These data, combined with recent studies examining the roles of other members of the ZMYM family, suggest that ZMYM4 may be acting as a pioneer factor during early specification of the PPE and NCCs. As the PPE gives rise to the otic placode and later, the sensorineural epithelium of the inner ear, we hypothesize that ZMYM4 may be involved in cell fate specification within the inner ear. More specifically, as it has been linked to neurodevelopment and loss of ZMYM4 results in an expansion of the neural ectoderm, it is possible that ZMYM4 may play a role in the specification of the neural components of the inner ear, the vestibular and spiral ganglion (SG) neurons.

Methods: We collected mouse embryos at specific time points between embryonic day 12.5 and adulthood and performed immunohistochemistry on both whole mount and cryo-sectioned tissue to establish a temporospatial timeline of ZMYM4 expression throughout the inner ear.
Results: ZMYM4 is expressed within the otic sensorineural epithelium from the earliest stages examined and is maintained in the population of cells that delaminate from the otic epithelium to form the vestibular and SG neurons. At P0, expression of ZMYM4 is largely confined to the nuclei of the SG neurons and to a small number of support cells adjacent to the outer hair cells. Interestingly, analysis of single cell RNA-seq datasets available on gEAR suggest that Zmym4 mRNA expression decreases between E12.5 and P0, while the immunohistochemical data indicates the ZMYM4 protein is maintained at relatively consistent levels throughout ontogeny. This suggests that ZMYM4 may function as either a pioneer factor or as a bookmarking factor, binding to DNA and maintaining critical sites of gene transcription in an open configuration.
Conclusions: These data represent the first investigation of the possible functions of ZMYM4 during inner ear development. In the future, we plan to investigate possible DNA binding sites of ZMYM4 using CUT and RUN assays and changes in epigenetic states following inhibition of ZMYM4 using ATAC-seq. Phenotypic changes in inner ear and SGN development will also be determined following ZMYM4 inhibition or over-expression. Overall, ZMYM4 is an exciting candidate for future research as it may play a key role in regulation of the epigenetic landscape during sensorineural development within the ear.

T82. The Role of Cilia in the Development, Survival, and Regeneration of Hair Cells

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Category: Development: Cellular/Systems
Background: It has previously been shown that mutations in cilia genes lead to a decrease in the number of hair cells in the lateral line of zebrafish. While in the inner ear, this decrease appears to be due to the death of hair cells via apoptosis the cause of this decrease in the lateral line has not been thoroughly investigated. It is also unknown what role if any cilia play in the regeneration of hair cells. In this work, we investigated hair cell development, survival, and regeneration in the lateral line in zebrafish mutants of two genes impacting cilia the anterograde IFT gene, ift88, and the retrograde IFT gene, dync2h1.
Methods: Experiments were conducted in zebrafish larvae aged 4-7 days post fertilization (dpf). We used an HCS-1 antibody to label hair cells, a Sox2 antibody to label supporting cells, and a cleaved-caspase3 antibody to label cells undergoing apoptosis. EdU was used to label proliferating cells and JC-1 was used to measure mitochondrial membrane potential. To investigate hair cell regeneration fish were treated with gentamicin for 24 hours to kill hair cells and trigger regeneration.
Results: We failed to find evidence for alterations in hair cell development in our cilia gene mutants with proliferation during development and supporting cell number not being significantly different between wild-type siblings and mutants. We did forever find evidence of impacts on hair cell survival as there were cleaved-caspase 3 positive hair cells in both cilia gene

mutants. However, the number of these cells was considerably smaller than the difference in hair cell number between wild-type siblings and mutants. We see something similar in mutants that impact hair cell activity but not cilia formation, cdh23 mutants, but the distribution of apoptotic hair cells at different developmental time points is altered. We also found evidence of mitochondria dysfunction in our cilia gene mutants as they showed a slight reduction in mitochondrial membrane potential and differentially responded to RU360 treatment as compared to wild-type siblings. Lastly, we found that cilia gene mutants could regenerate their hair cells. There were slight decreases in proliferation during regeneration in mutants and the number of hair cells postregeneration was lower than wild-type sibling controls, but interestingly cilia gene mutants that had regeneration triggered via gentamicin treatment had significantly more hair cells than age-matched controls that had not been treated with gentamicin.
Conclusions: We found that mutations in cilia genes lead to impairments in hair cell survival while we failed to find impacts on hair cell development in these mutants. Additionally, cilia gene mutants are still able to regenerate their hair cells.

T83. The Role of FGFR2b Ligands in Spiral Ganglion Neuron Subgroup Specification and Survival

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Category: Development: Cellular/Systems
Background: The afferent innervation to the cochlea is composed of the spiral ganglion neurons (SGNs) which transmit mechanosensory input from the hair cells centrally to the cochlear nucleus. Two populations of SGNs can be distinguished in the mammalian cochlea: Type 1 SGNs which constitute 90-95% of the total population, and form contacts with inner hair cells (IHCs); and Type 2 SGNs which constitute the remaining 5-10% of the total population and form contacts with outer hair cells (OHCs). The Type 1 population can be further divided into 3 molecularly distinct subgroups, termed 1A, 1B and 1C. In the mouse, unspecified SGNs initially split into two lineages leading to either Type 1A/Type 2, or Type 1B/1C precursors by gestational day (GD)14, with all 4 groups identifiable by GD18. However, relatively little is known about which genes are important in specifying and maintaining the different Type 1 and Type 2 SGN subgroups. We have previously identified the transcription factor Etv4 as expressed, and likely active, in the Type 1A/Type 2 branch of SGN development. As Etv4 is a downstream pathway effector of FGF signaling we sought to understand if FGF signaling might direct SGN precursors towards a Type 1A/Type 2 fate.
Methods: To do this we employed a mouse line in which signaling by FGF3/FGF10 signaling is inhibited by expression of a doxycycline inducible dominant-negative FGFR2b ectodomain. Inhibition was initiated at GD11, and continued through tissue collection and fixation at GD18. We confirmed that at GD15 Etv4 expression is lost from SGNs when FGF3/FGF10 function is inhibited.
Results: At GD18 there is a severe reduction in the number of SGNs in the cochlea following FGF3/FGF10 signaling inhibition. However, of the SGNs present, there was a significant

increase in the proportion of neurons which were Type 1C, and a decrease in the proportion that were Type 1A. In addition, there was an almost complete loss of Type 2 neurons.
Conclusions: These results indicate that FGF3/FGF10 signaling is either (1) involved in the specification of Type 1A/Type 2 SGNs, or (2) required for Type 1A/Type 2 SGN survival. To differentiate between these two hypotheses we are assessing markers of the Type 1A/Type 2 lineage at GD14 and 16 when SGNs are first differentiating. We are also assessing markers of cell death at these ages to determine when SGNs are lost. Lastly, we are assessing SGN subgroup markers in Etv4 knockout mice as we predict that these mice may have a similar phenotype.

T84. Cochlear Extension and Patterning Require Myosin II and Cadherin 1

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Category: Development: Cellular/Systems
Background: The elongated sensory epithelium of the cochlea, the mammalian auditory organ, is comprised of an exquisite mosaic of mechanosensory hair cells and non-sensory supporting cells. Correct patterning of this mosaic is necessary for auditory function and requires the coordination of multiple processes of cell growth, migration, and rearrangement. We have previously shown, using a combination of live imaging, in vitro explants, and a pharmacological inhibitor of Myosin II activity, that non-muscle Myosin II is required for multiple aspects of cochlear extension, including tissue extension, migration of hair and supporting cells toward the cochlear apex, luminal hair cell expansion, and luminal constriction of supporting cells.
Methods: To determine the cell-type specific roles of Myosin ll, we expressed a conditional dominant-negative allele of Myosin II (Myh10-DN) in specific subsets of cochlear epithelia cells in the developing mouse cochlea using a Cre-LoxP approach. We observed both cell- and non-cell-autonomous roles for Myosin II activity. We have also examined the effect of loss of the cell-adhesion molecule Cadherin-1 (E-cadherin, Cdh1), which is normally expressed in the lateral portion of the cochlear epithelium and has been shown to interact with Myosin II in other systems, especially in epithelial tissues.
Results: In pillar cells, which form a boundary between the medial and lateral domains of the cochlear epithelium, Myosin II is required non-cell-autonomously to maintain cellular organization in the lateral outer hair cell region. Expression of Myh10-DN in hair cells causes a failure of both the normal increase in hair cell size, which contributes to later stages of cochlear elongation, and the accompanying decrease in supporting cell luminal surface area. Loss of Myosin II activity in Hensen's cells, lateral to the outer hair cell region, disrupts formation of the hair cell-supporting cell mosaic and leads to gaps between the outer hair cell rows. In contrast, the overall length of the cochlea is unchanged when Cdh1 is removed from the cochlear epithelium. However, loss of Cdh1 leads to a marked decrease in the luminal surface area of some supporting cells, such that hair cells are often found directly adjacent to each other, appearing to form cell-cell contacts. Cdh1 mutant cochleae also have defects in patterning, including loss of outer hair cells and misalignment of rows.
Conclusions: The interaction of Myosin II and Cadherin 1 is known to be important in many different contexts of epithelial remodeling. These results suggest that in the cochlea, Myosin II

and Cadherin 1 may act in a complementary manner to control the size of hair and supporting cells and the junctions between them, which must be regulated to ensure proper patterning of the cochlear sensory epithelium. We plan to further explore this interaction using both live imaging and super-resolution microscopy.

T85. Post-Developmental Mechanisms May Restrict Mammalian Hair Cell Regeneration

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Category: Regeneration

Background: The capacity to regenerate auditory and vestibular hair cells (HC) after injury is unevenly distributed across the vertebrate lineage. In mammals, HC regeneration is activated in response to damage in neonates but then lost in mature animals. Although adult mammals lack significant HC regenerative potential, non-mammalian vertebrates can regenerate HCs throughout life. The mechanisms suppressing mammalian HC regeneration are not fully understood. Using molecular, genetic, and pharmaceutical approaches acting at the signaling and transcriptional levels, multiple groups have increased HC differentiation and post-ablation regeneration in mouse. However, these experiments do not produce mature, functional HCs, suggesting that HC regeneration is more intricately silenced.

Suppression of mammalian HC regeneration may be better understood with a cross-modality study on the initiation of suppression after development. In the P0/P1 neonatal mouse cochlea, there is minor HC regeneration after ablation, but this response is lost when ablation is induced after the first postnatal week. The cochlea's apical turn, containing HCs that differentiated just prior to birth, retains regenerative capacity longer than the medial and basal regions that experienced earlier HC differentiation. The neonatal utricle has a similar, transient regenerative response to HC ablation. These observations suggest a rapid suppression of mammalian regenerative capacity following auditory and vestibular HC differentiation. We aim to identify multi-modal gene regulatory changes driving this suppression in mammals.

Methods: To characterize gene regulatory changes following HC differentiation, single-nuclei transcriptomes and epigenomes of mouse cochlea and utricle will be obtained from prenatal HC differentiation, partially regenerative neonatal, and non-regenerative juvenile timepoints. Prospective regeneration-suppressive changes will be resolved with pair-wise comparisons between prenatal, neonatal, and juvenile transcriptomes and epigenomes. Studying regenerative non-mammalian vertebrates during development may also reveal gene regulatory changes specific to mammals and involved in regeneration suppression. Thus, our observations will be further compared to single-nuclei transcriptomes and epigenomes from zebrafish auditory and vestibular organs at developmentally matched timepoints. These experiments will be followed by validations of multi-omic data and assessment of candidate suppressors.

Results: This project is in the initial stages, but our experimental plan is summarized in the methods section.

Conclusions: By investigating developmental mechanisms that restrict mammalian HC regeneration, evolutionary differences that drive distinct injury responses across the animal

kingdom can be better understood. Our observations may further contribute to regenerative therapies for select hearing disorders.

T86. Dissecting the Role of GSK3β and Wnt/β-Catenin Signaling in Supporting Cell Proliferation in the Damaged Neonatal Mouse Utricle

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Category: Regeneration

Background: The mammalian utricle displays limited spontaneous hair cell (HC) regeneration and proliferation. Wnt/β-catenin pathway plays diverse roles in the inner ear, including proliferation and hair cell differentiation. Previous studies showed that overexpression of β-catenin, the central mediator of canonical Wnt signaling, increases proliferation and hair cell regeneration in the neonatal utricle. GSK3β functions to disassemble β-catenin and renders Wnt/β-catenin pathway inactive, but it also acts on numerous other pathways. Previous research showed that a combination of factors (including GSK3β inhibitors) increased proliferation in the neonatal cochlea and utricle, but whether GSK3β inhibitors exert effects through the Wnt/β-catenin pathway is unknown. Here, we examined the effects of combining growth factors with the GSK3β inhibitor (CHIR) on proliferation in neonatal mouse utricles in vitro, focusing on the interplay between GSK3β and the Wnt/β-catenin pathway.

Methods: Utricles were dissected from postnatal (P) 3 C57BL/6 wildtype mice in aseptic conditions. Whole organs were cultured in serum-free media with seven factors (7F) including CHIR, EGF, bFGF, IGF-1, VPA, pVc and TGFβi. Neomycin was added for 24 hours to induce hair cell loss, followed by 5 days of culture with EdU to label proliferating cells. Tissues were stained for EdU, Myosin7a, Sox2 and costained with DAPI. Furthermore, UBCCreERT2/+; Ctnnb1fl(exon3)/+ and UBCCreERT2/+; Ctnnb1flox/flox mice utricles were treated by 4-hydroxy-tamoxifen.

Results: Neomycin treatment resulted in the loss of 60%-70% of type II HCs and 98%-99% of type I HCs. The combination of 7F robustly increased EdU+ Sox2+ supporting cells in the whole neomycin-damaged utricles. Removing CHIR from the 7F completely abolished proliferation throughout the utricle. Removing EGF, VPA, or TGFβi from 7F significantly reduced proliferation in the extrastriola while maintaining proliferation in the striola. On the other hand, removing bFGF, IGF-1, pVc did not significantly change the degree of proliferation, suggesting that these three factors are dispensable. Notably, CHIR alone was insufficient to induce proliferation. The 4F combination (CHIR, EGF, VPA, and TGFβi) promoted proliferation to the extent similar to the 7F treatment. Moreover, replacing CHIR with Wnt agonist that directly activates TCF in the 7F combination failed to induce proliferation. Finally, overexpression of β-catenin in UBCCreERT2/+; Ctnnb1fl(exon3)/+ utricles with EGF, VPA, and TGFβi treatment did not induce proliferation, while robust proliferation was noted in UBCCreERT2/+ utricles treated with the 4F combination. Furthermore, β-catenin deletion in UBCCreERT2/+; Ctnnb1flox/flox utricles did not prevent 4F-induced proliferation.

Conclusions: The combination of CHIR, EGF, VPA, and TGFβi is sufficient to induce supporting cell proliferation in neomycin-damaged neonatal mouse utricle. Furthermore, GSK3β

inhibition via CHIR is required but insufficient to induce supporting cell proliferation, and that CHIR may act via a β-catenin independent manner.

T87. Single-Cell Analysis of Adult Mouse Utricles After Targeted Ablation of Hair Cells With Diphtheria Toxin

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Category: Regeneration

Background: The vertebrate inner ear houses hair cells, which are specialized mechanosensory receptors essential for our ability to detect and encode sound, head motion, and gravity. In humans and other mammals, hair cells are only made once during development and destroying or damaging hair cells causes permanent impairments to hearing and balance. In contrast, non-mammalian species, including fish, can regenerate hair cells from their surrounding supporting cells throughout life. The ability of supporting cells to respond to regenerative cues (e.g. hair cell loss) greatly diminishes with maturation and little to no hair cell regeneration is observed in adult mice with functional improvements. We hypothesize that evolutionarily conserved and species-specific gene networks control supporting cell quiescence, reactivity, and trans-differentiation. This study seeks to identify the gene regulatory networks controlling supporting cell reactivation (or their lack of) in mammalian injury contexts by leveraging genetics and genomics to discover evolutionarily conserved and species-specific signals following hair cell damage.

Methods: To study hair cell damage in adult mice, we use Pou4f3DTR transgenic mice that express the human diphtheria toxin receptor gene under the control of a hair cell specific Pou4f3 promoter. Treatment of adult Pou4f3DTR mice with two intramuscular injections of diphtheria toxin (DT), administered spaced two days apart, results in widespread ablation of hair cells in the vestibular organs (utricles) by 14 days post injection. Using fluorescently-tagged phalloidin to visualize adult inner ear hair cell bundles, Myosin VI/VIIa antibodies for hair cell bodies, and Sox2 antibodies for supporting cells, we are assessing hair cells and supporting cells in untreated and DT injected mouse utricles. Utricles will be dissected 7,14,28, and 60 days post-DT injection for 10X Chromium Multiome ATAC and Gene Expression experiments. Using the Seurat toolkit for single-cell genomics, this study will compare gene expression and chromatin accessibility changes between hair cell-ablated utricles and controls at single-cell resolution, identifying the consequences of hair cell ablation.

Results: This data will help re-construct the gene regulatory networks (GRNs) for hair cell regeneration in mammalian injury contexts. Cross species analysis of this data to previously identified GRNs in regenerating zebrafish inner ears will identify evolutionarily conserved and species-specific responses in supporting cells following hair cell ablation.

Conclusions: This study aims to identify the key genes essential for inducing the transdifferentiation of supporting cells into new hair cells in mammals and to uncover the factors contributing to the deficient process, which is otherwise successful in non-mammalian vertebrates.

T88. Single Cell Transcriptomics Reveals Damage Induced Mitotic Response in the Neonatal Mouse Utricle

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Category: Regeneration

Background: The utricle is an inner ear vestibular sensory organ that depends on mechanosensitive hair cells (HCs) for detecting linear acceleration. In newly born mice, hair cells are generated from supporting cells (SCs) and continue to be added for the first 7 postnatal days as part of the developmental process. Non-mammalian species regenerate hair cells, however, loss of hair cells in mammals is permanent because of limited regenerative capacity. There are currently no therapies that regenerate lost hair cells in mammals. Here, we elucidate the crucial molecular factors and pathways involved in the regeneration of murine utricular HCs following damage in vivo.

Methods: We used an established in vivo hair cell ablation model (Pou4f3-DTR) to understand the molecular mechanism of hair cell regeneration following injury using the utricle as a model. Damage was induced on postnatal day 1 (P1) to selectively ablate HCs. To address mitotic regeneration specifically, we utilized a triple transgenic model (Pou4f3DTR; Ki67CreERT2; Rosa26mTmG) to fate map proliferating cells. Droplet-based cell capturing platform was used for single cell RNA seq (scRNAseq) following lineage tracing. Utricles were harvested at P2, P3, P4, P5, P6, and P9 for both histology and scRNAseq.

Results: Early time point experiments confirm loss of HCs beginning at P2. Analysis of the damaged tissues shows a wave of proliferation at P3-P6 using Ki67 as a marker. Newly dividing cells begin to increase at P3 and peek at P6 (p LESS THAN 0.0001). Histologic data using our triple transgenic model (Pou4f3DTR; Ki67CreERT2; Rosa26mTmG) show cells that undergo mitosis permanently switch color to GFP. We have collected scRNAseq data in both undamaged and triple-transgenic lineage traced damaged utricles at early postnatal ages (P3 through P9). Our preliminary analysis demonstrates high quality cells with expected clusters of hair cell sub-types, supporting cells, and transitional epithelial cells. A total of 28,809 cells passed all quality control metrics. A group of presumed damaged activated cells were identified from the scRNAseq data. Our sequenced scRNAseq data also contains fate mapped cells to infer trajectory of mitotic cells.

Conclusions: Our results reveal insights into the molecular mechanism of post-injury mitosis within the inner ear, paving the way for in vitro and in vivo manipulation experiments for hair cell regeneration. Understanding mitotic regeneration within the neonatal mammalian utricle could lead to the discovery of new tools to regenerate hair cells for hearing and balance disorders.

T89. Robust Regeneration of Hair Cells by Co-Expression of Gfi1, Atoh1 and Pou4f3 in the Adult Mouse Cochlea

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Category: Regeneration

Background: The loss of cochlear hair cells (HCs) is the main cause of hearing impairment. In non-mammals, sensory cell regeneration is spontaneous, but neither regeneration nor proliferation occurs in the mature mammalian cochlea. Previous work has shown that Atoh1 can promote HC regeneration in the neonatal cochlea, but its efficacy is limited as a singular factor in the adult organ. Recent studies showed that co-expression of Gfi1, Pou4f3, and Atoh1 (GAP) using the Rosa-GAP mice can robustly induce ectopic hair cells outside the organ of Corti in the mature cochlea. To achieve spatiotemporal control of this approach in supporting cells (SCs) adjacent to HCs, we engineered and characterized several mouse lines in which cochlear SCs express tamoxifen-inducible Cre recombinase. Furthermore, we used the Pou4f3-DTR mice to determine the effects of HC ablation on GAP-induced HC regeneration.
Methods: We examined the following mouse lines: Prox1-CreERT2, Fgfr3-iCreER, Sox9-CreERT2, Rosa-GAP, Rosa-tdTomato, and Pou4f3-DTR. Tamoxifen was given intraperitoneally at P21 and diphtheria toxin (DT) at P20. Cochleae were collected at 2, 4, 8 weeks after tamoxifen injection and prepared as whole mounts. Immunofluorescence staining was performed to label native HCs (Myosin7a+, Sox2-), regenerated HCs (Myosin7a+, Sox2+), outer hair cell-like cells (Myosin7a+, Sox2+, Prestin+) and inner hair cell-like cells (Myosin7a+, Sox2+, vGlut3+). Each cochlear turn is separately analyzed, and cells of interest were quantified.
Results: Sox9-CreERT2; Rosa-tdTomato; Rosa-GAP mice had high mortality, likely because of GAP expression outside the inner ear. Prox1-CreERT2; Rosa-tdTomato; Rosa-GAP mice survived but did not show significant tdTomato expression in the cochlea. Fgfr3-Cre; Rosa-tdTomato; Rosa-GAP mice survived at least 8 weeks and had GREATER THAN 50% tdTomato+ SCs. After tamoxifen injection to Fgfr3-Cre; Rosa-tdTomato; Rosa-GAP mice, we observed new HCs in all turns at 2, 4, 8 weeks. We also observed loss of native outer hair cells, probably due to trans-differentiation of SCs to HCs. After ablation of native HCs by DT, robust regeneration of HCs (Myo7a+, Sox2+) was observed in all three cochlear turns at 2, 4, 8 weeks, with the numbers being the highest in the apical turn and increasing with time. Most regenerated hair cells were observed in the supporting cell layer, with some spanning the whole epithelium and few migrated to the hair cell layer. At 4 weeks, regenerated hair cells expressed the outer hair cell marker Prestin, but not the inner hair cell marker Vglut3. Ongoing experiments will investigate the degree of maturation in these regenerated outer hair cell-like cells and the effects of regeneration on native HCs.
Conclusions: We have characterized a transgenic model allowing spatiotemporal control of robust hair cell regeneration in the adult mouse cochlea, with almost all regenerated hair cells becoming Prestin+ outer hair cell-like cells.

T90. Transcriptomic Analysis of Newly Regenerated Avian Auditory Hair Cells

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Category: Regeneration
Background: Inner ear sensory hair cells (HCs) are essential for transducing sound and balance stimuli. In mammals, lost HCs are not regenerated, leading to permanent sensory deficits. However, non-mammalian vertebrates, such as birds, have the remarkable ability to regenerate

HCs. This regenerative process occurs as supporting cells either proliferate and differentiate into new HCs or directly transdifferentiate into new HCs. Newly regenerated HCs in the avian hearing organ (the basilar papilla) differ from mature HCs by lacking hair bundles and innervation. We hypothesize that the transcriptomic profiles of these nascent HCs are distinct and reflect an intermediate phase of HC identity that precedes full cytomorphological and functional maturation.
Methods: Our laboratory previously generated single-cell transcriptomic profiles of cells from the chicken basilar papilla at various time points following sisomicin-induced HC loss at post-hatch day 7¬. The transcriptomes of nascent HCs at four days post-sisomicin treatment (PST) were compared with those of undamaged HCs to identify marker genes. The expression patterns of these nascent HC markers were further validated using qPCR and in situ mRNA detection techniques.
Results: Single-cell RNA sequencing analysis revealed significant differences in gene expression profiles between nascent HCs at four days PST and baseline undamaged HCs. Validation of these distinct marker genes showed that newly regenerated HCs, although formed over several days, exhibit a coordinated onset of a nascent gene expression pattern, followed by a gradual transition toward a gene expression profile indicative of HC maturation.
Conclusions: Our findings reveal a unique transcriptomic signature in nascent regenerating HCs in the chicken inner ear, characterized by the presence of genes usually not expressed by HCs and the absence of some genes that one would expect to be active in HCs. These genes may be required for HC fate commitment and differentiation, specifically during regeneration, and their expression may differ from that seen during normal development. Understanding the molecular mechanisms of HC regeneration in birds could provide valuable insights for developing reprogramming strategies aimed at inducing HC regeneration in mammals.

T91. EDNRB2 is a Novel Marker for the Precursor State and Involved in Differentiation into Hair Cells in the Avian Auditory Epithelium

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Category: Regeneration
Background: In contrast to mammals, the avian auditory epithelium, basilar papilla (BP), is capable of hair cell (HC) regeneration through direct conversion or mitotic division of supporting cells (SCs). To elucidate the mechanism of avian HC regeneration, we previously performed single-cell RNA sequencing using an explant culture model (Matsunaga et al., 2023), in which a 48-h exposure to streptomycin induced total HC loss followed by HC regeneration via SC direct conversion (Matsunaga et al., 2020). The results of pseudotime trajectory analysis suggested that SC was differentiated into HC through a precursor stage, and endothelin receptor beta 2 (EDNRB2) was the most differentially expressed gene in the precursor stage. The aim of this study is to clarify the functional role of EDNRB2 in the avian cochlea during HC regeneration.
Methods: Post-hatched day-1 (P1) and embryonic days (E) 4, 7, 9, and 11 chickens were used as experimental animals. P1 chick cochlear ducts were provided for explant cultures. Explants were exposed to streptomycin for 48 h to induce complete HC loss. Histological evaluation was performed by immunostaining or in situ hybridization (ISH). BQ788 was used for the

pharmacological inhibition of EDNRB signaling. Bulk RNA sequencing was performed to examine differentially expressed genes in explant cultures resulting from the suppression of EDNRB signaling.
Results: ISH for EDNRB2 in chick embryos revealed that the expression of EDNRB2 was detected only in the precursor cells during cell fate determination at E7 and E9. On the other hand, the expression of EDNRB2 was not observed in common progenitor cells at E4 or nascent HCs at E11. These results indicate that EDNRB2 is a specific marker of precursor cells in BPs. Pharmacological inhibition of EDNRB signaling during HC regeneration of BP explants resulted in a significant decrease of regenerated HCs, suggesting that EDNRB2 may regulate the differentiation of precursor cells into HCs. RNA sequencing of BP explants with or without exposure to an EDNRB inhibitor demonstrated that inhibition of EDNRB signaling downregulated the expression of genes associated with HC differentiation and cell migration. Conversely the cell cycle-related genes were upregulated by inhibiting EDNRB signaling. These findings indicate that EDNRB2 plays a role in regulating the cell cycle of precursors and migration to the HC location during HC differentiation.
Conclusions: EDNRB2 is a novel marker of precursor cells in the chicken auditory epithelium and may be involved in the cell cycle and migration of precursors to the HC position, which is crucial for SC direct conversion to HC and maturation of nascent HCs.

T92. Sox2 Binds to Loci Associated With Hair Cell Regeneration Genes

Theresa Mai*[1], Erin Jimenez[1]

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Category: Regeneration
Background: Hearing loss affects over 430-million people worldwide, with more than 90% of individuals affected by sensorineural hearing loss, the result of damage to hair cells within the inner ear that relay sound to the brain. It is predicted that in the next 40 years, the hearing loss epidemic will impact over 30 million adults, highlighting the significant need for a long-term solution. Intriguingly, non-mammalian vertebrates such as the zebrafish spontaneously regenerate hair cells after loss or damage, making these organisms attractive models for studying hearing regeneration with potential therapeutic applications. We have shown that the adult zebrafish hair cell regenerative capacity is controlled by regulatory elements within the genome that interact with a network of Sox and Six transcription factors. Among these transcription factors, Sox2 is of interest due to its known importance in cell fate specification during inner ear development. However, the specific genomic targets of Sox2 in the regenerating adult zebrafish inner ear have not been explored.
Methods: To study hair cell damage and regeneration in adult zebrafish, we use Tg(myo6b:hDTR) transgenic animals that express the human diphtheria toxin receptor (hDTR) gene under the control of the hair cell specific myo6b promoter. Treatment of adult Tg(myo6b:hDTR) zebrafish with an injection of diphtheria toxin (DT) leads to widespread ablation of hair cells in the auditory and vestibular organs 5 days post injection. To identify the direct genomic binding targets of Sox2 during hair cell regeneration, we isolated inner ear sensory epithelia at consecutive timepoints after ablation followed by immunoprecipitation of epitope tagged Sox2 protein along with its bound DNA. DNA was sequenced to determine the

genomic regions that were bound by Sox2. To identify regeneration specific Sox2 genomic targets, we removed common peaks shared between untreated and treated samples leaving only regeneration specific genomic targets of Sox2. This data was integrated with previous single-cell ATAC-seq and RNA-seq regenerating zebrafish sensory epithelia to identify potential Sox2 dependent transcriptional targets.
Results: Several strong peaks were located within genetic loci of transcripts with known functions during adult zebrafish hair cell regeneration, including Sox2, Atoh1a, and Six4b. Enrichment of known Sox motifs and de novo motifs were significantly present within peaks. Finally, we identified Sox2 binding at over 4000 conserved non-coding elements.
Conclusions: This study elucidates the gene regulatory networks that govern hair cell regeneration to identify potential therapeutic candidates that can be leveraged to promote regeneration. To better understand the mechanism by which Sox2 regulates transcription of nearby genes, our future work will examine the consequence of Sox2 conditional loss of function on bound loci gene expression as determined by scRNA-seq experiments in regenerating adult zebrafish inner ears.

T93. Investigating cAMP and PKA Signaling in Avian Supporting Cell Proliferation

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Category: Regeneration
Background: Hearing loss is a global health issue affecting millions of people worldwide, with no available restorative treatments. Mammals, including humans, cannot regenerate lost inner ear sensory hair cells, leading to permanent hearing loss. In contrast, non-mammalian vertebrates, such as birds, possess the ability to regenerate hair cells and restore hearing, a process driven by supporting cell division. Previous work from our lab identified an essential signaling pathway for supporting cell S-phase entry and hair cell regeneration in vivo, but only after hair cell damage induced by an ototoxic drug, sisomicin. To deepen our understanding, we aim to explore additional signaling pathways that regulate mitotic quiescence in the undamaged basilar papilla and promote supporting cell proliferation after hair cell ablation. Previous studies, conducted in vitro, found that cyclic AMP (cAMP) activators stimulate proliferation in the undamaged auditory sensory epithelium, a response blocked by cAMP-regulated protein kinase A (PKA) inhibitors. Our in vivo paradigm serves as a robust model to validate these findings.
Methods: We pharmacologically elevated cAMP levels in the undamaged basilar papilla by directly infusing cAMP activators into the lateral semicircular canal of post-hatch day 7 (P7) chickens. Supporting cell proliferation was assessed through EdU incorporation 48 hours post-infusion. We also tested these activators in the damaged basilar papilla by co-infusing them with sisomicin. To assess the role of PKA, we used PKA inhibitors to reduce cAMP levels in combination with sisomicin and/or cAMP activators to determine their effect on supporting cell proliferation.
Results: Preliminary results show that elevating cAMP levels with 8-Bromo-cAMP and 3-isobutyl-1-methylxanthine (IBMX) in the undamaged basilar papilla resulted in a minimal increase in supporting cell proliferation, as indicated by the presence of EdU incorporation.

Surprisingly, co-administration of cAMP activators with a PKA inhibitor still resulted in supporting cell proliferation. However, introducing PKA inhibitors alone or with sisomicin did not significantly reduce the proliferative response observed with cAMP activation and sisomicin treatment. These results suggest that PKA may play a complex role in supporting cell proliferation.
Conclusions: Our results reveal additional signaling components that may play an important role in regulating supporting cell proliferation in vivo. The cAMP pathway appears to be capable of driving supporting cells into S-phase without damaging the avian basilar papilla. These insights enhance our understanding of sensory hair cell regeneration in birds and lay the groundwork for future translational studies for hearing loss in mammals.

T94. Exploring the Role of Cochlear Immune Response in Acsl4 and Pex3 Mutant Mice With Age-Related Progressive Hearing Loss

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Category: Immunology
Background: Acsl4 converts polyunsaturated fatty acids (PUFAs) into fatty acyl-CoA esters, modulating eicosanoids that influence inflammation and resolution. Pex3 is essential for peroxisome formation, affecting PUFA metabolism, ROS control, and inflammation. Our previous study (Kochaj et al., 2022) demonstrated progressive hearing loss in Pex3 mutants. Similarly, Acsl4 mutant mice exhibit normal hearing at 2 weeks but show rapid deterioration by 3 weeks. Since these genes are involved in lipid pathways regulating inflammation, this project aims to determine whether cochlear immunity contributes to hearing loss or responds to cochlear dysfunction by comparing immune responses before and after hearing loss onset in these mutants.
Methods: Flow cytometry was used to quantify cochlear immune cell populations. Two cochleae from each mouse were dissected, and immune cells were isolated and processed to identify various cell types, including leukocytes, resident and recruited macrophages, and neutrophils. Macrophages were labelled with an Iba1 antibody, and confocal imaging was performed at specific cochlear regions.
Results: At 3 weeks old, post-onset of hearing loss, flow cytometry revealed an increased number of leukocytes and immune cells in Acsl4 mutant mice. A distinct morphological gradient of macrophages was observed along the cochlear duct, with Acsl4 mutants showing predominantly ameboid (activated) macrophages, while controls had mostly dendritic (resting) macrophages. Similarly, preliminary data from 4-week-old Pex3 mutants showed ameboid macrophages throughout the cochlea, in contrast to the dendritic morphology seen in controls. Preliminary data from 2-week-old Acsl4 mutants, before hearing loss onset, showed no significant differences in cochlear macrophage morphology compared to controls.
Conclusions: In both Acsl4 and Pex3 mutants, cochlear macrophages transitioned to an activated, ameboid state following hearing loss onset. Identifying common features of macrophage activation across different mutants with progressive age-related hearing loss may enhance therapeutic strategies by revealing key targets for drug intervention.

T95. Macrophage Heterogeneity in Cochlea: Implications for Auditory Function in Healthy and Aged Mice

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Category: Immunology

Background: Macrophages orchestrate homeostatic, inflammatory, and reparative activities. However, these activities are tissue-dependent, making them a highly heterogenous population. Presence of macrophages have been reported in the cochlea of the inner ear, however, their dynamics and functions remain relatively unexplored. Understanding their heterogeneity is crucial to deciphering their roles in both normal and diseased states. Through single-cell RNA sequencing, the Ajami lab has identified three macrophage populations at the protein level expressing Tmem119 (coMGL) and the two other populations (coMacs), both expressing CD206, but only one of the populations expressing CD163, in healthy adult mice. We hypothesize that these populations have specific functional roles in the cochlea.
Methods: Immunohistochemistry was used to confirm the presence of these macrophage populations in both young and aged mice, as well as in different compartments of the human cochlea. Preliminary auditory brainstem response (ABR) tests were conducted in FMS-intronic regulatory element (FIRE) FIRE mice, which lack the Tmem119+ population, and Lyve1-CRE mice, which lack the CD206+CD163+ population. Additionally, a noise-damage model was developed in healthy mice to investigate the functional differences among macrophage populations following noise exposure.
Results: Improved hearing levels were observed in the FIRE mice compared to controls. Quantification of coMacs after noise damage revealed compartmental differences in macrophage populations compared to control mice.
Conclusions: The findings emphasize the importance of understanding the heterogeneity of cochlear macrophage populations in order to elucidate their specific functions under normal and pathological conditions. Future studies will focus on applying the noise-damage model to FIRE and Lyve1-CRE mice to explore how the absence of these macrophage populations impacts the response to noise-induced damage.

T96. Circadian Modulation of NLRP3 Inflammasome Activation in Macrophages Exacerbates Noise-Induced Hearing Loss: Insights From Single-Cell RNA Sequencing

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Category: Immunology

Background: Noise-induced hearing loss (NIHL) is a leading cause of acquired sensorineural hearing loss (SNHL), with over 1 billion people at risk due to excessive noise exposure. While past research has mainly focused on mechanical and metabolic damage in the cochlea, recent studies have highlighted the importance of immune and inflammatory responses in noise-induced cochlear damage. The cochlea is now recognized as a dynamic immune environment, with mononuclear phagocytes, particularly macrophages, playing key roles in responding to stress. However, the detailed immune landscape in noise-exposed cochleae remains incompletely understood. This study aims to profile immune and non-immune cells in the cochlea before and after noise exposure and investigate the role of the NLRP3 inflammasome in NIHL, particularly during nighttime.
Methods: We conducted single-cell RNA sequencing (scRNA-seq) on cochlear samples from male CBA/CaJShjh mice exposed to noise at different Zeitgeber times. Immune and non-immune cells were analyzed using UMAP clustering. Mononuclear phagocyte populations were identified and re-clustered, and gene set scoring was applied to assess macrophage activation. Multiplex immunohistochemistry and western blotting were used to evaluate NLRP3 inflammasome activation in macrophages. CY-09, an NLRP3 inhibitor, was tested to reduce noise-induced inflammation and macrophage activation. Auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE) were measured to assess hearing function.
Results: The analysis revealed that macrophages and monocytes are the primary immune cell types responding to noise exposure, showing significant enrichment post-exposure. In particular, NLRP3 inflammasome signaling was notably amplified in macrophages during nighttime noise exposure, leading to increased levels of pro-inflammatory cytokines such as IL-1β and IL-18. This inflammatory response was associated with enhanced cochlear damage, including glutamate-induced neurotoxicity and synaptic loss. Among non-immune cells, spiral ganglion neurons (SGNs) showed the most significant changes following noise exposure, although overall non-immune cell populations remained relatively stable across conditions. Treatment with the NLRP3 inhibitor CY-09 effectively mitigated the inflammatory response in macrophages, suggesting its potential as a therapeutic approach.
Conclusions: This study highlights the central role of macrophages and NLRP3 inflammasome activation in driving noise-induced cochlear damage, particularly during nighttime noise exposure. The amplification of NLRP3 signaling exacerbates inflammation and cochlear injury, contributing to heightened neural excitability and synaptic loss, while NLRP3 inhibitors like CY-09 show promise in reducing these effects. These findings provide new insights into the immune dynamics of NIHL and suggest potential therapeutic strategies to protect against inflammation-related cochlear damage, not only in NIHL but also in other forms of SNHL.

T97. Open Board

T98. Fractalkine Signaling Regulate the Levels of Several Chemotactic Cytokines After Acoustic Trauma

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Category: Immunology
Background: Fractalkine (CX3CL1) is a chemokine produced in spiral ganglion neurons (SGNs) and binds to its receptor, CX3CR1, expressed by macrophages in the cochlea. Mice lacking CX3CR1 show decreased macrophage density and increased SGN loss after cochlear injury due to selective hair cell ablation, aminoglycoside ototoxicity, or acoustic trauma. These findings suggest that intact fractalkine signaling is required to regulate macrophage density and SGN survival in the injured cochlea, yet the mechanisms remain unknown. This study aimed to examine the effects of fractalkine signaling on cochlear inflammation by measuring cytokine levels after acoustic trauma to understand how fractalkine plays a neuroprotective role in the injured cochlea.
Methods: We utilized mice with intact fractalkine signaling (wildtype) and that were deficient in either CX3CL1, CX3CR1 or express CX3CR1-I249/M280 human polymorphic variant instead of the murine counterpart (CX3CR1hM280). 5-6 weeks aged mice per genotype were exposed to 112 dB SPL noise at 8-16 kHz for 2 hours or unexposed. Animals were euthanized following phosphate-buffered-saline intracardial perfusion; cochleae were isolated at 1-, 7-, and 15-days post noise exposure (DPNE). Protein was extracted from pooled cochleae from 3-4 mice per experimental group. Samples were loaded in triplicate at an equal concentration of 50 µg/µL and read on a Luminex xMAP system. The 26 cytokines screened were, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12, IL-13, IL-15, IL-17, IL-18, IL-22, IL-23, IL-33, M-CSF, IFNα, IFNβ, IFNγ, TNF-α, CXCL1, CXCL2, CXCL10, CCL2, CCL3, CCL4, CCL7. Data was analyzed with ProcartaPlex software, normalized to total protein (pg/mL) with 4PL/5PL logistic fit. Analytes that showed a fold change (FC) of ≥1.0 after acoustic trauma were included. Each analyte was compared among the four genotypes per recovery period after acoustic trauma. A two-way ANOVA was performed to determine the statistical significance among the genotypes.
Results: Cytokine levels were comparable between unexposed wildtype and fractalkine signaling mutant mouse models. Cytokines and chemokines like IL6, CXCL1, CXCL2, CXCL10, CCL2, CCL7, CCL3, and CCL4 showed a significant elevation (FC ≥ 1.5) at 1 DPNE in all the genotypes with their levels gradually returning to baseline by 15 DPNE without significant difference among the genotypes. Notably, at 1 DPNE, chemotactic cytokines like CXCL1, CXCL2, CXCL10, CCL2, CCL7, CCL3, and CCL4 were found to be significantly reduced in mice lacking CX3CR1 receptor compared to wildtype mice.
Conclusions: These data suggest that such acoustic trauma causes acute inflammation due to spontaneous resolution of elevated cytokine levels. Importantly, fractalkine signaling regulates the levels of several chemotactic cytokines for monocytes, macrophages, and neutrophils after acoustic trauma, suggesting its role in the chemotaxis of immune cells in the noise-injured cochlea. Future studies will address whether infiltration of blood-circulating immune cells influences SGN survival after acoustic trauma.

T99. Single-Cell Multiomics Supports Different Immune Profiles in Migraine, Vestibular Migraine and Menière Disease

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Category: Immunology
Background: Menière Disease (MD) is an inner ear condition that is characterized by episodes of vertigo, tinnitus and sensorineural hearing loss (SNHL). Migraine (MI) affects about 20% of the world population, and subset may show concurrent vestibular symptoms and classify as Vestibular Migraine (VM). The goal of this study is to analyze mononuclear cells of MI, VM and MD individuals using single-cell multiomics to define immunophenotypes and potential druggable molecular targets.
Methods: Nuclei from peripheral blood mononuclear cells from 18-patient Spanish individuals with MI (5), VM (5), MD (7) and 6 healthy controls were isolated using the Chromium 10x platform. After library preparation and sequencing, scRNAseq and scATACseq FASTQ files were processed using 10X's software Cell Ranger ARC and were aligned with GRCh38 reference genome. Downstream analysis was done using Seurat and Signac R packages, and plot visualization was performed using ggplot2.
Results: Migraine and VM did not show any differences in their transcriptome, consequently, they were regarded as a single cluster (MI+VMc). In migraine patients, polarization of NK cells was driven by the release of IL-2, IL-12, IL-15, IL-18 from immune lymphoid cells type 1 (ILC-1). Two phenotypes are observed in MD, one that inactive, and one driven by monocyte genes. Monocytes on MD "Monocyte-driven" cluster (MDMc) are polarized by Stem Cell Factor, Vascular Endothelial Growth Factor, and Hepatocyte Growth Factor.

Immune related pathways are active in both MI+VMc and MDMc. Specific biochemical pathways in MI+VMc were cellular response to metal ions, whereas MDMc enriched pathways were response to biotic stimuli.

Conclusions: The immune response differs in MI+VMc and MDMc. These findings support NK cells polarization by ILC-1 cytokines in migraine patients, and two phenotypes in MD individuals, one inactive and one "Monocyte-driven".

T100. The Effect of L-Ergothioneine on Gap-In-Noise ABR Temporal Coding in Middle-Aged CBA/CAJ Mice

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Category: Hearing Loss: Consequences and Adaptation
Background: Age-related hearing loss (ARHL) stands as the most prevalent form of hearing impairment and represents the third most common chronic health condition among the elderly population. The degenerative processes affecting the peripheral and brainstem auditory systems may begin in middle age, so, a key therapeutic window for preventative interventions of ARHL may exist. L-ergothioneine (EGT), a naturally occurring amino acid, is a potentially novel approach for attenuating ARHL progress through timely intervention strategies. Using the middle-age CBA/CaJ mouse, a strain which mirrors many aspects of human ARHL, we

investigated whether or not long-term EGT treatment could improve auditory temporal processing. Our study provides novel insights for exploring if EGT's antioxidant and anti-inflammatory properties can slow down the progression of ARHL.
Methods: Thirteen middle-aged CBA/CaJ mice (16~19 months old) were categorized into two groups: the control (N=7) and EGT-treated group (N=6, treated with 35mg/kg EGT administered subcutaneously for 7 days, and then 70mg/kg EGT once/week until testing end date). A Gap-in-noise (GIN) ABR was used to assess temporal processing. For ABR GIN, a silent gap was inserted in the center of two wideband noise (WBN) bursts, NB1 and NB2, at 80 dB SPL, each with a repetition rate of 21/s, duration of 25 ms and rise-fall times of 0.5 ms. Each averaged response was obtained from 100 stimulus presentations and then replicated, with gaps-in-noise having durations of: 0, 1, 2, 4, 8, 16, 32 to 64 ms. ABRs were acquired over a period of 150 msec using the TDT System 3 and BioSig.
Results: GIN ABR amplitudes and latencies were measured in control and EGT groups following two-months of treatment. The GIN ABRs amplitudes in the EGT-treated group showed no change for NB1 P4 and NB2 P1 between baseline and 2 months post-treatment. The EGT treated group showed a decrease in the the NB1 wave 1 (P1) ABR latency, approximately ~0.2 (ms) at 2 months of EGT treatment.
Conclusions: Since GIN ABRs are considered a biomarker for auditory temporal processing, our results suggest that EGT treatment may have the potential to slow age-related temporal processing declines in middle-aged mice. This highlights a critical time window for preventing neurodegenerative disorders associated with aging. For future research, conducting longer-term experiments with EGT-treated mice (e.g., 4 or 6 months) could provide a clearer understanding of EGT's effects on ARHL, particularly in the context of early intervention in the aging process of the central auditory system.

T101. Transcriptome Analysis of the Mouse Cochlear Nucleus in Response to Auditory Input

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Category: Hearing Loss: Consequences and Adaptation
Background: he cochlear nucleus (CN) serves as the first relay station in the central auditory nervous system, responsible for receiving cochlear input. Sensory experience is known to modify the structure and function of neural circuits via activity-dependent neuronal plasticity, including cell type specification, axon/dendritic arborization, and formation of synaptic connection. Previous studies have shown auditory activity-dependent development of CN and plasticity of synapses between auditory nerve and CN neurons; however, which CN cell types and how global expression of genes are affected by the auditory input remain unclear.
Methods: In this study, we combined snRNA-seq, snATAC-seq and congenital hearing loss animal models to generate a comprehensive molecular change of the cochlear nucleus of normal and hearing-loss mice.

Results: 1) Based on comparison of transcriptomic profiles between normal mice and three types of hearing-loss mouse models (Vglut3-/-, Otof-/- and Ush1c-/- mice) as well as hearing-restored mice (Vglut3-/- gene therapy mice, Vglut3-/-+GT), we identified that the Spp1+ -bushy cell is the major cell type that exhibited most pronounce changes in transcriptomic profiles.
2) Among all hub genes showing hearing-dependent downregulation in hearing-loss mice, only Spp1 gene was shared among all three hearing-loss models.

3) Spp1 has the potential to impact the functionality of the auditory processing through its co-expression network, including Nefh, Nefm, Nefl, et al. This elucidates the mechanisms underlying activity-dependent cell plasticity transition.

Conclusions: Spp1+-bushy cells are the primary cell type affected by auditory input, and Spp1 plays an important role in maintaining bushy cell functions in response to auditory input.

T102. Emergence of Tonotopically Organized Spontaneous Activity in the Brain After Genetic Disruption of MET Channel Function

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Category: Hearing Loss: Consequences and Adaptation
Background: Hearing loss is pervasive and associated with other hearing-related disorders such as hyperacusis and tinnitus, and can precipitate cognitive decline and dementia. However, the mechanisms that link hearing loss to these brain pathologies are poorly understood. In tinnitus, the neural activity responsible for the perception of phantom sounds appears to be generated in the brain, but we have a limited understanding of the nature of this activity or the circuit level changes responsible.
Methods: To determine how hearing loss changes neural activity patterns in the brain, we developed an inducible model of complete hearing loss by deleting Tmie, which encodes a MET-associated protein critical for hair cell mechanoelectrical transduction, in 4–8 weeks-old Gfi1-P2A-GFP-CreERT2;Tmiefl/fl mice (Tmie cKO mice). We expressed genetically encoded Ca2+ indicators in these mice and defined the spatiotemporal response characteristics of neurons in the inferior colliculus (IC), a central hub of information processing in the auditory system. The response of neurons to sinusoidal amplitude modulated pure tones (3 – 32 kHz at 25 – 105 dB SPL; 10 Hz modulation) was recorded using wide-field single-photon (1P) and high-resolution, two-photon (2P) microscopy in awake mice at different times after Tmie deletion.
Results: Genetic inactivation of MET function resulted in complete loss of sound-evoked responses (thresholds GREATER THAN 90 dB SPL for ABR and Ca2+ imaging) within seven days. This loss of sound sensitivity was accompanied by the emergence of sound-independent (SI) neural activity in the IC. Remarkably, this SI activity included large-scale events aligned to the same isofrequency domains evoked by pure tones in control (hearing) mice. Two-photon imaging revealed that large scale coordinated events in Tmie cKO mice reflected simultaneous firing of groups of adjacent neurons, which were distinct from the sparse and uncorrelated spontaneously firing of hyperactive neurons that also emerge after deafening. SI activity in Tmie cKO mice was independent of cochlear input, as bilateral cochlea ablation did not diminish their frequency, suggesting these tonotopic-like activity patterns are generated within the brain.

Highly correlated SI activity was also observed one week after bilateral cochlear ablation in wild-type mice, suggesting that this phenomenon results from the absence of acoustic input, rather than other possible aspects of Tmie inactivation.
Conclusions: Here, we describe a new model of deafness achieved through inducible, genetic inactivation of the MET channel associated protein TMIE, providing reproducible, temporal control of cochlear inactivation. Inactivation of MET function in hearing mice led to the emergence of correlated neuronal firing aligned to isofrequency domains, resembling sound-evoked response patterns in hearing mice. The reproducible nature of this phenomenon holds great promise for uncovering the circuit level changes responsible for induction of SI activity following hearing loss that may be perceived as sound.

T103. Immune Response in the Spiral Ganglion Following Cochlear Hair Cell Loss

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Category: Hearing Loss: Consequences and Adaptation
Background: Spiral ganglion neurons (SGNs) of the cochlea slowly die after neonatal aminoglycoside-induced hair cell loss in rats, resulting in the death of GREATER THAN 80% of all SGNs over the course of ~14 weeks. We have previously shown that macrophages increase in abundance and activation by postnatal day 21 (P21), well prior to the start of significant SGN death, which is first apparent at P39. Additionally, concurrent with the start of SGN death, there is an increase in lymphocytes in the ganglion, including CD4+ helper T cells, CD8+ cytotoxic T cells, and CD161+ NK cells. To determine whether the lymphocyte component of this inflammatory response is causal to SGN death, we evaluated SGN survival after hair cell loss in two immunodeficient rat strains: 1) RNU nude rats lacking T cells and 2) SRG rats lacking T, B, and NK cells.
Methods: Sprague-Dawley (SD), Crl:NIH-Foxn1rnu/rnu (RNU nude, T cell deficient), Crl:NIH-Foxn1rnu/+ (RNU+, normal T cells), and Rag2em2heraIl2rgem1hera/HblCrl (SRG, T, B, NK cell deficient) rats were intraperitoneally injected 1x/day with kanamycin from P8 to P16. Cohorts of deafened and hearing control rats were euthanized at various ages and cochlea were sectioned for immunohistochemistry to label hair cells, neurons, leukocytes, macrophages, T cells, B cells, and NK cells.
Results: We found that SGN survival in RNU nude rats is not significantly different than in RNU+ rats after deafening, indicating that T cells are not required for SGN death. Preliminary data indicate that genetic ablation of T, B, and NK cells in SRG rats does not prevent SGN death post-deafening, however, SGN survival is moderately improved in the basal half of the cochlea of SRG rats compared to deafened wildtype controls. For all strains evaluated, we also found that macrophage number is significantly increased in the kanamycin treated groups compared to hearing controls.
Conclusions: We directly assessed the role of T cells in SGN death after deafening using T cell deficient RNU nude rats and found that T cells are not required for SGN death after hair cell loss. Preliminary results using the T, B, and NK cell deficient SRG strain indicate that lack of all lymphocytes improves SGN survival in the basal, but not apical, half of the cochlea compared to

hair cell ablated controls. Further investigation is required to determine the role of lymphocytes, specifically B and NK cells, in the cochlea after hair cell loss. As we have previously reported, the increase in macrophage number and activation within the cochlea precedes significant neuronal death, indicating that macrophages are not simply responding to increased need for phagocytosis of neuronal debris, but rather may be casual to the death of SGNs. Future studies are planned to assess the role of macrophages in SGN death after hair cell loss.

T104. Noise-Induced Hearing Loss Impairs Auditory and Visual Decision-Making Task Learning

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Category: Hearing Loss: Consequences and Adaptation

Background: Hearing loss (HL) has consequences that include both sensory and cognitive processing deficits. Previous work has shown HL can impede auditory learning and task acquisition (von Trapp et al., 2017). However, the neural basis of HL-related deficits to non-auditory processing are unclear. Here, we developed a behavioral paradigm to assess auditory and visual decision-making task acquisition under normal hearing (NH) and noise-induced HL (NIHL) conditions. We examine whether NIHL impairs learning across different sensory modalities.

Methods: Separate groups of adult gerbils were trained to perform a single-interval alternative forced-choice auditory or visual decision-making task. Gerbils initiated trials by placing their nose in a nose poke on one end of the test cage. They were then required to discriminate between slow (4-Hz) versus fast (12-Hz) presentation rates of amplitude- modulated (AM) noise (Auditory task) or light-emitting diode (LED) flashes (Visual task) by approaching the left or right food tray located on the opposite side of the test cage. For a subset of gerbils in each group, we induced permanent HL by exposing them to loud noise (~120 dB SPL) during a single 2-hour session. We quantified hearing sensitivity by measuring auditory brainstem responses (ABRs) across pre- and post- noise exposure time points.

Results: Exposing gerbils to loud broadband noise permanently decreased hearing sensitivity. Specifically, we found a significant increase in ABR thresholds ranging ~30-60 dB SPL for clicks and tones that persisted for more than two weeks post-noise exposure. NH gerbils learned the auditory and visual discrimination tasks at similar rates ($p=0.98$). However, gerbils with NIHL exhibited a significantly slower rate of auditory task acquisition (average total trials = 4424) compared to NH gerbils (average total trials = 2402) ($p=0.0003$). For the visual task, gerbils with NIHL also exhibited a significantly slower rate learning (average total trials = 4141), compared to NH counterparts (average total trials = 2414) ($p=0.002$). In general, gerbils with NIHL displayed a lack of motivation during task acquisition, which can be explained by their limited number of daily trials compared to NH counterparts. Gerbils exhibited different strategies during learning according to hearing status. Typically, NH gerbils displayed a strong side bias during initial training sessions that diminished throughout training. Gerbils with NIHL did not exhibit initial side biases and showed signs of balanced guessing. This may indicate a lack of perseveration under conditions of NIHL, which delays long-term learning. We found no

significant difference in reaction times between NH vs NIHL gerbils on auditory (p=0.78) and visual (p=0.99) task performance.
Conclusions: These findings suggest that NIHL delays the learning of both auditory and visual decision-making tasks. Future experiments will examine the neural basis of these effects.

T105. Small Arms Fire-Like Noise Induced Hearing Loss (NIHL) May Possess Distinct Diagnostic Profile From Previously Studied Models of NIHL

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Category: Hearing Loss: Consequences and Adaptation
Background: Noise exposure is the second most common cause of hearing loss, behind aging. Small arms fire-like (SAF) noise is an acute form of noise exposure found in military and law enforcement occupations and recreation. Clinically, SAF noise induced hearing loss (SAF-NIHL) is often diagnosed and treated similarly to other forms of hearing loss by addressing loss of hearing sensitivity through hearing amplification strategies. However, SAF exposure may differ from other forms of NIHL. To develop SAF specific diagnostics and therapeutics, it is imperative to investigate the pathophysiology of SAF-NIHL. In 2019, Altschuler et al. found increased thresholds, reduced wave 1 auditory brainstem amplitudes, and a reduction in cochlear synapses 12-15 weeks after noise exposure. While these measures characterize peripheral damage, they do not provide information about more central auditory changes or responses to complex sounds. Our study aims to identify SAF-NIHL biomarkers responsible for auditory processing throughout the peripheral and central auditory systems to inform a progression map of damage post-SAF exposure. We hypothesize SAF exposure disrupts temporal processing through damage to hair cells and ribbon synapses leading to downstream neurotransmitter imbalance and neuroinflammation throughout the auditory brainstem.
Methods: Rat subjects (3-6 months) were exposed to SAF noise (50 rounds of 12 biphasic 0.3 ms pulses, 1 round every 3 s) at either 120 dBpSPL (SAF group; n=8, F=4) or 60 dBpSPL (sham group; n=4, F=2). We analyzed distortion product otoacoustic emissions (DPOAEs)(4, 8, 10 kHz), auditory brainstem responses (ABRs)(click, 8 kHz), and auditory evoked potentials (AEPs) of complex stimuli at baseline and post exposure days (7, 14, 28, 56). Thresholds were found using click and 8 kHz ABRs. Complex AEP stimuli included dynamically amplitude modulated sweeps (dAMs)(8 kHz or noise carrier amplitude modulated exponentially) and speech tokens of a male voiced “Purdue” (8 kHz or noise carrier modulated by speech envelope).
Results: Thresholds were persistently elevated by 10-15 dB. DPOAE signal-to-noise ratio decreased in all post-exposure days at 8 and 10 kHz. ABR waveform analysis demonstrated an overall decrease in amplitude with a greater decrease in wave 5. dAM response energy decreased at all days for frequencies between 8-120 Hz. Speech token neural responses demonstrate an overall decrease in response to all frequencies, except for a heightened response seen at the onset of the stimulus for frequencies between 500-2500 Hz. All measures demonstrated a general trend of gradual damage (days 7-14) and minor recovery (days 28-56).
Conclusions: Our findings suggest the diagnostic profile of SAF-NIHL may differ from the previously studied models of NIHL. Future work will identify mechanisms of damage at

different time points post-exposure through anatomical imaging of biomarkers including neurotransmitter, neuron-glia interaction, hair cell integrity, and synaptic ribbon puncta.

T106. Mouse Facial Grimace Can Be Used to Assess Auditory Pain During Sound Exposure and Requires Cochlear Mechanotransduction

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Category: Hearing Loss: Consequences and Adaptation

Background: Pain hyperacusis is a debilitating condition in which normally tolerated levels of sound are perceived as painful. This aSects many facets of life and can result in patients withdrawing from the world (Fackrell et al., 2019). Currently, no treatments exist for pain hyperacusis beyond sound avoidance (Jahn and Koach, 2023). A mouse model of auditory pain is needed to study the cause of pain hyperacusis and eventually develop treatments. We previously presented an assay for auditory pain by measuring facial grimace using continuous sound that increased in level over time from 70 to 120 dB SPL (Kohler et al., ARO 2024). Here, we show progress on this assay by 1) interleaving sound presentation with silence and 2) demonstrating that auditory pain relies on cochlear detection of sound.

Methods: Two cohorts, 6-week-old C57BL/6 (wildtype) mice and TMIE-/- mice with littermate controls (TMIE-/+, Zhao et al., 2014), were exposed to sound (2-minute periods of white noise, 2-20 kHz) at sound pressure levels (SPL) varying from 70 dB to 120 dB interspersed with 2 minutes of silence. Animals were contained in custom-made 9 x 5 x 5 cm clear polycarbonate boxes with air holes on one end to encourage a profile presentation for video recording. A deep neural network (DeepLabCut) was trained to mark features of the mouse head in profile on each frame of video (Moëne and Larsson, 2023). The facial parameters of eye ratio, ear ratio, ear angle, ear tip tilt, snout position and mouth position were used to assess the presence of a facial grimace response to sound, as previously reported (Kohler et al., ARO 2024). Nose tip position was used as an additional measure of mouse motion. ABRs were performed to assess hearing of TMIE-/- mice and littermate controls, as the knockout mice are deaf due to lack of mechanotransduction in inner ear hair cells (Zhao et al., 2014).

Results: Facial parameters were significantly diSerent in wildtype mice as a function of SPL. In addition, nose position showed reduced variance during periods of sound. The interspersed 2-minute intervals of silence showed no correlation with sound level and were not significantly diSerent from the first 2-minute period before sound exposure. Thus, changes in facial grimace and movement occurred in response to sound exposure. TMIE-/- mice lack hair cell mechanotransduction, however, their middle ear mechanics are unaSected. TMIE-/- mice do not grimace or change their movement patterns due to increased levels of sound as wildtype mice do.

Conclusions: As facial grimace and reduction in movement are hallmarks of an animal experiencing pain, we conclude that the measured auditory pain experience in response to sound exposure relies on the cochlear detection of sound.

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T107. Modeling Normal and Impaired Hearing With Deep Neural Networks Optimized for Ecological Tasks

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Category: Hearing Loss: Consequences and Adaptation

Background: Computational models that perform real-world hearing tasks using cochlear input could help link the peripheral effects of hearing loss to real-world perceptual consequences. Deep artificial neural networks, optimized separately for sound localization and recognition tasks, have been shown to account for many aspects of normal hearing behavior. Here, we extend this approach to model the behavioral consequences of hearing loss using a network jointly optimized for multiple tasks.

Methods: We trained a single deep neural network model to localize and recognize speech, voices, and environmental sounds using simulated auditory nerve representations of naturalistic scenes. Once trained, we compared the model's spatial hearing and speech recognition performance to that of humans. We also measured the model's psychoacoustic thresholds (tone detection in quiet/noise, temporal gap detection, and spectral/temporal modulation detection) by training linear classifiers to make binary judgments using the model's learned features. These classifiers are intended to represent the decision rules that humans use to perform simple hearing tests, relying on relatively fixed internal representations that were plausibly optimized for ecological tasks over longer timescales. To investigate the perceptual consequences of hearing loss, we altered the model's peripheral input and measured the resulting effects on behavior. Different types of hearing loss were simulated by manipulating the number and functionality of inner hair cells (IHCs), outer hair cells (OHCs), and auditory nerve fibers (ANFs).

Results: When equipped with healthy cochleae, the model accounted for several aspects of binaural speech perception in humans with normal hearing, reproducing the effects of noise, reverberation, and spatial separation between speech and noise. When healthy cochleae were replaced with damaged cochleae, the model's performance resembled that of humans with hearing loss: speech recognition deteriorated (especially at low SNRs) and spatial release from masking was reduced. Psychoacoustic thresholds measured from the model similarly reproduced patterns of normal and impaired human hearing. Despite never being fit to human data, the model replicated the effects of noise carrier bandwidth, modulation rate, and masker modulations on human amplitude modulation detection thresholds. Simulations of plausible and idealized hearing loss phenotypes (i.e., combined OHC and ANF loss vs. isolated OHC or ANF loss) suggest that both OHC and ANF loss contribute to real-world hearing difficulties, but they produce distinct behavioral outcomes in psychoacoustic listening tests.

Conclusions: The results provide a normative account for fundamental aspects of human hearing, suggesting phenomena like spatial release from masking and modulation frequency selectivity can be understood as consequences of optimization for ecological tasks. Machine-learning-based models that generate behavior from simulated auditory nerve input can predict aspects of hearing-impaired behavior and may help disentangle the perceptual consequences of different types of hearing loss.

T108. Mind the Gap Among Aural Performance, Language Perception and Listening Comprehension of Short Stories in Children Using Cochlear Implants

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Category: Hearing Loss: Consequences and Adaptation
Background: Listening comprehension is an important aspect of oral communication. Previous studies on listening comprehension in cochlear implanted (CI) children focused on the comprehension of words and sentences. Therefore, little is known about the contribution of cognitive and linguistic factors to complex-text (e.g., stories) comprehension, which becomes increasingly important as children age. We examined the associations among listening comprehension, speech perception, memory, and language abilities.
Methods: 42 children aged 6-10 years participated in the study so far: 27 with normal hearing (NH), and 15 bilateral CIs users. Data collection from children with CIs is ongoing. Children were tested on 2 stories, and speech perception, memory and language tasks. Their parents evaluated their aural skills with the PEACH questionnaire (Parents' Evaluation of Aural Performance of Children).
Results: Preliminary results indicate that children with CIs were rated significantly lower on aural performance in noise than NH children based on parental questioners. They also had poorer word perception and phonological processing. In contrast, there were no significant differences in listening comprehension between the groups assessed by either comprehension questions or the ability to re-tell the stories. Phonological processing was the only significant predictor of listening comprehension in either group.
Conclusions: Contrary to our expectations, listening comprehension was not reduced in the current sample of CI users. This suggests that despite poorer aural performance children with CI may be able to compensate for the perceptual disadvantages they have while listening to short stories. Phonological processing was the only predictor of listening comprehension, suggesting that language is key to comprehension skills both for NH and CI children. Another implication is that the commonly used listening comprehension task we chose likely does not represent the difficulties CI children experience daily, especially in adverse listening conditions.

T109. Testing of Auditory Reaction Time to Explore Value of Robotic Stop of Cochlear Implant Insertion

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Category: Clinical Otolaryngology & Pathology
Background: Risks of cochlear implantation include damage to the microstructures of the cochlea or translocation of the electrode array into the scala vestibuli. Robotics-assisted insertion attempts to avoid trauma by addressing human kinetic limitations by facilitating slower and more controlled insertion rates than achieved by manual insertion. Performing electrocochleography

during insertion provides feedback regarding the status of the physiological system. Although both robotics and electrocochleography have been utilized in the same surgery, hearing preservation outcomes remain variable. A possible explanation that has not yet been explored is the time delay between an electrocochleography Signal Drop Alert and stopping electrode insertion. Average human response time to auditory stimulation is 0.5s. During the time of this reaction, the array is allowed to travel additional distance, possibly resulting in permanent damage to the cochlea by over insertion that could have been avoided if reactions were faster/instantaneous. A combined "AIMBOT" system has been developed by coupling iotaMotion's "iotaSOFT" robotic-assistance device and Advanced Bionics' "AIM" electrocochleography system. This novel system is intended to assist the surgeon to sense, interpret, react to, and mitigate cochlear insertion trauma in real-time. Benchtop response times to detect and respond to ECochG feedback were measured for human subjects and compared to the automated AIMBOT system.
Methods: Auditory reaction time of 8 subjects was tested by asking each to perform an action in response to a sound. Using AIMBOT, each subject was asked to lift their foot off the foot pedal in response to an AIM Signal Drop Alert. Human reaction time and AIMBOT stop time were measured from the time of the AIM Signal Drop Alert to the stop of the motor in the iotaSOFT drive unit.
Results: The average reaction time of the test subjects in this experiment was 590±85ms. The average stop time of the AIMBOT system was found to be 272±28ms making the robotic stop faster on average by 318ms (46% faster).
Conclusions: The prototype combination of robotic-assisted insertion and electrocochleography successfully stops the forward motion of the electrode array significantly faster than humanly possible, meeting the need of improving this human-limited factor. Considering that 1.6mm/s is the average speed a human inserts an array at a steady rate, the additional distance the array would travel is 944µm. Traveling at a rate of 0.1mm/s with the iotaSOFT, the average additional distance the array will travel after a Signal Drop Alert is 27.2µm when being stopped by the system compared to 59µm when stopped by a surgeon. Considering thickness of the basilar membrane being 0.55-1.16µm, a faster reaction time and therefore shorter over travel by the array could mitigate translocation. Though, further research is needed to determine how exactly improvement of reaction time translates to patient outcomes.

T110. A Sex-Specific Distribution of Meniere's Disease in a Murine Model of X-Linked Hypophosphatemia

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Category: Clinical Otolaryngology & Pathology
Background: Our recent unpublished data demonstrate a high coincidence of Meniere's disease (MD) and X-linked hypophosphatemia (XLH), a rare X-linked dominant disorder of phosphate homeostasis caused by loss-of-function mutations in the PHEX gene. In our human cohort, we identified a male-specific distribution of MD among XLH patients, suggesting a dosage-sensitive

effect of the PHEX gene on the inner ear. A Phex-deficient XLH mouse model has been shown to mimic the functional inner ear degeneration of end-stage MD, but no sex-specific distribution has been reported. Here, we investigated whether a Phex-deficient XLH murine model recapitulates the sex-specific distribution of hearing loss and endolymphatic hydrops of MD in XLH humans.
Methods: We correlated phenotypic auditory dysfunction with the underlying histopathology in Phex-deficient and wildtype (WT) mice. Hemizygous (-/Y) males, heterozygous (+/-) females, and homozygous (-/-) females were compared to WT male/female mice. Cochlear function was characterized (5.6-44.07 kHz) via distortion product otoacoustic emissions (DPOAEs) and auditory brainstem responses (ABRs) at 30 days postnatally. Temporal bone specimens were embedded in methyl methacrylate resin (Technovit 9100) and the cross-sectional area of scala media between the spiral ligament and Reissner's membrane was measured in mid-modiolar sections (EHarea) using Dragonfly image analysis software. Endolymphatic hydrops was quantified as a ratio of EHarea to an "ideal EHarea", which was calculated as the surface area of scala media between the spiral ligament and an "ideal" (non-distended) Reissner's membrane, defined as a straight line from its attachment at the limbus to its attachment on the lateral wall. Scala media from four half turns of the cochlea were analyzed for each specimen.
Results: Hemizygous male mice (n=12) generated variable, but significantly elevated DP and ABR thresholds across the range of test frequencies (p LESS THAN 0.001), and histologic evidence of severe endolymphatic hydrops, compared to WT male mice (194%+37.2% ideal area). Heterozygous female mice (n=4) demonstrated a much less severe phenotype, with only moderately elevated DP and ABR thresholds at several frequencies, and minimal to no endolymphatic hydrops on histology. The phenotype of homozygous female mice (n=3) resembled that of the hemizygous males, with significantly elevated DP and ABR thresholds across the range of frequencies (p LESS THAN 0.001), and histologic evidence of severe endolymphatic hydrops (132%+24.4% ideal area). Notably, the overall magnitude of threshold elevation was similar for DPs and ABRs for all mutant mice.
Conclusions: The Phex-deficient murine model of XLH exhibits the sex-specific distribution of MD seen among the human XLH patients we have reported previously. This development of poor cochlear function and severe endolymphatic hydrops presents in Phex-deficient hemizygous male mice and Phex-deficient homozygous female mice, but not Phex-deficient heterozygous female mice. These findings suggest, for the first time, a dosage-sensitive effect of the Phex gene on cochlear function.

T111. The Clinical Phase 2a Prohear Study - A Randomized,Double-Blind, Placebo-Controlled and Split-Body Trial Testing the Otoprotective Potential of ACOU085 in Cisplatin-Treated TesticularCancer Patients

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Category: Clinical Otolaryngology & Pathology
Background: Cisplatin is a widely used chemotherapeutic agent causing dose-dependant

permanent ototoxic hearing loss in up to more than 70% of patients (Cheung et al., 2022). A recent metanalysis estimated almost half a million annual cases globally (Dillard et al., 2022), making prevention of cisplatin-induced ototoxicity an important medical need. Kv7.4 is a voltage-gated potassium channel expressed in the outer hair cells (OHCs) of the cochlea, mediating potassium efflux and maintaining OHC resting potential thereby maintaining functional hearing and OHC survival. We here report on the currently ongoing PROHEAR clinical Phase 2a trial, testing the otoprotective potential of the novel, proprietary Kv7.4 activator ACOU085 (bimokalner) against cisplatin-induced ototoxicity and hearing loss based on promising data from several preclinical models.

Methods: Testicular cancer patients 18-45 years old with planned cisplatin treatment (cumulative dose ≥300 mg/m2 administered in three chemotherapeutic cycles) and normal hearing in both ears according to current WHO criteria are randomly allocated to treatment intra-individually with ACOU085 (right or left ear) and Placebo (right or left ear) following confirmation of eligibility and obtained written informed consent. A full audiological assessment (Otoscopy; Air/Bone Conduction PTA; Tympanometry/Stapedius Reflex; OLSA tests in quiet/noise; Freiburger test w. SRT and SPL; DPOAE; Nystagmus test; DHI questionnaire; Tinnitus level/intensity; House-Brackmann index) is performed at visits 1-5 (days 1/22/43/64/150), before each chemotherapy cycle (visits 1-3), at the end of cycle 3 (visit 4) and after a 3-month follow-up (visit 5) along with standard clinical assessments. After audiological evaluation the patients receive ACOU085/Placebo via transtympanic injection in either ear on visits 1-3 (before each chemotherapy cycle) using a proprietary, thermoreversible extended-release formulation. Using the unique split-body trial-design, intra-individual changes of audiometric variables for ACOU085 and Placebo treated ears between visits 2-5 and visit 1 (baseline) are compared. A significant proportion of patients showing meaningful differences between ACOU085 and Placebo treated ears is considered evidence supporting a clinically relevant otoprotective effect of ACOU085 against cisplatin-induced ototoxicity and preservation of hearing in cancer patients. (EUCT 2023-503696-15-00; NCT06521190).

Results: Preliminary, blinded interim results from the first enrolled patients completing 3 treatment cycles during chemotherapy indicate a high (GREATER THAN 80%) incidence of ototoxicity with significant PTA changes (up to 35+ dB) at high and extended high frequencies (6-16 kHz). Indication of clinically relevant otoprotective potential of ACOU085 is supported by observed side differences in PTA of up to 25 dB.

Conclusions: The preliminary results of the clinical trial indicate more prevalent and severe cisplatin-induced hearing loss than generally expected for adult patients, perhaps revealed by the use of extended high-frequency audiometry. With continued enrollment of up to 40 planned participants in the PROHEAR Phase 2a clinical trial, we will provide a detailed presentation of the background and trial design, along with updated interim observations.

T112. Ototoxicity Profiles of Patients Receiving Low-Dose Versus High-Dose Cisplatin Administrations

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Category: Clinical Otolaryngology & Pathology

Background: Both the American Speech-Language-Hearing Association (ASHA) and the American Academy of Audiology (AAA), endorse routine ototoxicity management, including baseline auditory assessment and regular monitoring visits during and after cisplatin-based chemotherapy. Yet there currently is no widespread adoption of ototoxicity management protocols clinically. Perceived barriers to "real world" implementation include logistics relating to accessing and testing patients, limitations in personnel and institutional resources, but also the lack of public knowledge about the risk of cisplatin-induced hearing loss as it varies across different cancer types and treatment approaches. This study was designed to characterize the onset, incidence, and severity of hearing loss as influenced by different cisplatin treatment schedules, specifically, individual versus cumulative cisplatin dose, in adults treated with cisplatin-based chemotherapy. We hypothesized that individuals with reduced individual cisplatin doses would show later onset and reduced severity of hearing loss following their cancer therapy.

Methods: We conducted a large-scale, multi-site retrospective study to examine hearing thresholds from 739 adult cancer patients (587 (79.4%) male, 152 (20.6%) female) treated with cisplatin. Threshold shifts were calculated as the difference in behavioral thresholds obtained at baseline (pre-cisplatin therapy) and follow-up (during and after cessation of cisplatin therapy) visits. We applied change in hearing criteria, based on threshold shift data ranging from 1 to 8 kHz, defined by the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAEv5.0) and compared the onset, incidence and severity of cisplatin-induced hearing loss between patients receiving different cisplatin treatment schedules (high/bolus doses once every three weeks (≥75 mg/m2) versus low weekly doses(LESS THAN 70 mg/m2).

Results: Overall, a CTCAE grade ≥1 hearing loss was detected in 60.9% of patients treated with cisplatin. However, the incidence of hearing loss varied significantly according to cisplatin schedule with hearing loss observed in 70.7% of patients receiving a high dose schedule of cisplatin treatment compared to 43.8% of patients receiving a low dose schedule of cisplatin treatment. Moreover, the individual dose schedule significantly impacted the onset of hearing loss, with only 22.1% presenting with hearing loss after 100-200 mg/m2 cisplatin and 31.0% after 200-300 mg/m2 low-dose schedule of cisplatin treatment versus 37.9% and 47.7%, respectively.

Conclusions: Recommendations to and compliance with recommended ototoxicity monitoring may be enhanced with better understanding of the timing and severity of ototoxicity symptoms. Those requiring higher individual dose schedules of cisplatin administration are at greater risk for hearing loss and should therefore be monitored more closely to provide opportunities for either adjustments in treatment or audiology consultation.

T113. Epithelial Hyperplasia, Not Fluid Pressure-Induced Membrane Stretch, in Endolymphatic Hydrops Challenges the Classic View of Meniere's Disease

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Category: Clinical Otolaryngology & Pathology

Background: The generally accepted pathophysiological theory of Meniere's disease (MD) suggests that endolymphatic hydrops (EH), or excess endolymph fluid, directly triggers MD symptoms by increasing fluid pressure, causing stretching, distortion, and repeated ruptures of the cochlear and vestibular epithelia. However, this "endolymph hypertension" hypothesis has not been fully validated, and clinical interventions aimed at reducing inner ear fluid pressure have not proven effective in managing either acute episodic symptoms or long-term disease progression. In this study, we revisited the structural basis of EH with the goal of proposing a more accurate pathophysiological model of MD.

Methods: We analyzed archival digitized human temporal bone (TB) specimens from the Massachusetts Eye and Ear Collection, including MD cases (n=19) at various disease stages, secondary EH (n=8), focal EH (n=3), and controls without inner ear disease (n=23). We developed a machine-learning algorithm (U-NET; Dragonfly ORS) to quantify epithelial cells numbers and measure the internuclear distance between neighboring epithelial cells–an indicator of epithelial stretching–in cross-sections of Reissner's and saccular membranes. Immunohistochemical labeling for the epithelial sodium channel (ENaC) on tissue sections from hydropic (MD) and non-hydropic inner ears was performed to compare the functional differentiation status of cells in hydropic and non-hydropic epithelia.

Results: All hydropic inner ear epithelia, including those from MD, secondary EH, and focal EH cases, showed significantly increased epithelial cell counts, indicative of hyperplasia (cell proliferation), with no evidence of increased intercellular distances, ruling out fluid pressure-induced stretching. Notably, epithelial hyperplasia was present even in early-stage MD and in the presumed progression of focal EH. Quantitative analysis revealed no significant difference in total epithelial areas (i.e. cochlear duct plus endolymphatic sac) between MD cases and controls, suggesting that EH-induced epithelial expansion (hyperplasia) compensates for epithelial damage in the endolymphatic sac, potentially offsetting the structural impact. Immunohistochemistry indicated normal ENaC expression in most epithelial cells in hydropic membranes, suggesting they remain functionally fully differentiated.

Conclusions: Our findings identify epithelial proliferation as a key characteristic of hydropic inner ear epithelia, and support a novel "endolymphatic sac epithelial damage and compensatory hyperplasia" model as the underlying mechanism of EH in MD.

T114. Extended High Frequency Bone Conduction in Adults and Children: Clinical Instrumentation, and Preliminary Results

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Background: Behavioral pure tone audiometry (PTA) detects hearing loss, and measurement of air and bone conduction (AC and BC) thresholds differentiates sensorineural from conductive hearing loss types. Standard PTA measures AC through 8kHz, and BC through 4kHz. Extended high frequency (EHF) AC thresholds are now routinely tested through 16kHz; however, BC thresholds are not available above 4kHz due to limited transducer output, and it is not possible to differentiate hearing loss types above 4kHz, limiting treatment options. Using two novel BC transducers with improved high frequency output, we describe techniques for reproducible measurement of EHF BC thresholds in adult and pediatric subjects.
Methods: Two bone transducers, the Tascam® HP-F200, and Westra® KLH96, were used to test EHF BC thresholds in adult and pediatric subjects with normal hearing (standard frequency thresholds ≤25dB), or hearing loss (clinical history of SNHL, prior middle ear surgery, or CHL from otitis media with effusion). AC and BC thresholds were defined using ANSI standards and ASHA guidelines with Telephonics TDH39P earphones and Radioear B81 bone vibrators, followed by EHF AC testing from 8 to 16kHz with Sennheiser/DD45 circumaural headphones. EHF BC thresholds were obtained from the left or better hearing ear with two bone transducers calibrated according to Remenschneider et al. 2022. Test-retest absolute differences were assessed by serial thresholds measurements on the same day with removal and replacement of the Tascam and/or KLH96 transducer. Comparisons of force thresholds were performed between devices. The feasibility of masking at EHF using new BC transducers was also established.
Results: All subjects, including pediatric subjects (aged 5-11), tolerated testing well. Measures of intra-device reliability were similar for all subjects. 93% of measures had test-retest differences ≤5dB and ≥99% of measures had differences ≤10dB. In adults, the average absolute inter-device difference across EHF was 5dB which was similar to absolute average test-retest differences across EHF, which was 3dB. In pediatric subjects, the absolute average inter-device difference through 12.5kHz was LESS THAN 3dB but increased to GREATER THAN 5dB at 14 and 16kHz.
Of the subjects tested with either SNHL or prior middle ear surgery, the EHF AC and BC thresholds were consistent with a pattern of SNHL or CHL, respectively. CHL from otitis media with effusion resulted in a 40dB air-bone-gap (ABG) at 6 and 8kHz, and an average ABG of 25dB from 9kHz-16kHz. Patients with prior stapedectomy were found to have a 25-40dB EHF ABG.

Conclusions: The demonstrated test-retest reliability, small inter-device variability, successful pediatric testing, and initial outcomes across patients with known hearing loss types suggests EHF BC testing with either bone transducer can provide novel information about high frequency hearing as a part of routine clinical audiometry.

T115. OPEN BOARD

T116. A Novel Spurr Epoxy Embedding Method for Human Temporal Bones

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Category: Clinical Otolaryngology & Pathology
Background: Traditional methods for processing human temporal bones for histological analysis have advanced the study of hearing and balance disorders. However, these methods face challenges including long processing times, high costs, and difficulties in preserving DNA and proteins. A significant hurdle is the embedding process. Historically, celloidin has been used for its ability to penetrate bone tissue and preserve the inner ear's structural integrity. However, celloidin's slow penetration (6-8 months per bone), high cost, and limited availability are major drawbacks. Recent advancements in sectioning techniques, such as large diamond saws and laser microtomes, have opened up new possibilities for alternative embedding media. We identified Spurr epoxy as a promising alternative due to its excellent penetration, lower cost, widespread availability, and ease of removal for various staining techniques. Our study aims to present a new method for embedding large human temporal bones using Spurr epoxy.
Methods: Human temporal bones were harvested during autopsies using a plug saw and were similar in size (5cm x 4cm x 3cm). Given that previous Spurr epoxy techniques were designed for smaller specimens, we modified the method to ensure optimal penetration in our larger samples. The embedding protocol we developed is as follows: After procurement, temporal bones are drilled at the superior semicircular canal using a diamond burr, fixed in 10% formalin for two weeks, and then dehydrated through graded ethanol and acetone solutions. We use a vacuum pump during the final dehydration steps. Embedding involves a 50:50 mixture of acetone and Spurr epoxy, followed by three changes of 100% Spurr epoxy. In each step of the embedding, the samples are vacuumed for 5 minutes, seven times daily, and placed in an orbital shaker to enhance infiltration. To assess Spurr epoxy penetration, blocks were trimmed and opened with a diamond saw, and 20µm sections were obtained using a laser microtome.
Results: The total embedding time with Spurr epoxy ranged from 4-5 weeks, compared to the 6-8 months required with celloidin. The total embedding cost was significantly lower than celloidin ($170 with Spurr epoxy; $2500 with celloidin). After opening the block, we observed complete penetration of the embedding media. The 20µm sections obtained with the laser microtome showed optimal preservation of the inner ear's membranous and cellular architecture.
Conclusions: We have developed an improved protocol for embedding human temporal bones using Spurr epoxy. Not only is this method much faster and cheaper than celloidin, but it also achieves complete media penetration and excellent preservation of cochlear structures.

T117. Sound Quality and Music Perception of Custom Passive and Uniform Fit Electronic Musician's Hearing Protection Devices

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Category: Clinical Otolaryngology & Pathology
Background: Despite knowledge that hearing protection is designed to protect individuals from hazardous noise exposure, noise-induced hearing disorders (NIHD) persist as a public health concern. Musicians are at higher risk of NIHD due to their exposure to loud music. They are often unprotected, or under-protected, due to the lack of regulation of sound levels during live music events. Even though products developed specifically for musicians are available in the form of musician's hearing protection devices (MHPD), these products may still cause some

sound distortion as the attenuation still varies across frequencies. Previous research suggests level and uniformity of attenuation are significantly associated with ratings of overall sound quality, but the correlations are weak. This study builds on previous research by using custom-fit products (to remove differences in comfort as a confounding factor), recruiting musicians as well as untrained listeners, and adding music perception tests to the test battery.
Methods: Both musicians and untrained listener groups are asked to rate the sound quality of music and complete music perception tests while listening to music through custom-fit passive MHPDs (cMHPDs) and electronic uniform-fit MHPD (eMHPDs). Three attenuation filters are used for the cMHPD (9, 15, and 25 dB attenuations) and three attenuation settings are used for the eMHPD (9 and 15 dB, and an "off" setting).
Results: Data collection is ongoing. Data will be analyzed to test hypotheses related to changes in perceived sound quality and performance on music perception tests with increasing attenuation. We predict ratings of sound quality will decrease with measured increases in attenuation and decreases in the uniformity of attenuation. Additionally, we predict poorer scores on tests of music perception (mistuning and timbre) with increased attenuation and decreased uniformity of attenuation. Lastly, we hypothesize that these MHPDs will have larger effects on perception in the musician group compared to the untrained music listening group.
Conclusions: The results of this study will contribute to the understanding of barriers to the use of MHPD by musicians and music appreciators. Data from the music perception tests will reveal whether increasing attenuation compromises performance on pitch and timbre-related tests of perception. If test performance is not compromised, musicians may be more willing to use MHPDs. Findings of compromised performance would suggest a need for longer term studies as musicians may ear-train to devices with additional time and support. Taken together, these findings will help guide education on MHPD use by musicians and music appreciators.

T118. Novel Biomarker Identification in Plasma for Hearing Instability Disorders Using Targeted Proteomics Approaches

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Category: Clinical Otolaryngology & Pathology
Background: Hearing instability (HI), characterized by sudden shifts in hearing thresholds, occurs in various clinical conditions such as Meniere's disease, autoimmune inner ear disease, and enlarged vestibular aqueduct disorders. Despite its prevalence, the underlying mechanisms of HI remain poorly understood. We hypothesize that alterations in the immune protein profile may contribute to HI in many affected individuals.

Methods: To explore this, we utilized targeted proteomics to identify potential biomarkers in plasma from patients with HI. Plasma samples were collected longitudinally from HI patients and healthy controls enrolled in a clinical protocol at the NIH Clinical Center and analyzed using OLINK proteomics. We quantified 3,072 proteins across 8 OLINK panels, with a particular focus on pathways related to inflammation, immune response, and auditory function. Patients were categorized based on the presence of MRI-confirmed endolymphatic hydrops and other audiological factors, including hearing stability.
Results: Our analysis revealed distinct proteomic profiles linked to HI and identified candidate biomarkers with significant differential expression compared to controls. These biomarkers, once optimized, could serve as valuable diagnostic tools or therapeutic targets. Gene enrichment and gene ontology analyses were performed to further elucidate the pathophysiological processes underlying HI. The proteomic data was correlated with published single-cell RNA sequencing data from the inner ear to investigate the role of peripheral immune proteins in ear function.
Conclusions: Our findings indicate that HI patients with endolymphatic hydrops exhibit unique peripheral protein profiles that hold promise as biomarkers.

T119. The Relationship of Between-Ear Attentional Transitions With Otoacoustic Emissions

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Category: Otoacoustic Emissions
Background: To search an object in a cluttered environment, attention is moved from one place to another until the relevant object is found or the search is terminated. The attentional search has been investigated experimentally using a cueing paradigm. When exogenous attention to the right or left ear is induced by a cue, reaction time (RT) to the target presented at the cued ear was shorter than at the uncued ear immediately after the cue presentation, whereas this pattern reversed after a few hundred milliseconds. This inhibitory aftermath of orienting is called inhibition of return (IOR). IOR has been theorized to facilitate target search by discouraging attention from returning to the previously inspected space.
Although the contributions of multiple brain regions to IOR have been reported, the location where the modulation according to IOR first occurs is unknown. Given the abundant evidence for corticofugal projections to outer hair cells (OHCs), we examined whether the OHC motility changes accompanied with the attentional search by measuring otoacoustic emissions (OAE) during the cueing paradigm.

Methods: The stimuli consisted of a 500 Hz cue tone and subsequent target sound (two-tone complex), each of which was presented to the same or opposite ears, referred to the cued and uncued trials, respectively. The participant's task was to indicate the ear of target presentation as fast as possible, and RTs were measured. The cued and uncued trials were presented randomly with equal probabilities, and thus the participant was unable to predict the target ear based on the

cue. The stimulus onset asynchrony (SOA) between the cue and target was either 0.125 s or 0.725 s, to capture immediate exogenous orientation and IOR, respectively. We measured distortion product OAEs (DPOAEs) evoked by the target two-tone complex. The component frequencies were selected around 1 kHz for maximizing the DPOAE of individual participant.
Results: In line with previous studies, RT in the cued trials was significantly shorter than in the uncued trials for the short SOA condition, whereas this trend reversed for the long SOA condition (i.e., IOR). At the individual level, however, some participants did not exhibit IOR, i.e., no reversal between the short and long SOA.
In the long SOA condition, the participants with and without IOR exhibited a decrease in DPOAE on the uncued and cued trials, respectively. In other words, OAE at the attended ear (i.e., shorter RT) was attenuated in both groups in the long SOA condition. There was no significant effect of the cue in the short SOA condition.

Conclusions: The results suggest that OAE, i.e., OHC motility, does not change associated with immediate exogenous orientation but reflects attentional states for a later period, possibly reflecting individual search strategies: maintaining or reorienting to the ear of the focus.

T120. Stimulus Frequency Otoacoustic Emissions Extracted by Pharmacologic Blocking of Outer Hair Cells Shows That Extraction by High-Level, Near-Frequency Suppressors Reveals Nearly the Entire SFOAE

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Category: Otoacoustic Emissions
Background: Stimulus-frequency otoacoustic emissions (SFOAEs) are used to study cochlear amplification. Their interpretation requires understanding where the SFOAEs originate along the cochlear length. SFOAEs are typically measured by taking the ear canal pressure difference between (1) a low-level probe tone, which has sound from both the sound source and the SFOAE, and (2) the probe tone plus a second tone that is near the probe frequency and at much higher sound level, which is assumed to suppress the SFOAE. The difference between the two measurements, termed the subtraction "residual", is taken to equal the SFOAE. It has not been clear whether the SFOAE is fully extracted by this method, since the high-level suppressor tone may not fully suppress the SFOAE. Also, second-tone "suppressers" at frequencies much higher than the probe frequency produce large SFOAE-frequency residuals. This has been suggested to show that a substantial fraction of the SFOAE originates far-basal of the SFOAE best-frequency (BF) place.
Methods: We compared SFOAEs measured by two methods: (1) cochlear amplification reduced by a high-level, near-BF-frequency, suppressor-tone, and (2) a pharmacologic reduction of cochlear amplification (that did not require a high-level tone that might evoke new SFOAE-like residuals). In both cases, the SFOAE was calculated from the difference in the ear-canal measurements with and without SFOAE reduction. Pharmacologic reduction was done by perfusing an ototoxic solution (20 mM salicylate or 150 mM KCl) from the guinea pig cochlear apex to the cochlear aqueduct in the cochlear base. This perfusion removed cochlear responses

from the SFOAE characteristic frequency (CF) place prior to removing any responses that might originate basal to the CF place – which would make it easier to see SFOAE components originating from the base.
Results: SFOAE amplitudes obtained by pharmacologic extraction averaged slightly higher (~2.8 dB) than those obtained by acoustical extraction. The times at which the SFOAEs were reduced were similar in the two methods. Further, there was little or no additional reduction of the residual as the perfusion progressed along the region two octaves or more basal of the probe tone BF region, even though a second tone with a BF in this region produces large SFOAE-frequency residuals.
Conclusions: The slightly larger SFOAE values found with pharmacologic extraction suggests that the near-frequency suppressor in the standard SFOAE paradigm does not completely silence the SFOAE. The similarity of the SFOAE reduction times between the two extractions, and the lack of additional residual reduction as the solution flows far basal of the BF region, indicate that SFOAEs originate primarily from the region around the probe-tone BF. Overall, the results indicate that the standard SFOAE paradigm works sufficiently well for extracting the essential attributes of the SFOAE.
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T121. Innovative Use of Human Amniotic Membrane Allograft for Recurrent Epistaxis: A Case Report

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Category: Development: Human Subjects
Background: Epistaxis is a prevalent condition affecting an estimated 60% of adults. While most cases are benign, a small percentage require medical intervention, particularly cases of recurrent epistaxis that have been resistant to traditional treatments such as cauterization.
Methods: A 36-year-old female with chronic recurrent epistaxis and a nasal septal excoriation which was unresponsive to conservative measures and multiple cauterization treatments, was treated with a human amniotic membrane allograft (StemPatch). The patient was monitored in clinic for post-procedural outcomes and follow-up assessments.
Results: The application of the stem cell patch resulted in significant clinical improvement and improved quality of life for this patient. On follow-up the StemPatch was well-integrated, with no signs of recurrent bleeding or complications. The treatment effectively addressed the patient's recurrent nasal bleedings, demonstrating the potential of human amniotic membrane allografts as viable alternatives to traditional methods.
Conclusions: The use of human amniotic membrane allografts offers a promising alternative for managing refractory recurrent epistaxis. It's regenerative properties and ability to support epithelial repair present a viable option for patients with chronic nasal excoriations. Therefore, the use of human amniotic membrane allografts like Stempatch appears to be an effective and low-risk treatment for chronic recurrent epistaxis, particularly in cases where traditional methods have failed. Further research and clinical trials are warranted to establish its broader applicability and long-term efficacy.

T122. A Subcortical Model With Efferent Gain Control Explains Effects of Hearing Loss on Auditory Enhancement Under Simultaneous and Forward Masking

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Category: Psychoacoustics

Background: In auditory enhancement (AE), target detectability is manipulated by inclusion or exclusion of the target component in a multi-tone precursor. Two forms of AE have been demonstrated: In AE under simultaneous masking (AESim), the target "pops out" of a multi-tone masker when the target component is excluded from a precursor. In AE under forward masking (AEFwd), the "enhanced" target is a more effective forward masker of a subsequent probe tone at the target frequency. The mechanisms behind both effects remain unclear, and hearing loss effects these two forms of AE differently. This study tested the hypothesis that efferent control of cochlear gain plays a key role in shaping these phenomena.

Methods: The study replicated two psychoacoustic experiments (Kreft et al., 2018, JASA 143:901 and Kreft and Oxenham, 2019, JASA 146:3448). We investigated AE using a subcortical auditory model with a medial olivocochlear (MOC) system that receives inputs from wide-dynamic-range brainstem neurons and from modulation-sensitive inferior colliculus (IC) neurons.

For AESim, the stimulus is a 500-ms precursor followed by a 100-ms gap, then a 100-ms masker; the task is to select the interval with the target component in the masker. For AEFwd, the stimulus is similar to AESim, but followed by a 100-ms gap and then a 20-ms probe tone at the target frequency; the task is to select the interval with the probe. The masker and precursor consisted of four equal-amplitude, logarithmically spaced sinusoids centered at the target frequency. The target component was either present or absent in the precursor.

Thresholds for AESim and AEFwd were estimated using models with a range of audiometric hearing loss. Stimulus levels were varied to match the experimental conditions, and threshold equalizing noise was used to match the normal-hearing model thresholds to those of the model with hearing loss. The detection threshold for AESim was estimated based on the average-rate profile for a population of model IC neurons with characteristic frequencies spanning the stimulus spectrum. Probe-detection thresholds for AEFwd were estimated based on the response rates of model neurons tuned near the probe frequency

Results: The model with MOC efferent gain control showed enhancement consistent with the psychoacoustic studies. Key findings included: a) Enhancement under AESim for both NH and HI models. b) No enhancement under AEFwd for the HI model. c) Enhancement under AEFwd for the NH model varied with stimulus level, similar to experimental results. Models without the efferent gain control failed to capture these effects.

Conclusions: The model with efferent control of cochlear gain simulated the enhancement effects in both AE conditions, with and without hearing loss. These findings support the hypothesis that efferent gain control could underlie auditory enhancement.

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T123. Cochlear Frequency Selectivity and Extended High Frequency Hearing in Individuals With Normal Audiograms

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Category: Psychoacoustics

Background: The role of frequencies above 8 kHz (extended high frequencies, EHFs) in human hearing remains poorly understood. While EHF hearing loss is prevalent among young adults, it's perceptual and physiological implications are unclear. Despite clinically normal audiograms, some evidence suggests that poorer EHF hearing may indicate subclinical cochlear damage. Frequency selectivity is a fundamental aspect of cochlear function. A previous study found that reduced EHF hearing is linked to broader auditory filters, but it is unclear if this effect is limited to 2 kHz or extends to other frequency regions. Moreover, the simultaneous masking method used in that study may have overestimated filter bandwidths compared to forward masking paradigms.

Stimulus frequency otoacoustic emission (SFOAE) delays provide a non-invasive method to assess cochlear tuning. SFOAE estimates are consistent with psychophysical measures obtained using forward masking. The present study aimed to investigate the relationship between EHF hearing acuity and cochlear frequency selectivity in lower frequencies. We hypothesized that EHF hearing damage, even with normal audiograms, is associated with broader cochlear tuning. Broadened cochlear tuning can have significant implications for the neural processing of speech.

Methods: SFOAEs are non-stationary time-varying signals, and a spectral-based approach to characterize its delay may not accurately capture the characteristic place and could contribute to high individual variability in delay estimation. To address this issue, we applied time-frequency analysis using the stockwell transform. We validated this approach on the gammatone model and simulated SFOAEs. Swept-tone evoked SFOAEs at 40 dB probe for 0.5 to 4 kHz were measured using a suppressor-based paradigm from 40 young, healthy adults (19–30-year-olds) with clinically normal audiograms (≤20 dB HL; 0.25-8 kHz). The sharpness of cochlear tuning (QERB) was computed using an empirical model. We also measured wideband absorbance to account for potential middle-ear contributions. Linear mixed-effects models were applied to analyze the relationship between hearing thresholds at EHFs and QERB while adjusting for the effects of other confounding variables, such as hearing thresholds in the 0.25 to 8 kHz range.

Results: Preliminary analysis reveals an association between EHF hearing thresholds and QERB estimates. Furthermore, SFOAE estimates of QERB were consistent with forward-masking tuning curves, as reported in the literature. The results were discussed in the context of frequency effects and EHF hearing thresholds.

Conclusions: Findings have implications for understanding subclinical auditory damage and potentially explaining listening difficulties in listeners with normal audiograms.

T124. Estimation of the Hearing Thresholds for Distantly-Presented Bone-Conducted Ultrasound Using Adhesive-Vibrators

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Category: Psychoacoustics

Background: Ultrasound, high-frequency sound above 20 kHz, can be clearly heard by bone-conduction. This “bone-conducted ultrasound (BCU)” can transmit speech by using amplitude-modulation. Furthermore, BCU can be heard even when presented to distal parts of the body, such as the neck, trunk, and upper limbs. Previous studies have suggested that "distantly-presented BCU" is expected to be applied to a novel audio device that can selectively transmits sound information to a specific user who touches the vibrator. However, improving wearability is crucial. In previous studies on distantly-presented BCU, which focused on elucidating basic perceptual characteristics, the vibrator was fixed using an elastic band. However, this method was troublesome and unappealing. Therefore, for the practical application of distantly-presented BCU, it is necessary to develop a new fixation method that is easier to wear and more aesthetically pleasing. Nonetheless, no studies have yet investigated practical methods for fixing the vibrator in distantly-presented BCU vibrator. In this study, we propose an “adhesive method” in which the vibrator is fixed using double-sided tape, and compared the hearing threshold of BCU between the adhesive method and the conventional method in which the vibrator is fixed using an elastic band. In addition, we evaluated how parameters such as the protrusion height of the vibrating surface from the vibrator chassis and the adhesion area affected the hearing threshold.

Methods: The stimulus used was a 30-kHz tone burst, and hearing thresholds were measured using a 1up-2down method with a 3AFC procedure. The vibrator was inserted into a chassis made with a 3D printer, and stimuli were presented using both the conventional and the adhesive methods. The stimuli were presented at the mastoid, sternocleidomastoid, three points on the chest, and three points on the back. Additionally, at the chest and back, hearing thresholds were estimated using chassis with varying protrusion lengths and chassis diameters, and their effects were evaluated.

Results: BCU was perceptible at all positions where the vibrator were able to be securely fixed, whereas it was not possible to fix the chassis used in this experiment at the mastoid and clavicle. The hearing threshold of the adhesive method increased by 10-20 dB compared to the conventional method; however, the hearing threshold decreased as the vibrator protrusion and adhesion area increased. This result is thought to be attributed to the increase in protrusion length, which increased the pressed length and consequently the presentation pressure, as well as the increase in adhesion area, which augmented the system's rigidity and mitigated energy loss.

Conclusions: These findings provide useful information for the application of the distantly-presented BCU to a novel audio device.

T125. Physical Exertion as an Index of Listening Effort in Older Individuals With Hearing Loss

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Category: Speech Perception

Background: Listening effort refers to the allocation of mental resources with the goal of maintaining speech intelligibility under adverse circumstances. Listening effort is a crucial facet of hearing loss and thus important for evaluating efficacy of hearing interventions. Although various measures to quantify listening effort exist, each has its limitations.
Previous research has indicated that individuals are inclined to exert physical effort to simplify a forthcoming visual task. Analogous research in the auditory domain has revealed that participants exert more physical effort under challenging listening conditions, suggesting its potential as an objective index of listening effort. This approach uniquely encapsulates the motivational aspect of effortful listening as theorized in the Framework for Understanding Effortful Listening (FUEL), as it measures physical exertion prior to the trial. Research to date, though, has only investigated this index in a sample of typically hearing young adults. The present work thus aimed to replicate the paradigm in a sample of older individuals with mild to moderately severe hearing loss while also testing its sensitivity to hearing interventions.

Methods: Participants (N = 9) could improve the signal-to-noise ratio (SNR) of a subsequently presented sentence by exerting effort using the space bar on a keyboard, with more presses leading to greater SNR improvement. A progressive-ratio schedule was used, requiring the participants to incrementally increase the number of space bar presses to achieve the most favorable SNR in each successive trial within a block. Probe sentences were either high or low in context, the starting SNR was either of hard or medium difficulty, and participants completed the task aided and unaided.
Results: Results showed that participants pressed the space bar more frequently under adverse (low sentence context, hard SNR and unaided condition) than under the easier (high context, medium SNR and aided condition) conditions. This suggests that individuals were motivated to exert physical effort to render difficult listening tasks easier.
Conclusions: This work thus replicates previous findings in a population of older individuals with hearing loss, while also extending them, showing that the task is also sensitive to hearing interventions. While these initial results further underscore the potential of this novel listening effort index for inclusion in audiological outcome batteries, further work is needed to directly compare the present approach with pre-existing measures of listening effort.

T126. Speech-In-Noise Processing Across Age and Cognitive Function: A Preliminary Study

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Category: Speech Perception

Background: In daily life, the ability to selectively focus on important sounds amidst background noise is essential. Previous research indicates that understanding speech in noisy environments increases cognitive load across auditory, attentional, memory, and cognitive domains, thereby reducing overall speech processing efficiency. As auditory processing efficiency declines with age, this challenge becomes more pronounced in older adults and individuals with cognitive impairments, such as those with Mild Cognitive Impairment (MCI).
Methods: The present study aims to examine the differences in neural mechanisms of speech-in-noise between groups varying by age and cognitive function using Electroencephalogram (EEG). Participants included 8 young adults with normal hearing (mean age: 23 years), 9 older adults with normal hearing (mean age: 69.4 years), and 2 individuals with MCI (mean age: 72.5 years). EEG signals were recorded using a 64-channel system while participants listened to spoken narratives under two conditions (clean and speech-shaped noise; SSN). Participants were instructed to focus on the speech. Before the EEG session, an adaptive procedure was used to estimate each participant's speech recognition threshold (SRT), and the noise level was adjusted to correspond to 50% SRT. Neural responses to acoustic features were modeled using a forward encoding approach. Temporal response functions (TRF) were then constructed to compare peak latency and amplitude between groups.
Results: Preliminary results indicated that older adults and the MCI group exhibited delayed TRF peaks at 50ms and 100ms compared to the young adult group, with the MCI group showing higher peak amplitudes. Notably, a 200ms TRF peak was observed only in the young adults and was absent in older adults and the MCI group.
Conclusions: These findings suggest age-related and cognitive function-related differences in neural processing during speech-in-noise tasks. Further research is necessary to explore these neural discrepancies and their potential to inform the development of diagnostic tools and personalized rehabilitation strategies for auditory processing deficits in older adults and cognitively impaired individuals.

T127. Sound Degradation Type Differentially Affects Neural Indicators of Cognitive Workload and Speech Tracking

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Category: Speech Perception
Background: Hearing-in-noise (HIN) is a challenging task that is essential to human functioning in social, vocational, and educational contexts. Successful speech perception in noisy settings is thought to rely in part on the brain's ability to enhance neural representations of attended speech. In everyday HIN situations, important features of speech (i.e., pitch, rhythm) may be degraded in addition to being embedded in noise. The impact of these differences in sound quality on experiences of workload and neural representations of speech will be important for informing our knowledge on the cognitive demands imposed by every-day difficult listening situations.
Methods: We investigated HIN perception in 20 healthy adults using continuous speech that was either clean, spectrally degraded, or temporally degraded. Each sound condition was presented both with and without pink noise. Participants engaged in a selective listening task, in which a short-story was presented with varying sound quality, while EEG data were recorded. Neural

correlates of cognitive workload were obtained using power levels of two frequency bands sensitive to task difficulty manipulations: alpha (8 – 12 Hz) and theta (4 – 8 Hz). Acoustic and linguistic features (speech envelope, word onsets, word surprisal) were decoded to reveal the degree to which speech was successfully encoded.
Results: Overall, indices of cognitive workload increased significantly when noise was added across sound conditions, while prediction accuracy of speech tracking decreased, suggesting that more effort was required to listen, and that the speech was not as successfully encoded. Surprisingly, the temporal degradation resulted in the greatest indication of workload, possibly as a function of a compensatory mechanism to restore the important temporal information required for speech comprehension.
Conclusions: Our findings suggest a potential trade-off between cognitive workload and successful speech encoding during effortful listening, which may help to inform future interventions that aim to mitigate these every-day challenges.

T128. The Influence of Speaker Facial Features on Audiovisual Speech Perception in Age-Related Hearing Loss

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Category: Speech Perception
Background: During face-to-face communication, adults with hearing loss appear to rely on the visual modality to resolve uncertainty in auditory speech. For instance, adults with hearing loss are more likely to experience McGurk fusions during conflicting audio and visual speech, and (Rosemann and Thiel 2018, Neuroimage 175:425-37) and demonstrate slightly better speechreading ability for single words compared to age-matched typical-hearing peers (Tye-Murray et al., 2007, Ear and Hearing 28:656-668). However, this visual reliance in adults with hearing loss does not appear to translate to enhanced audiovisual integration or supranormal perception of audiovisual sentences (Spehar et al., 2008, JASA 123:2858-2866). It may be that a reliance toward visual processing is simply compensatory and does not involve a modification of visual behaviours or visual perception. Alternatively, a visual reliance could arise through increased overt attention to visemic cues. However, no research to our knowledge has examined how attention to specific speaker facial features facilitate audiovisual speech perception in adults with hearing loss. Here we explore the influence of masking a speaker's facial features (e.g., eyes, mouth) on audiovisual speech perception in adults with varying levels of hearing loss. We predict that adults with greater age-related hearing loss (ARHL) will perform worse when facial features are covered, and this effect will be largest when the speaker's mouth is obscured.
Methods: Participants aged 40 to 80 with untreated hearing loss or typical hearing participated in the study. All participants underwent pure-tone audiometry to 8 kHz, and QuickSIN measured speech-in-noise (SIN) listening. Participants first took the Canadian Digit Triplet Test to determine the level at which they could detect speech in noise with 50% accuracy. This speech detection threshold was used to establish a background noise level (speech-shaped noise) for each participant that was then applied during the audiovisual task. Participants were presented with a woman speaking monosyllabic audiovisual with her facial features covered across four conditions: upper-half hidden (e.g., eyes, forehead), lower-half hidden (e.g., mouth, jaw), full-

face hidden, and all facial features available (i.e., no masking). For each trial, participants verbally indicated the word they perceived. Responses were later transcribed verbatim and scored for accuracy.
Results: Data collection is ongoing.
Conclusions: Results, if consistent with our hypotheses, may suggest that adults with ARHL rely more on visual cues, such as facial articulation, to aid in speech perception. A reliance on visual information, particularly the speaker's mouth, would shed light on compensatory strategies individuals with ARHL use to maintain effective speech comprehension, especially in noisy environments.

T129. Unraveling the Paradox of Self-Voice Emotion: A Comparative Analysis

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Category: Speech Perception
Background: It is a common phenomenon for individuals to exhibit negative reactions to their own recorded voices. This discomfort may potentially lead to a lowering of self-esteem. However, some studies have reported cases where individuals do not feel uncomfortable or even find their recorded voices attractive. The mechanisms underlying these phenomena remain largely unknown. This study hypothesizes that both negative and positive emotions are evoked by self-recorded voices and that these emotions vary situationally, leading to this puzzling phenomenon.
Methods: All experiments were conducted online, and a total of 38 participants (25 males) were recruited via crowdsourcing. The participants were requested to articulate six sentences, and their voices were recorded. In rating the intensity of the emotions, participants were instructed to utilize a scale of 0-8. If the rating was 1 or higher, they were then asked to further rate the intensity of positive and negative emotions on a scale of 0-8. To examine the situational dependence of emotions towards self-recorded voices, participants were first asked to rate their emotions when listening to their own voice as they usually do (the recall condition). They were then asked to listen to and rate the recorded voice (the listening condition). To examine the relationship between personality and emotional responses, participants completed a self-esteem scale and a voice-related questionnaire, including voice preference, at the conclusion of the experiment.
Results: Our findings demonstrated that both positive and negative emotional responses are evoked by the presentation of self-recorded voice stimuli, and that these responses are influenced by a variety of factors. The positive emotional responses to self-recorded voices were elicited in a similar manner in both the recall and listening conditions and were positively correlated with self-esteem. The intensity of negative emotions was greater than that of positive emotions in the recall condition, whereas in the listening condition, negative emotions were reduced to a level comparable to that of positive emotions. No correlation was observed between negative emotions and self-esteem. The preference for the voice was found to be correlated with positive and negative emotions in both the recall and listening conditions.

Conclusions: The segregation of positive and negative emotional responses has facilitated a deeper understanding of the nature of emotions towards self-recorded voices. The findings of our study indicate that positive emotions are reflective of an individual's personality traits. Moreover, the reduction in negative emotions following listening suggests that participants may have underestimated their actual speech performance. Given the association between speech performance and self-efficacy, enhancing one's capacity for speech monitoring may contribute to an increase in self-esteem.

T130. Chirped-Speech Reveals Connection Between Brainstem Encoding and Speech-in-Noise Perception

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Category: Speech Perception

Background: Variability in speech perception abilities, especially in noisy environments, are common—even among those with normal hearing. One possible origin of these individual differences may lie in how auditory stimuli are encoded and processed in the brain. In this study, we evaluate speech recognition abilities under different noise conditions and explore the relationships between neural responses, speech perception performance, and subjective ratings of listening effort (task load).

Methods: We utilized "chirped-speech" (Cheech), using AzBio sentence lists, as a quick and reliable method to simultaneously measure behavioral speech recognition and neural encoding across the auditory pathway. Cheech embeds naturalistic speech stimuli with narrowband frequency sweeps (chirps) designed to elicit robust auditory evoked potentials (AEPs) from brainstem through auditory cortex (i.e., auditory brainstem [ABRs], middle latency [MLRs], and auditory late latency responses [LLRs]). Neural responses were measured with single-channel EEG recordings in normal-hearing, young adults while they listened to the AzBio lists and verbally repeated the sentences they heard. Cheech-modified AzBio lists were presented in quiet or a multi-talker babble masker resulting in three signal-to-noise ratios (SNR) conditions: ∞ (clean Cheech), -3, and +3 dB (3 lists, ~7.5 mins per condition). Participants also rated their subjective listening effort (task load) after each list block using the NASA Task Load Index.

Results: Decreased SNR (i.e., increased masker noise level) resulted in longer latencies and smaller amplitudes across all AEPs. Interestingly, individual speech-in-noise recognition performance was associated with more efficient neural processing in the brainstem (i.e., earlier ABR wave V latencies) as well as thalamo-cortical generators (i.e., MLR Pa latencies). In general, smaller amplitudes and prolonged latencies across all three ERPs were associated with ratings of higher subjective task load. To evaluate possible speech perception detriments due to Cheech processing, participants also completed a control speech recognition task using original, unmodified AzBio sentences presented at the same SNR levels. Despite some slight performance declines in noisy conditions compared to the original audio, Cheech-modified AzBio lists remained highly intelligible, including ceiling-level performance for sentences presented in quiet.

Conclusions: Our results suggest neural encoding within the brainstem, as well as higher levels in the auditory pathway, plays a key role for successful speech-in-noise listening. Collectively,

our study highlights the paradigm's potential for future applications to more accurately and efficiently assess an individual's speech and neural processing capabilities across the auditory hierarchy.

T131. Continuous and Concurrent Auditory TRFs Using Both EEG and MEG Reveal Processing Hierarchies During Natural Speech of Competing Speakers

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Category: Speech Perception

Background: Listening to speech in noise is an everyday occurrence made possible, if conditions allow, by complex neural processes. Specifically, listening to one person's voice in the midst of one or more competing voices is often called the cocktail party problem, and is a task that lends itself to experimental investigation because of its naturalistic nature and the ease of parametric control. Here, we record simultaneous EEG and MEG, as well as multiple behavioral measures, from normal hearing younger adults as they attend to one of two competing speakers, at several different signal-to-noise ratios (SNRs), reading a narrative text.

Methods: Using the temporal response function (TRF) paradigm with respect to the EEG and MEG responses, we analyze auditory responses from both the brainstem and cortex. Stimulus regressors are constructed to represent a hierarchy of auditory and language-based features from both the target and distractor speakers, from which multiple TRFs are calculated. These TRFs are also analyzed to determine which aspects of auditory brain responses are modulated by selective attention, and to what extent. EEG, which is sensitive to deep auditory sources, allows estimation of faster time-scale TRFs, calculated with regressors corresponding to a cochlea and auditory nerve model. MEG allows estimation of slower time-scale TRFs, calculated with slower regressors corresponding to features of the speech signal such as the envelope and envelope onsets, as well as linguistic features at the phoneme and word level.

Results: A hierarchy of speech-related TRFs and their corresponding sources is thus measured concurrently, including at the latencies of the auditory brainstem response (ABR, 0 – 15 ms), middle latency response (MLR, 15 – 60 ms), N1-P2 complex (60 – 200 ms), and slower linguistic responses (120 – 800 ms). We find little evidence that faster TRFs from early auditory areas depend on the speaker identity regressor (target vs. distractor), but can demonstrate that later auditory and linguistic cortical TRFs exhibit a wide range of levels of the effect of selective attention.

Conclusions: Some results in the recent literature are mixed with regard to attentional modulation of ABR- or frequency following response (FFR)-type responses; this novel approach that combines concurrent source-space EEG and MEG and separate families of TRF regressors for each speaker, may help to shed further light not only on how the cortex differentially tracks an attended speaker, but also on how the earlier auditory system may or may not do the same.

T132. Older Adults at the Cocktail Party: Is it Better With a Musical Background?

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Category: Speech Perception
Background: Older adults experience challenges when perceiving speech with competing speech in the background ('speech-on-speech', also known as the cocktail-party effect). Factors that contribute to this challenge include age-related hearing loss and age-related cognitive changes, both part of healthy aging. Previous studies have shown that a background of musical training may influence how well older adults perceive speech-on-speech. This may be due to music-related top-down changes in cognitive working memory processes reflected in oscillatory brain activity. Our own preliminary behavioral results show an emerging 'musician effect' in older adults during a speech-on-speech perception task. However, underlying group differences in real-time brain activity that may be occurring during speech-on-speech perception are not yet known. The goal of this research is to identify potential differences in brain activity of older adult musicians and non-musicians during speech-on-speech perception using electroencephalogram (EEG) methods. In particular, we will assess whether older musicians have more prominent working memory-related oscillatory activity during speech-on-speech perception compared to older non-musicians.
Methods: Participants will be 40 older adults (60+ years), who are either musicians (n = 20) or non-musicians (n = 20), as well as a group of younger adult non-musicians (n=20). All participants will have age-normal hearing. EEG will be recorded during a working memory test (n-back) and a speech-on-speech perception test (the Child-friendly Coordinate Response Measure test). Working-memory-related oscillatory isolated during the n-back will be identified during speech-on-speech perception and then compared across groups.
Results: Data collection is planned in November 2024.
Conclusions: Our results will provide knowledge on the effects of musical training on mechanisms of speech perception in the older adult population, in a social setting where older individuals have great difficulty hearing dialogue - the cocktail party setting. Such information could be used to further promote the presence of music in society, and encourage older adults to engage in musical activities. Moreover, this knowledge can be used to fine tune music training programs that target these specific mechanisms and hence increase the effectiveness of such training in older populations.

T133. The Consistency of Spectral Context Effects in Speech Perception by Cochlear Implant Users

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Background: Perception of any sound is influenced by the spectrum of its preceding context. In
Category: Speech Perception
spectral contrast effects (SCEs), a spectral peak in the context stimulus promotes perception of spectrally adjacent information in the target sound. In auditory enhancement effects (EEs), a spectral notch in the context stimulus promotes perception of that same spectral region in the

target sound (i.e. pop-out effect). In both basic auditory and speech perception, SCEs and EEs have been studied in isolation without much examination of a potential link between them. In previous work (Stilp 2019 JASA), normal hearing (NH) listeners' categorization of /d/-/g/ targets exhibited strong consistency in EE and SCE magnitudes (r = 0.67). Here, we extended this individual differences approach to cochlear implant (CI) users. Various studies reported SCEs and EEs in CI users' perception, but always in separate listener samples. We predicted that CI users would display consistency in the magnitudes of SCEs and EEs, but that SCE magnitudes would be larger, consistent with spectral smearing making spectral peaks broader (increasing SCEs) while making spectral notches less shallow (decreasing EEs).
Methods: Fifteen adult post-lingually deafened CI users were tested in a 2AFC task where target sounds from a 10-step continuum were categorized as /da/ or /ga/. The target syllable was always preceded by a context sentence whose spectral properties were filtered to elicit SCEs and EEs. To elicit SCEs, a bandpass filter preserved spectral energy in only the low-F3 region (1700-2700 Hz) or only the high-F3 region (2700-3700 Hz) to facilitate perception of /d/ and /g/ respectively. To elicit EEs, a bandstop filter removed all spectral energy from the same low-F3 region or high-F3 region, to promote perception of the opposite phonemes. Trials were blocked (bandstop contexts, bandpass contexts) and tested in random orders.
Results: CI users' responses showed poor categorization of the speech sounds in general, but still showed evidence of both SCEs (more "ga" responses when the context sentence comprised the high-F3 passband vs. the low-F3 passband) and EEs (more "ga" responses when the context sentence had a low-F3 notch vs. a high-F3 notch). Contrary to the first prediction, effect magnitudes were not consistent across context effect types (r = 0.09). Contrary to the second prediction, SCE magnitudes were not larger than EE magnitudes.
Conclusions: The consistency of EE and SCE magnitudes previously observed in NH listeners did not generalize to CI users. The loss of this consistency indicates another fundamental aspect of hearing that is affected by CI processing. This could potentially be alleviated by processing schemes that explicitly incorporate preceding context into electrode activation. Future research should consider limiting intrasample variability and testing target items that are distinguished by broader spectral details than /d/-/g/ tested here.

T134. Isolating Neural Correlates of the Speech-To-Song Illusion With Electrophysiology

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Category: Speech Perception
Background: The speech-to-song (STS) illusion is a perceptual phenomenon in which speech tokens, when repeated several times, sound increasingly like song rather than speech. The neural bases of the STS illusion have been previously investigated using fMRI (Tierney et al., Cerebral Cortex, 2013). This previous study introduced an original experimental design whereby speech tokens producing the illusion were contrasted with seemingly similar tokens that did not produce the illusion. Results showed that brain regions previously associated with pitch and song encoding increased their response after the illusion. Here, we use a similar paradigm but in an electroencephalography (EEG) setting. The better temporal resolution of the EEG allows to look

for neural correlates of the transformation on a repetition-by-repetition basis. Furthermore, it enables to test new hypotheses based on the relative balance of linguistic and musical expectations in the development of the STS illusion.
Methods: We record EEG responses when participants are presented with repetitions of spoken phrases that either elicit the illusion or not (28 illusion + 28 control stimuli, stimulus set of Tierney et al., 2023). Participants rate each repetition on a scale from speech-like to song-like. The first and last repetitions are rated after the stimulus presentation for subsequent group-contrast analysis. For all other repetitions, participants provide a continuous perceptual rating during stimulus presentation for a subsequent within-listener analysis. We use multivariate linear regression to model brain responses as a linear combination of stimulus features. These features include acoustics cues (e.g., fundamental frequency, amplitude envelope), linguistic content (e.g., phonemes, words), and expectations (e.g., linguistic and melodic surprisal). After the main experiment, EEG responses to passive listening of speech and music are collected to construct a within-listener model of speech and music encoding.
Results: We predict the encoding of acoustic cues relevant to music to become better encoded in the EEG responses as the stimuli undergo the speech-to-song transformation. Furthermore, we predict a reweighting of linguistic and melodic expectations as perception shifts from speech to song. Finally, the within-participant analysis, based on the real-time perceptual reports and individual forward EEG models, should provide neural correlates of the interindividual differences expected in the STS illusion.
Conclusions: To the best of our knowledge, this is the first study of the STS illusion using EEG, which enables us to investigate the temporal dynamics of brain responses to auditory stimuli and complement the previous findings obtained with fMRI. As different percepts are experienced for the exact same acoustic stimulus in the same listener, the STS illusion provides a particularly well-controlled opportunity to investigate the neural bases of speech, song, and music.

T135. Neural Signatures of Musical and Linguistic Interactions During Natural Song Listening

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Category: Speech Perception
Background: Song, poetry, infant-directed speech, and other expression modalities combining music and speech help us convey meaning and emotions beyond mere linguistic content. Such lyrics-tunes synergies hint at a common neural basis for their processing beyond a shared sensorimotor system. Yet, it remains unknown how music and speech neural processing interact to form a cohesive perceptual whole. Previous research pointed to areas of the human cortex sensitive to music, speech, and song, finding both shared and specialized sites. Yet, the interactions between tunes and lyrics processing when listening to songs remain poorly understood. Here, we probed auditory predictive mechanisms during song listening, which were previously only studied separately in speech and music.
Methods: To tackle this question, we probed neural auditory predictive mechanisms with electroencephalography (EEG). We compared the encoding of melodic predictions when N=20

participants were presented with songs or the corresponding hummed (speech-free) melodies. Similarly, we studied the encoding of linguistic predictions in EEG responses to songs and the corresponding spoken (melody-free) lyrics. Multivariate temporal response functions were then estimated with multiple lag linear regression to assess the simultaneous cortical tracking of linguistic and melodic properties and corresponding expectations for the three types of stimuli separately.
Results: We found that the concurrence of music and speech in songs alters their predictive signals as opposed to when they are processed separately. Furthermore, we found a trade-off in the neural encoding of melodic and phonemic expectations, with their balance depending both on who was listening (internal driver reflecting the listener's preference or skills) and how the song is composed and performed (external driver reflecting the salience of lyrics and tunes). Altogether, our results indicate that song involves parallel prediction processes competitively interacting for the use of shared processing resources.
Conclusions: This study probed the neural processing of speech and music expectations, providing evidence for their similarities, distinctions, and interactions. We found that the two predictive processes interact and compete for shared resources, which are allocated based on listeners' preference and expertise, and the balance of salience of the lyrics-tunes as established by the composer. Based on those findings, we propose a high-level music-speech integration model, accounting for expression modalities at their intersection, like poems and songs.
To further extend this model, we will also discuss the role that pitch-related auditory processing might play in the proposed model. Pitch is fundamental to extracting musical notes and phoneme categories and capturing the full expressiveness of both music and speech. Therefore, pitch represents a reasonable candidate for shared neural processing between speech and music.

T136. Influence of Enhancing Fundamental-Frequency Dynamics on Speech-on-Speech Intelligibility in Hearing-Impaired Listeners

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Category: Speech Perception
Background: Understanding speech in the presence of one or multiple interfering talkers is a challenging auditory task that occurs often in daily life. While young normal-hearing (NH) listeners can perform this task successfully, older hearing-impaired (HI) listeners often encounter severe difficulties in understanding speech in the presence of competing talkers, even when provided with hearing aids. Fundamental-frequency (F0) dynamics (i.e., the F0 dynamic range) of the competing voices and their differences between target speech and interfering speech (i.e., the F0-dynamic-range "contrast") have recently been shown to be beneficial for NH listeners, indicating that the enhancement of F0 dynamics and F0-dynamic-range contrast might also yield benefits for HI listeners. The only available study on this topic reported negligible benefits. However, that study used sentences whose short durations may have limited the opportunity for the listeners to "tune in" to the F0 dynamics of the competing voices and the differences between them. The present study explored the effects of enhancing F0-dynamics in the presence of competing voices for NH and HI listeners using longer speech segments.

Methods: Speech reception thresholds (SRTs) were measured in six NH and 26 HI listeners using a two-competing-voices experiment, where the F0 dynamic ranges of relatively long target and masking speech signals were compressed or expanded by means of a signal-processing method that modifies the F0 information in isolation from other acoustic features of speech. The target speech consisted of short sentences (used as queried sentences for measuring intelligibility) embedded in connected speech. The masker speech was also connected speech, spoken by a different talker of the same sex as the target talker.
Results: For NH listeners, on average, compressing the F0 dynamics of both signals had no effect on target-speech intelligibility. Target-speech intelligibility improvements up to 4 dB in SRT were observed when expanding the F0 dynamics of target or masker while compressing the F0 dynamics of the other signal, or when expanding the F0 dynamics of both signals. For HI listeners, on average, compressing or expanding the F0 dynamics of either or both speech signals reduced speech intelligibility. However, a large variability was observed across HI listeners, with some participants showing speech-intelligibility improvements of up to 5 dB in SRT as a result of increased target-F0 dynamics.
Conclusions: F0 dynamics enhancement in competing-talker scenarios might make F0-dynamics cues more salient, supporting speech intelligibility and providing a potential strategy for alleviating loss of speech-segregation ability for some HI listeners. Further research will be conducted to determine hearing-profile measures that are connected to the measured speech-intelligibility benefit in an effort to identify HI listeners that can benefit from F0-dynamics enhancement.

T137. Task Induced Changes in Listening Strategy Modulate Cortical and Subcortical Speech Processing

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Category: Speech Perception
Background: Listeners bin speech sounds into phonetic categories for successful speech perception. Some listeners achieve this with a more gradient/continuous listening strategy, retaining more within-category signal details, while others use a discrete/categorical strategy, discarding detail for a more abstract phonetic code. How neural responses change with listening strategy across the auditory system is unclear.
Methods: Here, we first assessed how individual differences in listeners' inherent perceptual strategy corresponded speech coding at cortical (event-related potentials – ERPs) and subcortical (frequency-following response – FFRs) brain levels. We then examined changes in speech FFRs/ERPs with real-time shifts in listening strategy as participants were cued to listen to the same speech tokens either categorically or gradiently.
Results: Behaviorally, we found listeners were able to switch between listening strategies on a trial-wise basis. Preliminary analyses of neural data reveal stronger ERP P2 responses when gradient listeners were cued for gradient listening. In contrast, those with a discrete predisposition had larger responses when cued for categorical listening. This finding demonstrates cueing congruent with a listener's inherent strategy magnifies their cortical speech coding in real time. At the brainstem level, FFR amplitudes to identical stimuli were larger under

cued gradient listening and in more gradient listeners, suggesting detailed listening strengthens subcortical speech coding on short- and long-term time scales.
Conclusions: Collectively, these results reveal an interplay between stable and real-time shifts in listening strategy that directly modulate speech coding at multiple levels of the auditory system.

T138. Modeling Continuous Speech Perception Using Artificial Neural Networks

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Category: Speech Perception
Background: Humans possess a remarkable ability to transform pressure waveforms entering the ear into meaningful linguistic representations. Despite decades of research in auditory perception, our understanding of speech perception remains limited. A fundamental computational challenge for speech perception is the lack of invariance in the speech signal. This challenge has driven the development of speech perception models; however, we still lack biologically plausible and fully stimulus-computable models of speech perception that replicate human levels of performance.
Methods: We developed a candidate model of human continuous speech perception by training an artificial neural network to generate sequences of American English phonemes from acoustic signals processed through a simulated cochlea. The model architecture includes six 2-dimensional convolutional layers for downsampling, followed by six bi-directional LSTM layers to capture temporal dependencies. The LSTM hidden states are mapped into phoneme space via a linear layer, and the model is trained using Connectionist Temporal Classification (CTC) loss. To address limited phoneme-labeled data, we used a pseudo-supervised training approach, employing a Grapheme-to-Phoneme model to transcribe phonemes from large-scale speech corpora, resulting in approximately 6 million transcribed utterances for training. For human-model comparisons, we conducted a behavioral experiment in which 100 participants transcribed 5000 nonwords, allowing direct comparison under identical conditions.

Results: Compared to existing automatic speech recognition systems, the model demonstrated competitive performance on unseen data and various transcription methods. In non-word recognition tasks, humans performed slightly better with an average phoneme error rate (PER) of 29%, compared to 33% for the model. However, at the phoneme level, the model exhibited a similar pattern of phoneme confusions as humans, both for consonants ($r=0.91$) and for vowels ($r=0.87$). The recognizability of individual phonemes was also highly correlated between humans and the model ($r=0.93$), highlighting the model's alignment with human perceptual patterns.
Conclusions: These findings collectively suggest that human-like speech perception emerges by optimizing for phoneme recognition from cochlear representations. This work lays the groundwork for systematic comparisons between human and model perception, including analyses of confusion patterns, categorical perception, auditory illusions, and context effects.

T139. Dynamics of the Multiple Demand Network Connectivity Under Varied Speech to Noise Ratios

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Category: Speech Perception

Background: When masked by background sound, speech listening appears to engage the domain-general multiple demand network (MDN) (e.g., Peelle, 2018), which includes the cingulo-opercular network (CON) and the frontoparietal network (FPN). The MDN's involvement doesn't appear to reflect operations required for speech understanding (Diachek et al., 2020), but rather a general increase in effort due to rising task demands (Duncan, 2010; 2013; Fedorenko et al., 2013). Within the CON, the anterior insula (AI) is thought to monitor performance and signal salience (Menon and Uddin, 2010; Shenhav et al., 2013) while the dorsal anterior cingulate cortex (dACC) appears involved in effort allocation. It has been hypothesized that, as listening conditions worsen, the AI signals the dACC, which in turn coordinates with the FPN to implement cognitive control mechanisms (Kerns et al., 2006; Dosenbach et al., 2008). This study examines how varying signal-to-noise ratio (SNR) during story listening alters how regions of the MDN functionally coordinate with each other, measured as dynamic functional coupling. This will contribute to a better understanding of the neural mechanisms involved in effortful listening.

Methods: Forty-four adults (ages 19-34 years) listened to three stories, each 10 to 13 minutes long, while undergoing functional MRI scanning. The stories were mixed with a 12-talker babble masker and the SNR changed pseudo-randomly every 30 to 33 seconds across five levels: clear speech, +14 dB, +9 dB, +4 dB, and –1 dB. Functional connectivity was measured among key regions of the MDN. A generalized psychophysiological interaction (gPPI) analysis was employed to investigate how functional connectivity between pairs of regions was modulated by the SNR during story listening. To understand whether varying SNR modulated brain connectivity similarly across participants, we estimated modulation of gPPI connectivity patterns across participants (i.e., seed region in one individual, target region in another). This allowed us to identify shared connectivity patterns across participants and how these patterns varied with SNR. We call this approach "intersubject gPPI" (IS-gPPI).

Results: Preliminary analysis revealed increased connectivity among key regions of the MDN as SNR decreased. Increased coupling with decreasing speech quality was observed between the right middle frontal gyrus, part of the DLPFC, and the right inferior frontal pars opercularis (IFGop). Enhanced connectivity with decreasing quality was also observed between the right IFGop and the right medial frontal region, which includes the dACC, as well as between the left AI and the right precentral gyrus.

Conclusions: Our findings demonstrate the critical role of the MDN in supporting effortful listening, with a set of regions, particularly in the right hemisphere, increasing their coordination as listening conditions become more challenging. These insights contribute to understanding how the brain responds to challenging listening environments and highlight the importance of further research into effortful listening.

T140. Attentional Disengagement Through External and Internal Distraction Reduces the Neural Tracking of Speech in Background Noise

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Category: Speech Perception

Background: Speech comprehension in background noise is often effortful. When this listening effort is experienced frequently by a listener, they may disengage from listening or 'zone out'. However, little is known about how speech is processed when a listener zones out and whether one can measure this zoning out based on a person's brain activity non-invasively. In three electroencephalogram (EEG) experiments, we investigated how the brain tracks speech in the presence of different degrees of background noise while the listener disengages from speech due to external distraction or internal distraction (mental imagination).

Methods: Overall, 72 adults (19-35 years) participated in the current study. In three experiments, participants listened to 24 spoken stories (1.5 – 2.5 min), presented under clear speech, or masked by a 12-talker babble noise at either +6 dB or -3 dB SNR, while 32-channel EEG was recorded. A temporal response function (TRF) approach was used to examine how well the brain tracks the acoustic envelope of the speech. Analyses focused on TRF weights and EEG prediction accuracy. In Experiment 1, participants attended to the spoken stories. This experiment was conducted to obtain an understanding of the neural tracking responses under different noise conditions. In Experiment 2, participants either attended to the stories or performed a visual distraction task (12 stories per condition). In Experiment 3, participants either attended to the stories or performed a mental imagination task, where they mentally wandered through specific life events.

Results: Across three experiments, neural speech tracking was consistently enhanced in conditions where speech remained highly intelligible (clear and +6 dB SNR), regardless of whether attention was allocated to the speech or diverted to a distraction task. Meanwhile, neural tracking was the weakest under the most challenging listening condition (-3 dB SNR), irrespective of whether listeners actively ignored the speech or attempted to follow it closely. Notably, neural tracking was significantly attenuated during both the visual distraction task (Experiment 2) and the imagination task (Experiment 3) compared to the attentive-listening condition, as evidenced by a marked reduction in the N1-P2 amplitude and decreased EEG prediction accuracy, suggesting that attention allocation externally and internally during speech listening modulates the strength of neural tracking.

Conclusions: In this exploratory study, we provide evidence that the neural representation to continuous speech can be reliably measured when listeners actively disengage from a listening task, with the effects of attention on neural tracking appearing independent of the background noise adaptation. Our findings are the first to demonstrate that internal attentional disengagement from speech affects the neural representations of speech similarly to external attentional distraction. These findings highlight that non-invasive recordings of brain activity can identify mental disengagement from speech during challenging listening.

T141. Comparison of Phonemic Restoration Between Young and Middle-Aged Adults

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Category: Speech Perception

Background: It is known that the ability to listening in the presence of competing sounds declines in middle age. Phonemic restoration plays an important role in listening in the presence of competing sounds. Phonemic restoration is an auditory illusion in which listeners hear a part of a word that has in fact been replaced by another sound. Previous studies comparing older and younger adults reported that a robust phonemic restoration was observed in older adults, and the effect was even stronger under certain conditions. Therefore, it is possible that the stronger phonemic restoration compensates for the age-related decline in perceptual and cognitive functions. Studies with younger subjects reported that the phonemic restoration is strongly activated when a task that assesses the phonemic restoration is combined with another task. Give that the amount of attentional resource reduces with aging, the enhancement of phonemic restoration in dual-task conditions would be larger in older adults. This study addresses this question.

Methods: Participants were presented with four-mora words in which a consonant of the second mora was either replaced with noise or had noise added to it, and discriminated between the replaced and added conditions. The participants then memorized a newly presented four-mora word. This series of procedures was referred to one span. After repeating 3 to 6 spans, participants wrote all the words they had memorized on a response sheet. The discrimination between the replaced and added conditions was also evaluated without the memory task.

Results: 1. The error rate of replaced speech, i.e., probability that participants labeled a presented replaced speech as the added condition, was higher than that of added speech, i.e., probability that participants labeled a presented replaced speech as the added condition for both the middle-aged and the young subjects.

2. In both groups, the error rate of the replaced speech tended to increase with the difficulty of the distractor task.

3. Only when the word familiarity was high, the error rate of the replaced speech for the middle-aged adult group was significantly higher than that for the younger group.

Conclusions: The result 1 supports that both middle-aged and younger subjects showed phonemic restoration. The result 2 is consistent with previous studies that phonemic restoration was more likely to occur in the presence of a distractor task. For words with high familiarity, phonemic restoration is expected to rely on linguistic skills and vocabulary. On the other hand, for words with low familiarity, acoustic information can only be used as a cue. Combined with this, the result 3 can be interpreted that the older listers tend to rely more on linguistic skills and vocabulary under high cognitive load.

T142. Decoding Auditory Attention With Time-Invariant Speaker Identity

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Category: Speech Perception

Background: Auditory Attention Decoding (AAD) is a promising approach to improve hearing devices by using brain signals to identify and selectively enhance the attended talker in multi-talker environments. Recently, Target Speech Extraction (TSE) has gained popularity as a technique for selectively enhancing the target speaker from a mixture of voices. Most current approaches utilize non-invasive brain recordings to decode time-varying features, such as the speech envelope, spectrogram or an encoding in a latent space, as cues for extracting the attended speech. However, invasive brain recordings using intracranial electroencephalography (iEEG) have revealed brain regions that are both speaker selective and modulated by attention, suggesting the possibility of decoding a time-invariant feature, such as the identity of the attended talker.

Methods: In this work, we introduce a novel method for target speech extraction that decodes time-invariant information about the attended talker's identity from iEEG recordings. The process involves clustering speaker features from speech data, training a brain predictor to classify speaker clusters using paired brain-speech data, and then using those clusters to train a speech extractor on a large corpus of speech-only data. During inference, the speaker clusters are decoded from the neural data, and this decoded speaker identity is then used as a cue for extracting the attended speaker.

Results: The study involved six human participants undergoing clinical treatment for epilepsy, implanted with subdural ECoG grids or sEEG depth electrodes, who listened to two simultaneous conversations while focusing on one. Neural data were processed using an recurrent neural network (RNN)-based brain predictor and a speech extractor, evaluated across multiple metrics focusing on acoustic quality and speech content. The results show that the use of speaker verification features obtained with deep neural networks (DNN) achieved the best cluster prediction accuracy and speech extraction performance. Our results demonstrate that it is feasible to reliably decode the attended talker's identity from brain waves and that using this identity as a cue for extracting the attended speech significantly improves performance compared to traditional time-varying feature-based approaches.

Conclusions: This study provides an alternative approach to brain-controlled speech enhancement models, introducing a new perspective for decoding speech and speaker-related information from neural data. Unlike most previous speech decoding methods, which primarily focus on the rapidly changing acoustic properties of speech, this method enables higher-level decoding related to the characteristics of the speech source, which remain constant over time as long as the speaker does not change. A significant aspect of this study is its use of intracranial recordings, which provide higher signal quality and signal-to-noise ratio (SNR). In future works, we will examine this model on more accessible non-invasive signals like scalp EEG.

T143. The Effects of Voice Onset Time on Dichotic Listening Using Persian Consonant-Vowel Stimuli in Young Adults With Normal Hearing

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Category: Speech Perception

Background: The dichotic listening test is often used to study the asymmetry of the cerebral hemispheres and to assess speech and language lateralization. The present study was conducted to investigate the role of Voice Onset Time (VOT) in understanding Consonant-Vowels during the dichotic listening test.
Methods: In this study, three 36-word lists were used, which were categorized into four classes based on VOT and presented in three attention states in a dichotic manner. After sorting the test items based on desired criteria and creating the lists, the words of each list were loaded dichotically into the personalized special software. Then a cross-sectional study was performed on 71 right-handed individuals with normal hearing (38 women and 33 men). The age of the participants ranged from 19 to 28 years with a mean age of 23.7 ± 2.46 years. The scores obtained by the participants were recorded in the software.
Results: There was a significant difference in the mean scores of correct responses between the right vs. left ear in various VOT modes (P LESS THAN 0.001). Both VOT and attention were significantly associated with the Laterality Index (LI) (P LESS THAN 0.001), and the VOT of stimuli had a significant impact on the individuals' rate of error (P LESS THAN 0.001); however, the attention variable did not have a significant impact on the error rate (P = 0.051).
Conclusions: The data obtained in this study, in accordance with those of previous experiments, suggest that differences between languages may have no considerable impact on the outcomes of the dichotic listening test. Also, using the VOT feature in speech stimuli can significantly deviate the Laterality Index to the right or left.

T144. Neural Modulation of Auditory Attention Across Anatomical Regions and Frequency Bands Using ECoG

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Category: Speech Perception
Background: Decoding auditory attention involves understanding how different brain regions and frequency bands process competing auditory signals. This study investigates the relationship between anatomical locations and neural frequency bands during attention shifts between speakers, aiming to pinpoint the regions and frequencies most relevant to this process. Using invasive neural recordings from epilepsy patients, we examine how attention modulates neural responses across both low- and high-frequency bands in the temporal lobe.
Methods: Electrocorticography (ECoG) and stereotactic EEG (sEEG) recordings were collected from eight epilepsy patients. Two patients had subdural ECoG grids implanted over the left hemisphere, and six had bilateral sEEG depth electrodes. We analyzed data from 373 electrodes positioned over the temporal lobe. Patients listened to pre-recorded stories from a male and a female speaker in both single- and multi-talker conditions, without spatial separation. Neural responses were compared between these conditions, and frequency bands (delta, theta, alpha, beta, gamma, and high gamma) were extracted for analysis.
Results: We observed a 'U-shaped' pattern of attention-related modulation, with the strongest effects in the delta and high gamma bands, and weaker modulation in mid-frequency bands. In

the lateral temporal lobe (e.g., superior, middle, and inferior temporal gyri), the anterior regionsshowed more modulation in low-frequency bands (delta through alpha), while posterior regions were predominantly modulated by high-frequency bands (gamma and high gamma). Modulation in the beta band spanned both anterior and posterior temporal regions. Medially, Heschl's Gyrus (HG) exhibited significant delta band modulation, differing from the lateral temporal responses.
Conclusions: Our findings highlight the critical role of both delta and high gamma bands in modulating auditory attention, particularly within the temporal lobe. Low-frequency modulation primarily occurs in anterior lateral temporal regions, while high-frequency modulation is more prominent posteriorly. The distinct delta band modulation in Heschl's Gyrus underscores its unique contribution to auditory processing. These insights deepen our understanding of the neural mechanisms underlying auditory attention and offer potential applications for neuroprosthetic devices. Future research should explore medial temporal structures and other cortical areas to build a comprehensive model of auditory attention.

T145. Distinct Roles of SNR, Speech Intelligibility, and Attentional Effort on Neural Speech Tracking in Noise

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Category: Speech Perception
Background: Robust neural encoding of speech in noise (SIN) enables speech comprehension in complex acoustic scenes. Various objective and subjective factors influence how the auditory cortex encodes noisy speech. Objective factors include the signal-to-noise ratio (SNR), representing the physical properties of the acoustic signal and its masking by background noise. Speech intelligibility, a subjective measure, reflects the listener's ability to recognize spoken words and depends on the listener's auditory processing capabilities and SNR. Attentional focus is another subjective factor that pertains to the listener's ability to selectively concentrate on the speech signal, filtering out unwanted sounds in complex auditory scenes. Another related yet distinct factor is attentional effort, which involves the cognitive resources expended to focus on the target speech while ignoring distractions and is influenced by listener engagement, fatigue, and overall listening task difficulty. While these factors are interconnected, they are mechanistically distinct. SNR is an external, quantifiable measure, whereas intelligibility and attention are subjective experiences that vary across individuals, even in identical acoustic settings. This differentiation underscores the complexity of auditory processing and the gaps in understanding how these elements collectively influence neural speech encoding.
Methods: In this study, fourteen native English speakers performed selective speech listening tasks at various SNR levels while EEG, gaze activity, and buzzer press responses were recorded. Attentional performance was assessed using a repeated word detection task, and attentional effort was inferred from gaze velocity. Utilizing a high-resolution range of SNR values, continuous behavioral measures, and multiple biophysical measurements allowed us to distinguish the unique contributions of each component to neural speech encoding.
Results: We demonstrate that neural tracking of target speech is influenced by both objective (SNR) and subjective (speech intelligibility, attentional performance and effort) factors in

distinct ways. As speech intelligibility increases, the positive effect of improving SNR on neural tracking of target speech diminishes. Specifically, in conditions where speech is highly intelligible, further increases in SNR decrease neural speech tracking. We propose that this decrease is caused by the reduced attentional effort required to focus on the target speech. Our findings show that gaze velocity, a measure proposed for quantifying attentional effort, effectively explains this reduction in neural speech tracking accuracy.
Conclusions: Our findings suggest a complex interaction between speech intelligibility and attentional effort mediated by SNR in shaping the neural representation of speech in noise. These findings advance our holistic understanding of noisy speech processing in the auditory cortex and have practical implications for designing auditory technologies, such as hearing aids, to improve speech perception under challenging listening conditions.

T146. Fluid Intelligence and Working Memory Are Differentially Recruited to Support Challenging Speech Perception

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Category: Speech Perception
Background: Listeners often face speech perception challenges such as acoustic ambiguity due to background noise (ambiguity of form) or semantic ambiguity due to homophones (ambiguity of meaning). Although listeners can use context to determine the appropriate word form or meaning, the underlying cognitive abilities recruited to overcome these different challenges may differ. Scores on cognitive tests correlate with one another due to a common underlying general cognitive factor known as 'g'. Scores on working-memory tests have been shown to correlate with speech-in-noise performance, but 'g' was not accounted for in these studies. Furthermore, the degree to which different cognitive factors are related to different challenges has not been explored. In this study, we use a pattern completion task as a proxy for general cognitive ability and examine how this and working memory predict individual differences in the intelligibility of ambiguous sentences.
Methods: Participants completed an online sentence transcription task with 112 sentences across four signal-to-noise ratios (SNR; clear, +6 dB, +4 dB, and +2 dB) and two semantic ambiguity conditions: high ambiguity (HA) containing homophones (e.g. "The shell was fired towards the tank") and matched low ambiguity (LA) sentences without homophones (e.g. "The secrets were written in her diary"). Participants were then invited to complete cognitive tasks including Raven's Advanced Progressive Matrices (RAPM), Reading Span, and auditory 2-back. RAPM is a pattern completion task (indexing general cognitive ability). Reading span is a complex measure of working memory requiring a semantic judgment on sentences, while simultaneously holding a list of letters in mind. The auditory 2-back test is another test of working memory in which participants monitor a sequence of spoken letters and indicate when the current letter matches the one presented two letters back.
Results: Intelligibility declined as SNR became less favorable and HA sentences were less intelligible than LA sentences. SNR and Ambiguity interacted such that the intelligibility difference between LA and HA sentences was greater at lower SNRs. When cognitive task scores were included as predictors, we found that higher RAPM scores predicted higher

intelligibility. The effect of RAPM performance on intelligibility was more pronounced at lower SNRs. Adding 2-back and reading span did not account for any additional variance in intelligibility beyond RAPM. Separate models for HA and LA sentences demonstrated that 2-back performance explained variance beyond that accounted for by RAPM in the high ambiguity condition only.
Conclusions: General cognitive ability robustly predicted the intelligibility of sentences in babble. The relationship observed in previous studies between Reading Span and speech-in-noise performance may be explained by general cognitive ability. Two-back performance, but not Reading Span, predicted the intelligibility of sentences with homophones, suggesting that working memory facilitates accurate meaning selection and context construction for the HA sentences, aiding intelligibility.

T147. Effects of Center Frequency Mismatch Between Ha and Ci Ears on Speech Perception in Simulated Bimodal Hearing

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Category: Speech Perception
Background: Bimodal hearing, the use of a cochlear implant (CI) in conjunction with a hearing aid (HA) in the contralateral ear, provides many bimodal users an improved performance with the combined use of a CI+HA compared to the use of the better ear alone (i.e., bimodal benefit). A critical factor influencing the bimodal benefit in speech perception is the degree of frequency mismatch between the HA and CI ears. This study aimed to determine whether the mismatched center frequency of each band between the HA and CI ears affects bimodal benefit in speech perception.
Methods: Ten adult subjects with normal hearing participated in this simulated bimodal study. Perception was measured in quiet and noise at -6, 0, and 6 dB SNR with HA alone, CI alone, and a combined CI+HA condition. Both acoustic and electric hearings were simulated with ten bands covering frequency ranges of 188 to 7938 Hz. Acoustic hearing was simulated using band-pass filtering for a high-frequency hearing loss beyond 1 kHz, with center frequencies of 243, 390, and 592 Hz for the first three bands. Electric hearing was simulated using a 10-channel sinewave vocoder with numerous input frequency ranges, resulting in different frequency mismatches across ears (i.e., one matched, and multiple mismatched conditions), relative to each of the three center frequencies in the HA ear. Two different frequency mismatch cases were tested: 1) clinical in which all ten center frequencies in the CI ear were shifted when any of the first three bands were adjusted to match with those in the HA ear, and 2) research in which only one center frequency was shifted when any of the first three bands were adjusted to match with those in the HA ear.
Results: The results reveal that bimodal hearing consistently produced the highest scores, followed by CI alone and then HA ear alone over SNRs. The research case outperformed the clinical case, in one-band condition, particularly in high SNR settings. The two-band condition showed enhanced speech perception under the research case, particularly in low SNRs. Lastly, the three-band condition demonstrated superior outcomes for the research case across SNRs.

Furthermore, in all configurations involving the adjustment of the band numbers, the matched condition provided more significant benefits compared to the mismatched conditions.
Conclusions: The results suggest that the match in the center frequencies of each band between HA and CI ears is the critical factor in affecting the bimodal benefit in speech perception improvement. Across all conditions, bimodal hearing exhibited the greatest improvement in speech perception with HA adjusted to three bands, followed by those with two bands. This evidence suggests that matching center frequencies may optimize bimodal hearing, especially for individuals facing bimodal interference under current clinical practices.

T148. Neural Tracking of Hierarchical Linguistic Structures in Second Language Acquisition

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Category: Speech Perception
Background: Listeners differ in their ability to process speech in a second language (L2). When listening to continuous speech, cortical activity from native speakers is entrained to the rhythms of linguistic structures at multiple levels, such as syllables, phrases, and sentences. In contrast, neural tracking of higher-level linguistic structures, like phrases and sentences, is weakened or absent in L2 learners and correlates with their proficiency. Therefore, the neural tracking of hierarchical linguistic structures may contribute to individual differences in L2 speech processing. However, it remains unclear when this neural tracking emerges during L2 acquisition and how it evolves with increasing L2 experience.
Methods: We used a frequency-tagging paradigm to assess neural tracking of hierarchical linguistic structures. Participants listened to synthesized 4-syllable Chinese sentences, with syllables, phrases, and sentences repeated at frequencies of 4/1.28 Hz, 2/1.28 Hz, and 1/1.28 Hz, respectively. Each trial consisted of ten 4-syllable sentences, with an additional one to three syllables padded at the end. Participants performed a syllable-counting task, in which they counted syllables cyclically from 1 to 4 and reported the final count. Cortical EEG responses at 4/1.28 Hz, 2/1.28 Hz, and 1/1.28 Hz were recorded and quantified to measure how well participants tracked syllabic, phrasal, and sentence-level structures, respectively. In a cross-sectional design, four groups of normal-hearing adults with varying levels of L2 Chinese proficiency participated: naïve English speakers with no experience in tonal languages (including Chinese), beginner L2 Chinese learners, advanced L2 Chinese learners, and native Chinese (L1) speakers.
Results: Preliminary results showed robust tracking at the syllabic, phrasal, and sentence levels in native Chinese speakers (n = 18), with reduced tracking observed in advanced L2 learners (n = 5). Data collection is ongoing for naïve English speakers, beginner L2 learners, and additional advanced L2 learners. Additionally, a longitudinal design is underway to follow neural tracking in a group of novice L2 Chinese learners over multiple semesters of classroom-based Chinese language instruction.
Conclusions: This study will enhance our understanding of the developmental trajectory of neural tracking during L2 acquisition and the relationship between language proficiency and the

neural oscillations underlying speech perception. The findings will offer insights into the neural mechanisms behind individual differences in L2 processing and acquisition.

T149. How Does Hearing Loss Affect Cognitive Influences on Speech-In-Speech Perception?

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Category: Speech Perception

Background: Understanding speech among competing speech ("speech-in-speech") is particularly difficult for people with peripheral hearing loss, which can affect quality of life and health outcomes. For people without hearing loss, speech-in-speech intelligibility is improved by cognitive factors such as advance knowledge of talker identity ("who"), semantic context ("what"), and spatial location ("where"). However, we do not fully understand how hearing loss affects the relative use of these factors. This study aims to test how the use of these three cognitive factors for speech-in-speech perception differs between young adults with and without hearing loss.

Methods: In total, we aim to recruit 48 participants (aged 18–35): 24 with bilateral mild-to-moderate hearing loss and 24 with normal hearing. Participants were first familiarised with the voice–name pairings of three male talkers. Then, in the speech intelligibility task, participants heard two concurrent sentences on each trial (one target and one masker), and repeated the target sentence aloud. The two concurrent sentences were spoken by different (male) talkers, had different topics, and were spatially separated. Before the sentences began, participants were visually cued to the talker identity (e.g., John), topic (e.g., Animals), or spatial location (e.g., Left) of the upcoming target sentence, or saw an uninformative cue (baseline condition). Performance in the baseline condition was matched across participants.

Results: The study is ongoing, and the results will be presented at the meeting. We predict that, compared to participants without hearing loss, participants with hearing loss will derive a greater benefit to intelligibility from semantic context and talker identity cues, but a smaller benefit from spatial cues.

Conclusions: Our findings will improve our understanding of how hearing loss interacts with cognitive influences on speech-in-speech perception. Possibly, some auditory cognitive processes could be degraded by hearing loss, while others may be preserved and could help compensate for hearing loss. This pattern of results would imply that the three cognitive factors are underpinned by (partially) distinct cognitive processes, and may interact with hearing loss via different neural mechanisms and/or pathways. Our findings may help towards understanding why people with hearing loss have particular difficulty understanding speech in noisy environments.

T150. Real-time Spatial Auditory Attention Decoding from Single-Trial EEG

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Category: Speech Perception

Background: This research aims to develop a real-time auditory attentional decoder (AAD), which identifies a listener's spatial attention within one-second latency from neural responses using electroencephalography (EEG) signals. The decoder outputs a probability between the two locations which can be read as the direction of the attended speech in two competing speech streams.

In the context of the cocktail party effect, which is the phenomenon that people can selectively make their auditory attention to a target speech even in a noisy room, it will be useful information to provide a better sound to the listener in noise, if the direction of the attended speech can be decoded by analyzing the individual listener's spatial attentional modulation.

Methods: We developed the real-time attentional decoding model using a combination of publicly available datasets and our datasets. We trained a convolutional neural network (CNN) model using the pairs of EEG data, recorded when the listener attended to the target speech and the target label. Previous studies showed developing real-time AAD using machine learning had three big challenges: (1) dataset dependency, (2) latency, and (3) data configuration compatibility.

For (1), this challenge is related to overfitting. Since EEG patterns vary significantly, even a model that performs well on one dataset may struggle with other datasets. To enhance its generality, we applied batch normalization layers to the conventional model. To solve (2), our model didn't include the speech envelope as input so it was solely based on spatial attentional modulation recorded in EEG. It allowed a very short latency, so we applied the smoothing function which allowed the AAD model to smoothly track the direction of the listener's spatial attention within a one-second window. To resolve the data configuration mismatch in (3), we combined the pre-existing dataset of 64-channel recordings from BioSemi with our dataset of 21-channel recordings from DSI-24, which was also used for real-time experiments. To standardize this, we aligned the channel configurations, reducing the data to 18 channels. This approach significantly reduced the overall data size and training time by over two-thirds while still maintaining accuracy in the results.

Results: The real-time decoding experiments were conducted in a speaker playback setup with independent continuous speeches from left (-45 degrees) and right (45 degrees). Participants followed visual cues (an arrow) directing them to the target sound, while the system continuously tracked and displayed their decoded spatial attention, and the decoded attention from single-trial EEG and the target were closely matched during the experiments.

Conclusions: We plan to expand our AAD model to handle more general situations, such as tracking spatial attention across various sound source directions. To achieve this, we will collect new training data using a paradigm that assesses whether participants are maintaining sufficient auditory attention.

T151. Comparisons in Vowel Confusion Patterns Between Bimodal Users With Greater and Lesser Bimodal Advantages

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Category: Speech Perception

Background: Improving speech perception for individuals with bimodal hearing is an ongoing challenge due to several influencing factors. These individuals face unique complexities in processing auditory signals due to receiving simultaneous input from both cochlear implants (CI) and hearing aids (HA). In this study, we compared confusion patterns in vowel recognition between two groups: those with greater bimodal advantage (GBA) and those with lesser bimodal advantage (LBA). The bimodal advantage is referred to as an improved performance for the combined use of a CI+HA compared to use of the better device alone. Unlike percent corrects, confusion matrices offer more specific insights into speech perception. The purpose of the study was to identify key differences in vowel recognition between the GBA and LBA.

Methods: Confusion matrices were monaurally and binaurally measured from 22 adult bimodal users (12 female, 10 male) in quiet and noise at 5 dB and 10 dB SNRs. The subjects were classified into GBA (n=7) and LBA (n=15) categories using a bimodal distribution with a six percent cutoff point. Vowel recognition was measured using a 12-alternative forced-choice paradigm in three listening conditions: HA alone, CI alone, and bimodal in quiet and noise.

Results: Average data showed that bimodal listening condition provided significantly higher recognition scores compared to those using CI alone or HA alone in both groups. CI alone outperformed HA alone in both groups as well. Confusion pattern analysis showed a significant difference in bimodal integration between the groups. The GBA group demonstrates a higher level of effective bimodal integration, indicating that they benefit greatly from combining auditory input from both CI and HA. While some cases in the GBA group show a CI-ear dominance, the LBA Group exhibits a more pronounced CI-ear dominance. This dominance in the LBA Group has hindered the effectiveness of bimodal integration, leading to less significant improvements in speech perception. In some participants, the LBA Group even experienced bimodal interference. which completely reduces speech perception in bimodal mode. Overall, the LBA group is characterized by a greater reliance on the CI ear, as evidenced by the more pronounced CI-ear dominance. The GBA group demonstrated significantly higher vowel recognition accuracy when you compared to the LBA.

Conclusions: The study highlights three key elements: bimodal integration, bimodal interference, and CI ear dominance. Bimodal integration is the most significant, supported by evidence of CI ear dominance. Bimodal interference has a limited direct impact on speech perception and processing. These results emphasize the importance of bimodal integration and highlight the need for personalized rehabilitation strategies to improve vowel perception, taking into account individual auditory processing differences.

T152. Cortical Processing of Phonemic Contrasts Across Two Languages in Bilingual and Monolingual Speakers

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Category: Speech Perception
Background: When listening to speech, our brains process a complex interplay of acoustic signals attempting to wean out what may be sounds that convey a message, therefore interpreting and giving meaning to them. Bilingual speakers often encounter more difficulties comprehending speech in acoustically degraded conditions than monolingual individuals. The potential impact of such differences on testing, treatment, and aural rehabilitation strategies in audiology is a significant concern. However, studies regarding differences in cortical speech processing between bilingual and monolingual speakers have been limited, and even those few studies have used English minimal pairs as the auditory stimuli in their experimental design. We propose using a bilingual Spanish/English minimal pair as candidate auditory stimuli to reveal distinct cortical patterns of bilingual speakers' speech processing in noise
Methods: Mismatch negativity (MMN) was recorded from 64 electrodes in line with the international 10-20 layout in young, normal-hearing adults, in response to six sets of English/Spanish minimal pairs. A passive auditory oddball paradigm, featuring an interstimulus interval of 750 ms and a total of 256 standards and 44 deviants, was conducted in quiet using three minimal pairs that shared the same meaning ('Gas,' 'Plan,' and 'Plus') and the other three that differed in meaning ('Son,' 'Ten,' and 'Tic'). For this preliminary analysis, data obtained from auditory channels were used, and epochs (-200 to 600 ms in reference to sound onset) bandpass-filtered between 3 and 15 Hz were used to obtain event-related potentials to standard and deviant stimuli.
Results: MMN was derived by comparing auditory responses to standard and deviant stimuli and quantified using the peak negativity between 150 and 300 ms. Our preliminary results revealed that among six minimal pairs, the word “plan” (English [pl’æn] vs. Spanish [pl’an]) elicited the most distinct MMN, showing an earlier latency when English was the deviant stimulus than when Spanish was.
Conclusions: This finding determined which English/Spanish minimal pair generated a distinct MMN response. Using MMN in response to the minimal pair “plan,” we will further investigate how Spanish/English bilinguals process speech in noise differently than English monolingual speakers. Our preliminary findings with source analysis will be discussed in the presentation.

T153. Using Generative Artificial Intelligence to Automate Speech-Comprehension Scoring of Naturalistic Speech Across Languages

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Category: Speech Perception
Background: Many people over 60 experience difficulties understanding conversations in noisy situations. Standard hearing tests do not capture such difficulties well because they assess verbatim word-report for short, disconnected words or sentences that poorly resemble ongoing conversation, which is context-rich and follows a coherent narrative. Moreover, tests are typically only available in a country’s dominant language (e.g., English), leading to inaccurate scores for native speakers of other languages. A major barrier to implementing naturalistic, narrative-like speech in multiple languages for hearing assessments is the absence of an accurate,

time-efficient approach for evaluation. The current research leverages large language models (LLMs) and modern artificial-intelligence (AI) based speech synthesizers to 1) automate the creation of narrative-like speech in different languages, 2) test a novel assessment approach for narrative-like speech, and 3) automate speech-comprehension scoring.
Methods: One group comprised 13 native English speakers. A second group comprised 12 native speakers of a different language, including French, Danish, Russian, and Spanish. Participants listened to 6 stories (~2 min; three in clear, three in babble noise at 2 dB SNR). Stories were created and translated to different languages using LLMs, and converted to auditory speech using modern AI synthesizers. After each story, participants freely recalled the story by speaking into a microphone. Participants listened and recalled in their native language. Analyses leveraged high-dimensional numerical vectors that represent semantic information across languages (LLM text-embeddings). Recall was scored as the correlation between embedding vectors of story sections and the recall. The similarity between recall in English and other languages was investigated using inter-subject correlation.
Results: Recall was similar for native English speakers and speakers of other languages, showing that recall scoring in any language leads to similar results. Participants recalled stories in the temporal order in which stories were played, providing an important metric to assess comprehension failures. The data further suggest that story recall is slightly lower for stories in babble than under clear conditions, for both groups. Direct comparison of recall data among participants of the same group versus participants from the other group revealed high similarity. That is, recall, including the temporal order of recall, was highly comparable among participants, regardless of the language in which they heard and recalled the stories, pointing to the generalizability of the automated scoring approach.
Conclusions: The current results provide a completely automated approach to generate high-quality speech materials and score speech comprehension data in different languages using generative AI. The work shows that investigations of speech comprehension do not require restrictions to specific native-speaker groups, as comprehension data can be mapped across languages with high consistency. The work provides an important step towards assessments of comprehension of naturalistic speech that are highly applicable in clinical contexts due to full automation.

T154. Phonological Processing in Cochlear Implant Users: A Functional Near-Infrared Spectroscopy (fNIRS) Study

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Category: Speech Perception
Background: Cochlear implants (CIs) have revolutionized auditory rehabilitation for individuals with severe sensorineural hearing loss (SNHL), enabling access to speech. However, speech perception outcomes after implantation vary widely, and the underlying mechanism of this large variability is still unclear. Researchers suggested that phonological processing abilities in cochlear implant users were related to speech perception. This study utilized functional Near-Infrared Spectroscopy (fNIRS) to identify brain activity related to phonological processing in

cochlear implant users (CIU) compared to their age- and sex-matched normal hearing peers (NHP).
Methods: We recruited 17 CIU (age: 69 ± 16 y, 8 male) and 16 age- and sex-matched NHP (age: 68 ± 8 y, 6 male). All participants underwent fNIRS scanning while performing a visual rhyming task1 designed to assess their phonological processing ability. FNRIS data were first preprocessed to remove motion artifacts and superficial physiological artifacts using Satori. The general linear model (GLM) was used to compute group results (p LESS THAN 0.05) for the contrast map of visual word rhyme GREATER THAN symbol matching condition. The Pearson correlation test assessed the correlation between speech perception scores (CNC Quiet) and beta values (two-tailed with unequal variance).
Results: Both groups only had significant activation in the left hemisphere covering Broca's area, dorsal-lateral prefrontal cortex, and middle temporal gyrus. CIU showed larger changes in oxygenated hemoglobin in the left temporal parietal junction (Wernicke's area). A significant correlation was found between the CNC word scores measured in quiet and the oxygenated hemoglobin concentration changes in the left anterior prefrontal cortex.
Conclusions: These findings suggest that the visual rhyme task can be a valid task for studying phonological processing in cochlear implant users since it does not rely on the quality of the peripheral hearing inputs. Understanding the underlying neural mechanisms of phonological processing can help us design optimal pre-surgical evaluations to individualize auditory rehabilitation for CI users to enhance speech perception outcomes in this population.

T155. The Role of Pitch Variability in Recognition and Intelligibility of Trained Voices

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Category: Speech Perception
Background: Listeners typically show a 10-15% improvement in speech-in-speech intelligibility for familiar voices compared with unfamiliar ones, both for naturally familiar and lab-trained voices. However, the role of specific speech features in voice learning is currently unclear. This study examined whether exposure to within-speaker variability in pitch (operationalised as the fundamental frequency, f0) during training contributes to explicit voice recognition and the familiar-voice intelligibility benefit.
Methods: Three groups of participants (N = 20 per group) were trained to recognise three novel voices (~1 hour of training in total). Group 1 were trained with monotonized sentences in which f0 was adjusted to the voice's median f0 across all speech samples, thereby removing the within- and across-sentence variability in f0 but preserving the average f0 for each voice. For Group 2 , each sentence's pitch contour was shifted such that the median f0 of the sentence matched that voice's median f0 across the entire sentence set. This manipulation removed across-sentence variability in f0 but maintained information about the natural f0 contour within a sentence (within-sentence variability) and the average f0 for each voice. Group 3 were trained using sentences that retained the natural variability in f0: to ensure these sentences were still manipulated, we swapped the median f0 between sentences for each voice, which retained information about both average f0 and the variability in f0 for each voice. All participants then

completed voice recognition and speech-in-speech intelligibility tests. Stimuli were only manipulated during training: testing used novel, natural speech materials.
Results: Group 1, who were trained on materials with minimal within-sentence and across-sentence f0 variability, showed poorer explicit recognition of trained voices than both other groups ($p = .02$). However, recognition did not differ between Groups 2 and 3. All three groups showed a significant intelligibility benefit for trained compared to novel voices. The average intelligibility benefit was ~11%, and the magnitude of this benefit did not differ significantly between groups.
Conclusions: Our results demonstrate that exposure to within-sentence pitch variability (i.e. f0 contour) is critical when learning to identify a speaker from their voice. In contrast, learning about how pitch varies across speech samples does not appear to be beneficial for voice identity learning. Consistent with previous studies, we show that the speech-in-speech intelligibility benefit can be achieved with short durations of voice training. Yet, unlike voice recognition, exposure to pitch variation in a speaker's voice is not advantageous for the improvement in speech intelligibility for trained voices—and participants gain an intelligibility benefit for trained voices even when they are not exposed to the voices' natural f0 variability. These findings contribute to a growing body of evidence that familiar-voice recognition and intelligibility rely on a different balance of speech features.

T156. Long-Term Memory for Voices Frees Up Cognitive Capacity to Enhance Intelligibility of Masked Speech

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Category: Speech Perception
Background: In noisy environments, speech is better understood when it is spoken by someone familiar than unfamiliar. If this benefit occurs due to long-term memory for voices freeing up cognitive capacity, visual distraction should disrupt speech-in-noise intelligibility of a familiar voice less than that of an unfamiliar voice. We examined the hypothesis that familiar voices are more efficiently processed (i.e., less resource intensive) than unfamiliar ones, by comparing their intelligibility when masked by a competing talker, while also manipulating concurrent cognitive load.
Methods: Participants (N = 30, 17 female) heard two sentences spoken concurrently in different voices (familiar-unfamiliar or unfamiliar-unfamiliar) and reported the content of one (target) while ignoring the other (masker). Two different unfamiliar talkers of the same sex as the familiar talker were used as maskers and targets. While listening to the target-masker pair, participants either tracked and reported on a subset of four moving dots among 12 distractors on a screen (dual task) or ignored these (single task).
Results: In both single- and dual-task conditions, target word-report accuracy was highest when the target voice was familiar, replicating the previously observed intelligibility benefit for familiar voices. Target word-report accuracy was lowest when the masker voice was familiar in that participants erroneously reported words from the familiar masker, suggesting that familiar voices capture attention when task-irrelevant. The effect of the dual task on word-report accuracy depended on the familiarity of the target voice in that the dual task reduced word-report accuracy

compared to the single task for unfamiliar, but not familiar, targets. This effect suggests that attention on, and intelligibility of, a familiar voice consumes fewer cognitive resources than an unfamiliar voice, thus freeing up cognitive capacity and reducing cognitive demand.
Conclusions: These results provide compelling evidence that 1) familiar voices enhance selective attention when task-relevant but can distract when task-irrelevant and 2) familiar voices free up domain-general cognitive capacity to enhance real-time intelligibility of masked speech. As people age, the acoustics of speech alone are no longer adequate to drive perception. Familiar voices may be a powerful tool to help individuals with hearing impairment capitalize on what they know to augment what they hear.

T157. Development of Acoustic Startle Response and Prepulse Inhibition in Fragile X Syndrome Mice

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Category: Binaural Hearing & Sound Localization
Background: Autism spectrum disorder (ASD) is a neurodevelopmental disability, affecting how a person communicates and interacts with each other and their environment. A large part of communicating effectively is being able to accurately and acutely process and localize sounds. The most common monogenic cause of ASD is Fragile X Syndrome (FXS), which is caused by a mutation of the Fmr1 gene that encodes Fragile X Ribonucleoprotein (FMRP). Auditory dysfunction is thought to be caused by an imbalance of excitatory and inhibitory neurological imbalances in the auditory brainstem, where FMRP is highly expressed. We aim to better understand the development of auditory processing in our knock-out (FXS) and wild-type mice at different critical time points: P8, P14, P18, P21, and adulthood (85-90 days old), where P is denoted as postnatal followed by how many days old.
Methods: To measure auditory development, we will be using acoustic startle response (ASR) and prepulse inhibition (PPI) measurements, where ASRs measure whole-body responses to a startle stimulus and PPI precedes the ASR with a certain cue (prepulse), that inhibits the ASR. This will be done at the developmental timepoints stated above.
Results: We expect robust startle to develop at P14 and for our KO group to have less of an overall startle response compared to the WT.
Conclusions: Therefore, PPI is a measurement of sensorimotor gating, and having improved or diminished responses to startle stimuli will ultimately show if the animal has any disruptions in the auditory pathway. Additionally, comparisons between KO and WT mice will be analyzed and provide insight into different processing mechanisms.

T158. Post-Auricular Orientation of Auditory Attention in Sound Field Versus Virtual Sound Space

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Category: Binaural Hearing & Sound Localization
Background: The post-auricular muscle (PAM) in many animals adjusts ear orientation to improve hearing for important sounds. In humans, while this muscle exists and responds to sound, we cannot similarly move our ears. This study aimed to measure PAM activity during a speech-in-noise listening task, with controlled speaker and noise orientations. The goal was to see how PAM signal-to-noise ratio changes with different presentation modes (real vs. virtual sound space) and azimuths (speaker and noise co-localized at 45˚ vs. separated at 135˚ and 45˚). It was hypothesized that PAM activity would be reliably recorded in about two-thirds of participants, and there would be no significant difference in PAM activation between real and virtual conditions. Maximum PAM engagement was expected with speech at 135˚ and noise at 45˚.
Methods: PAM activity was recorded using four electrodes around the ears, as well as electrodes on the outer canthi (to monitor eye movements) and neck (to track neck tension). Participants completed a listening test where target speaker and noise locations were controlled in both real and virtual sound spaces.
Results: PAM response was reliably recorded across all participants. A significant effect of electrode channel and an interaction between presentation mode and channel were observed. No significant differences were found between presentation modes for any other muscles. Additionally, there was no significant effect of azimuth.
Conclusions: The study demonstrates consistent PAM activation across all participants during a speech-in-noise task. This activity sometimes coincided with engagement of the anterior-auricular muscle and, to a lesser extent, neck and ocular muscles. PAM activation varied among participants, with some showing more engagement for speech at 45˚ and others at 135˚. Notably, there was no significant difference in PAM engagement between real and virtual sound conditions. This suggests that PAM activation is linked to spatial attention, even when ear orientation doesn't affect auditory input in virtual sound environments.

T159. Aging Impairs Temporal and Binaural Processing, and Spatial Hearing, While Increasing Synaptopathy in the Mongolian Gerbil

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Category: Binaural Hearing & Sound Localization
Background: Aging can lead to problems in spatial hearing abilities such as speech in noise recognition, often while sparing hearing thresholds. The exact mechanisms of this dysfunction are unknown, but they are thought to involve the auditory brainstem, the first site of binaural and spatial processing in the auditory pathway.
Methods: Here, we combine auditory brainstem responses (ABRs), envelope following responses (EFRs), spatial hearing behavior, in vivo electrophysiology and cochlear histology in the Mongolian Gerbil (Meriones unguiculatus) to look at mechanisms of age-related dysfunction in binaural processing. We performed ABRs, EFRs, and in vivo recordings in a cohort of young (2-10 month) and aged (GREATER THAN 30 month) gerbils to assess physiology of the auditory brainstem. From ABRs, we calculated the binaural interaction component (BIC), a biomarker of spatial hearing abilities.

Results: We found that aged animals have reduced ABR wave amplitudes, indicating impaired synchronous firing in the brainstem as a result of aging. We also find a reduction of BIC amplitude and less BIC modulation by interaural time differences (ITDs) in aging animals, indicating deficiencies of binaural processing. However, aged animals do not have reduced hearing thresholds indicating these changes are not solely due to peripheral hearing loss. Additionally, aged animals showed impaired neural synchrony in the EFRs, indicating temporal deficits at the level of the brainstem. This corresponded with a reduction of synchrony to amplitude modulated stimuli recorded in vivo from bushy cell axons and medial nucleus of the trapezoid body (MNTB) neurons. To see if these changes correlated with impaired spatial hearing behaviors, we also ran our cohorts of gerbils through spatial hearing tasks utilizing prepulse inhibition of the acoustic startle response (PPI). In our first set of experiments, we used gaps in noise of variable lengths to measure the auditory temporal processing abilities of the gerbils. We found that young gerbils could detect faster gaps than aging gerbils. We then measured spatial acuity by presenting broadband noise that swapped speaker locations, acting as a prepulse, prior to presenting a startle stimulus. PPI of the startle response increased monotonically with wider angles of speaker swaps in young gerbils, but not in all aging gerbils. Lastly, to assess a potential mechanism of this dysfunction, we performed immunohistochemistry stains on cochleae from our young and aging cohorts to assess levels of cochlear synaptopathy. We found that our aging gerbil cohort had reduced synapses on inner hair cells compared to our young cohort.
Conclusions: Collectively, this data shows aged gerbil have impaired auditory brainstem physiology, which impairs temporal and binaural processing and leads to dysfunction of spatial hearing behaviors, which is caused in part by increased synaptopathy. [Supported by NIDCD 1F31DC021622-01]

T160. Relationship Between Natural Head Orientation and Unaided and Aided Spatial Hearing Outcomes

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Category: Binaural Hearing & Sound Localization
Background: People naturally move their heads to listen better in group conversations. Recently, there has been growing interest in using head movement data to improve hearing aid algorithms. Previous studies have shown that orienting the head 30 degrees away from the target speaker can improve speech reception thresholds (SRT) by 1-3 dB in the presence of spatially separated interferers (Grange et al., 2018). However, the effects of natural head orientation during competing speech, particularly in aided listening, remain underexplored. This study aims to identify natural head orientation/movement tendencies across four listening configurations (target at the front, target at the side, co-located, and separated) both with and without two different directional microphones (Omni and Directional). It also seeks to examine the impact of unaided versus aided hearing on 'functional spatial boundaries (FSB)'—the spatial separation required to segregate competing speech effectively.
Methods: This study recruited adult listeners with symmetrical moderate to moderately severe hearing loss. We measured SRTs under both head-fixed and head-free conditions, with the target

and masker co-located at the front and side. Additionally, we determined the FSB required to achieve 9 dB of Spatial Release from Masking (SRM) by adaptively adjusting the angular separation between the target and masker in both head-fixed and head-free conditions. These tests were performed under unaided, aided-omni, and aided-directional microphone conditions. During testing, head orientation and movement (in pitch, yaw, and roll), were tracked with an optical motion capture system.
Results: There was no effect of head orientation/movement when the target and masker were co-located. However, when the masker was separated from the target at the front, both head orientation and variance significantly increased (p LESS THAN .05), with a weak tendency to orient their head between the target and masker. Conversely, when the target was presented from the side and the masker was separated from the target, no significant difference in head orientation was observed compared to the co-located condition. For the FSB with the target located at the front, a negative correlation was found in the unaided condition (p LESS THAN .05), a positive correlation in the omnidirectional mode (p LESS THAN .05), and no correlation in the directional microphone mode. Moreover, head orientation and movement in roll were significantly greater in the directional microphone condition compared to the omnidirectional mode (p LESS THAN .05).
Conclusions: These findings suggest that natural head orientation and movement can differentially impact hearing outcomes depending on whether a hearing aid is used, the target location, and the type of directional microphone employed. Additionally, there appears to be an interactive influence, as the type of hearing aid directionality also affects head orientation.

T161. A Retrospective Study for Binaural Speech Perception Trends in Listeners With Hearing Aids and Cochlear Implants

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Category: Binaural Hearing & Sound Localization
Background: A nuanced exploration into the individualized facets of binaural advantages and disadvantages remains an under-explored terrain in both research and clinical practice. In other words, a profound understanding of binaural and monaural benefits holds the potential to enhance various aspects of binaural speech perception significantly. This study aimed to explore binaural perception trends in hearing aid (HA) and/or cochlear implant (CI) users by using retrospective data analysis of their monaural vs. binaural speech perception scores.
Methods: Retrospective data from 172 CI users and 82 HA users in two audiology clinics in Kentucky (University of Louisville Hospital and Heuser Hearing Institute) were collected. They were divided into three groups: those who used two HAs (bilateral HA), those who used two CIs (bilateral CI), and 3) those who used bimodal stimulation (a CI with a contralateral hearing aid: bimodal CI). Subjects' demographic information (age, sex, duration of hearing loss, amount of residual hearing, duration of hearing device use), pure tone audiometry, and speech perception score (AzBio in quiet) were examined. In particular, the subjects' monaural and binaural speech perception scores were analyzed to explore binaural speech perception trends.

Results: The results showed that four distinct binaural speech perception trends were observed in all three subject groups: 1) binaural benefit; 2) binaural averaging; 3) binaural interference; and 4) better-ear dominance. Within the bilateral HA group, the binaural benefit trend was the most dominant at 55%, the better ear trend was 27%, the binaural averaging trend was 17%, and only 1% of subjects exhibited binaural interference trends. Within the bimodal CI group, the binaural benefit and better ear trends were similar at 37% and 34%, respectively, the binaural averaging trend was 17%, and an increased binaural interference trend (8%) was observed. Within the bilateral CI group, the better ear trend was relatively dominant (40%), and 27% binaural benefit, 20% binaural averaging, and 12% binaural interference trends were observed. Neither subject demographic data (i.e., residual hearing) nor audiometric thresholds did not explain those binaural speech perception trends within or between subject groups.
Conclusions: The presence of both binaural averaging and binaural interference trends implies that listening on two devices is not always better than one. Increased understanding of how binaural disadvantages (binaural averaging and binaural interference) affect speech perception for hearing-impaired users is clinically essential for the future design of training- and device-based rehabilitative strategies to increase the benefits of binaural processing for speech perception in quiet and noise. In addition, delving into the intricacies of individual binaural benefits is crucial for refining treatment strategies and tailoring services to the unique needs of each recipient, consequently bolstering speech perception and spatial hearing abilities. Such insights hold promise for addressing suboptimal outcomes, notable in mitigating instances of binaural interference.

T162. AC\BC: Sound Source Localization During the Use of a Bone Conduction Headset

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Category: Binaural Hearing & Sound Localization
Background: Bone conduction (BC) defines a mode of hearing separate from the conventional air conduction (AC) pathway. BC devices stimulate the cochlea via vibration of surrounding bone and through secondary and tertiary conduction pathways that are also engaged when the head vibrates. In both clinical (e.g., BC hearing aid) and non-clinical (e.g., BC headphones) contexts, intracranial BC signals may be superposed with AC signals at one or both cochleae, leading to complex interactions that may degrade transmitted acoustic information and constrain auditory performance. Here, we sought to examine impacts of BC communications device use on the localization of external AC sound sources in a cohort of adult human listeners with audiometrically typical hearing. In a subset of conditions, subjects also wore earplugs to capture performance in contexts (e.g., military operational settings) in which hearing protection and access to communications signals are both required.
Methods: A simple quantitative simulation was developed to predict peripheral interactions of AC and BC signals. Spatial acoustic cues associated with an AC source were quantified across variations of BC signal amplitude and timing, intracranial BC transmission properties (including transcranial attenuation and delay), and AC source location. A psychophysical stimulus battery was then developed. In a darkened hemianechoic chamber, human subjects localized brief

broadband noise bursts presented through loudspeakers while listening to a concurrent speech signal presented through a BC headset. The AC signal was varied in azimuth, elevation, amplitude, and timing relative to the BC signal. Subjects completed the task with open ears and with bilateral earplugs. In each case, baseline performance without the BC signal was also assessed. Localization errors were quantified across conditions and compared to simulation results.
Results: Interactions of AC and BC signals at the cochleae are predicted to result in an AC-BC sum with amplitude and phase characteristics that (1) differ from those of both AC and BC component signals and (2) differ between the ears in a manner that depends jointly on interaural disparities associated with AC signal and on BC transcranial transmission properties that are independent of the AC signal. In the context of an AC localization task, the predicted result is that a concurrent BC signal distorts task-relevant AC spatial cues and degrades localization performance, with worsening impacts as the relative level of the BC signal increases. Consistent with these predictions, preliminary data suggested that localization performance decreased during concurrent AC and BC stimulation, particularly with earplugs, compared to AC-only and non-current AC+BC stimulation conditions.
Conclusions: While AC and BC signals arrive at the cochleae through separate pathways, direct AC-BC interactions can lead to task-dependent impacts on auditory performance. Ongoing and future work will evaluate signal processing approaches for the mitigation of AC spatial cue distortion in particular.

T163. Effects of Binaural Unmasking on Subcortical and Cortical Responses to Continuous Speech

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Category: Binaural Hearing & Sound Localization
Background: Binaural unmasking, the process of using interaural cues to separate target signals from interfering noise, plays a critical role in speech understanding in noisy environments. Numerous human electrophysiological studies have explored the neural mechanisms underlying binaural unmasking, demonstrating its influence on cortical and subcortical responses. However, the findings for subcortical responses have been less consistent. These investigations have primarily relied on repetitive, isolated stimuli, leaving the effects of binaural unmasking on the neural processing of non-repetitive, continuous stimuli less understood (e.g., Dieudonné et al. 2024, bioRxiv). This study addressed this gap by examining how binaural unmasking affects subcortical and cortical responses to natural, continuous speech.
Methods: Binaural unmasking was assessed by presenting audiobook stories mixed with speech-shaped noise at a fixed signal-to-noise ratio (SNR) of -2 dB under two conditions: S0N0 (both speech and noise presented interaurally in-phase) and SπN0 (speech presented interaurally out-of-phase, with noise in-phase). The SNR was determined based on a pilot behavioral study that explored different SNR levels. A third condition involved presenting the stories in quiet. Trials from the three conditions were pseudo-randomized to ensure that the same condition was not repeated consecutively. Normal-hearing younger adults listened to the audiobook stories while EEG responses were recorded. After each trial, participants answered comprehension questions

about the story. They also rated how well they understood the gist of the story and the effort required to understand it. Cortical responses to continuous speech were estimated using temporal response functions (TRFs), which predicted EEG responses from a gammatone spectrogram (representing the speech envelope). Subcortical responses were estimated using TRFs based on auditory nerve model-derived speech predictors.
Results: Preliminary data revealed that, at the behavioral level, the SπN0 condition, compared to the S0N0 condition, was associated with higher story comprehension accuracy, better ratings of understanding the gist of the story, and lower ratings of effort required to comprehend the story. At the neural level, cortical TRFs showed reduced and delayed responses from the quiet condition to the SπN0 condition, with further reductions in the S0N0 condition. In the quiet condition, subcortical TRFs displayed a prominent peak at approximately 8 ms, indicative of subcortical response origins. This peak was disrupted in both the SπN0 and S0N0 conditions. Prediction accuracy for cortical and subcortical TRFs was highest in the quiet condition, followed by the SπN0 condition, and lowest in the S0N0 condition.
Conclusions: The preliminary results appear consistent with previous electrophysiological studies investigating binaural unmasking effects on cortical and subcortical responses to repetitive, isolated stimuli. These findings have potential implications for objectively assessing binaural processing abilities using naturalistic speech stimuli.

T164. Spectral and Binaural Cue Reweighting Contributions in Sound Localization in Reverberant Environments

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Category: Binaural Hearing & Sound Localization
Background: The auditory system uses binaural cues to determine the sound source location. The cues are weighted mainly depending on the frequency content. For low-frequency (LF) sounds, the interaural time difference (ITD) is the dominant cue. For high-frequency (HF) sounds, the interaural level difference (ILD) is the dominant cue. The transition between these frequency regions is at around 1500 Hz, where both ITD and ILD contribute in different proportions to sound localization. A previous study (Spišák et al., ARO Abstract #PD117, 2019) showed that visually guided training on HF vs. LF components in a real reverberant environment induces spectral reweighting, i.e., an increase in the weight of either the HF or LF component, when that component is reinforced. However, when testing the generalization of this reweighting to binaural cues in a virtual anechoic environment, an increase in the ILD weight was observed independent of the reinforced cue. Here, follow-up experiments were performed in a virtual anechoic environment and in a real reverberant environment without training to test whether simple exposure to reverberant environment prior to anechoic binaural weight testing is sufficient to induce the increase in ILD weight.
Methods: Two groups completed two test sessions (no training), performed on different days, either with only a virtual binaural weight measurement in an anechoic environment (Aonly group), or with both a virtual binaural and a real spectral weight measurement (AR group). In the real reverberant environment testing, stimuli consisted of 2 or 4 one-octave noise bands, together

covering the range 0. 7 – 11. 2 kHz, presented from neighboring speakers selected from a range of 11 speakers spanning the angles of -56° to 56°. In the virtual anechoic environment testing, narrowband noise stimuli (Fc = 2.8 kHz) had ITD/ILD combinations corresponding to one of 40 possible positions in the horizontal plane ranging from -70.2° to 70.2° and ITL/ILD separation of up to 25.2°. Participants' task was to localize the auditory stimuli. The relative weight of HF vs. LF components and ITD vs. ILD components was derived from the response locations re. the component locations.
Results: The AR group showed no change in spectral weighting , as expected because no training of HF or LF components was present. Contrary to our hypothesis, no reweighting was observed in the binaural testing in either group, even though an increase in ILD weight was expected for the AR group.
Conclusions: The ILD weight increase observed in Spisak et al. (2019) cannot be explained by a previous exposure to the reverberant environment, at least not if that exposure does not include active training. However, what aspect of the training is important is still unknown.

T165. Biomechanical Activation of the Utricle by Sound and Vibration in Mouse, Rat, Guinea Pig, Toadfish, Sheep and Human

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Category: Vestibular: Basic Research & Clinical
Background: The utricle is positioned just behind the stapes making it vulnerable to activation by loud sounds, acoustic blast waves, and temporal bone vibration. Most experiments examining sensitivity of the utricle to air conducted sound (ACS) and bone conducted vibration (BCV) use rodents or other small animal models with relatively small inner ears and utricles. In the present work we examined how differences in utricular morphology would be expected to introduce differences in sensitivity to ACS and BCV in utricles of mouse, rat, guinea pig, toadfish, sheep and human.
Methods: A two-degree-of-freedom model was used to describe motion of the sensory epithelium relative to the temporal bone, and motion of the otoconial layer relative to surface of the sensory epithelium. The epithelium was immersed in an endolymph-filled membranous sac, which was immersed in perilymph. Responses to BCV were simulated using classic base-support vibration with linear acceleration of the temporal bone (TB), and responses to ACS were simulated using stapes acceleration in perilymph. The model was validated using laser vibrometer recordings of macula motion in guinea pigs. There are only 2 parameters the model that vary with utricle size, allowing us to easily scale parameters determined for guinea pig to predict responses for other species. Simulations were carried out in the frequency domain in response to sinusoidal accelerations, and in the time domain in response to stimuli of various strengths with either constant peak acceleration or constant peak jerk (rate of change of acceleration).
Results: We determined previously in guinea pig that the key mechanical variable driving synchronized action potential firing in sensitive calyx-bearing afferents is hair bundle shear rate, which is proportional to the velocity of the otoconial layer minus the velocity of the epithelium. For short duration TB stimuli the shear rate is proportional to TB acceleration, but for longer

duration stimuli the shear rate is proportional to TB jerk. The critical duration that switches the adequate stimulus from TB acceleration to jerk corresponds to the corner frequency of the mechanical response, which is ~500Hz in guinea pig, 400Hz in rat and 100Hz in human. Hence, a pulsatile stimulus lasting ~1.5 ms in mouse or rat or guinea pig will evoke synchronized utricular afferent responses in proportion to linear jerk, while the same stimulus in toadfish, sheep or human will evoke responses in proportion to linear acceleration. Analogous findings apply to ACS stimuli.
Conclusions: Synchronized utricular afferent responses in rodents and other small animal models evoked by BCV and ACS stimuli (e.g. VsEPs and VEMPs) are unlikely to be directly reproduced in large animals or humans. Results suggest the BCV and ACS stimulus waveforms must be adjusted in amplitude and frequency content based on utricle size to match responses between sepcies.

T166. Rapamycin Reduces Noise-Induced Vestibular Loss and Improves Walking Speed in Noise Exposed Rats

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Category: Vestibular: Basic Research & Clinical
Background: Rapamycin acts on mammalian Target of Rapamycin (mTOR) which influences multiple functional signaling pathways involved in cell protection and survival. It has been demonstrated that rapamycin, added to diet, significantly reduced hearing loss following noise exposure. Using an established noise exposure paradigm that causes permanent hearing loss as well as changes in innervation and function of the vestibular periphery, we explore how treatment with rapamycin may protect the vestibular periphery leading to smaller changes in vestibular evoked short-latency potentials (VsEP) and balance beam crossing performance in noise exposed rats. Therefore, the goal of this work was to assess benefits of rapamycin treatment in noise-exposed rats.
Methods: Rats were trained to cross a 1-meter balance beam before receiving a single 6-hour 120 dB noise exposure at 5-months of age. After noise exposure, rats were tested on the balance beam until they reached 1-year of age. After completion of balance beam experiments, vestibular short-latency evoked potential (VsEP) responses were measured and ears were collected. A commercially prepared pelleted chow diet containing encapsulated rapamycin or vehicle was given to rats at a dose of approximately 42 mg/kg body weight per day. Diet was started one week prior to noise exposure and continued for 8-weeks after noise exposure.
Results: Rats that received the rapamycin and vehicle diets showed an initial effect of noise exposure, with an approximately 25% increase in balance beam crossing time. However, rats that received rapamycin showed improvement over the next two weeks and had only a small elevation in balance beam crossing time within 3-weeks of the noise exposure that returned to baseline within 4-weeks of noise exposure. This effect persisted after discontinuation of the diet. Furthermore, rats that received the rapamycin diet following noise exposure crossed the balance beam at a speed similar to baseline at one year of age versus vehicle treated rats which had significantly slower balance beam crossing times. VsEP amplitude was significantly larger in

rats that received the rapamycin diet versus rats that received the vehicle diet. Last, rats that consumed the rapamycin diet had calyx-only terminal counts in the saccule and utricle that were comparable to age-matched control rats. Rats that were fed the vehicle diet had significant reductions in calyx-only terminal counts in, both, the saccule and utricle suggesting that rapamycin was successful in protecting calyx-only terminals in the otolith organs from significant noise-induced damage.
Conclusions: These results demonstrate a protective effect on the vestibular periphery and a significant improvement in walking speed, both shortly after noise exposure and for months after discontinuation of the rapamycin diet. Taken together, these results demonstrate a measurable effect of rapamycin that may have long-term benefits for mobility and vestibular function that can be observed at the cellular level.

T167. Peripheral Vestibular System Pathology Secondary to Otitis Media in the Chinchilla Model

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Category: Vestibular: Basic Research & Clinical
Background: Otitis media (OM) complications, including labyrinthitis, can impact the peripheral vestibular system, leading to inflammation or infection within the inner ear. The aim of the proposed study was to assess the histopathological alterations in the peripheral vestibular system resulting from OM induced by Streptococcus pneumoniae in chinchilla model.
Methods: This study utilized 51 chinchilla temporal bones from the archival collection at the Paparella Otopathology and Pathogenesis Laboratory, University of Minnesota. The temporal bones in the experimental group (n=45) obtained from the animals previously inoculated with S. pneumoniae type 23 while the control group (n=6) had received saline injections for comparative analysis. Euthanasia was performed at different time points (3, 7, 14, 21, 28 days), and the temporal bones were extracted for further histopathological examination. The sensory epithelium cell densities and quantitative assessment of vestibular ganglion cells were performed by using a differential interference contrast microscope.
Results: The study revealed consistent and statistically significant decrease in average counts of type I and type II cells, starting from the 7-day infection period and persisting throughout the disease progression. Significant reductions in mean transitional cell density were observed by the 14th day, while dark cells showed decreased counts in the 21-day group. Furthermore, the 28-day group exhibited severe pathological changes and decreased mean density of vestibular ganglion cells.
Conclusions: This study highlights a significant decline in peripheral vestibular cells and ganglion neuron loss in cases of persistent OM compared to controls. These findings emphasize the significance of the chinchilla model and provide valuable insights into the histopathological consequences of prolonged OM, particularly highlighting clinical observations related to peripheral vestibular system pathology.

T168. The Effects of Aging on Gravity Receptor Function in Gerbils

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Category: Vestibular: Basic Research & Clinical

Background: The vestibular system helps maintain spatial orientation and balance and is known to progressively deteriorate with age. It consists of gravity receptor otolith organs of the utricle and saccule, semi-circular canals, all of which project to the central nervous system. The contribution of these individual systems to overall loss of vestibular function due to aging is not apparent. We have used vestibular evoked-potential responses (VsEP's) to changes in linear acceleration (i.e. jerk) stimuli to specifically study the change in function of gravity receptor organs due to aging in Mongolian gerbils.

Methods: Each measurement consisted of responses to series of alternating (256 positive and 256 negative) jerk stimuli to the head with a maximum intensity of about 10 dB (ref: 0 dB – 1 g/ms, g – acceleration of gravity), decreasing by 3 dB step levels in the naso-occipital axis. The responses were recorded using two subdermal recording electrodes placed behind each pinna of the two ears and a reference electrode placed at the caudal position of the head. At first, the recording from each electrode was obtained as an averaged response to positive and negative stimuli. The final response is determined as an average of responses from the two recording electrodes.

Results: The VsEP response consisted of a series of alternating positive and negative peaks whose amplitude reduced with the stimulation intensity. The preliminary analysis showed that the first VsEP responses occurred between 1.5 to 2 ms of the onset of stimulus for adult gerbils (n=8; P35-68) and between 1.5-2.5 ms for aged gerbils (n=4; P777-1138). Methods for objective determination of the VsEP threshold based on normalized cross-covariation and variance ratios of adjacent level stimuli presentations are being explored. Initial analysis showed that the threshold of the aged gerbils increased significantly compared to the adult gerbils.

Conclusions: While preliminary, the results suggest that aging in gerbils contributes to significant loss of gravity receptor function. Future studies will incorporate gerbils of various ages to determine the progression of gravity receptor function loss due to aging and correlate it to the physiological properties such as morphology and function of hair cells and afferents of the receptor organs.

T169. Balance Function Analysis in Stat1 Knockout Mice

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Category: Vestibular: Basic Research & Clinical

Background: Signal transducer and activator of transcription 1 (Stat1) knockout (KO) mice with immunodeficiency have been used frequently in viral infectious disease models. Our goal was to determine the baseline balance function in Stat1 KO mice to assess whether they are suitable for use in vestibular function studies.

Methods: Male and female wild type (WT) 129Sv/Ev mice (n=5 each) and male and female Stat1 KO mice (n=5 each) at 3, 11, and 24 months of age were compared. Motor coordination and balance were assessed using the rotarod and balance beam tests. In the rotarod test, latency, distance traveled, and rotations per minute (RPM) were recorded. The experiment was performed on three consecutive days with a total of nine trials per mouse. In the balance beam test, time to traverse the beam, number of paw slips, and weight of the mouse were recorded at 3 and 24 months of age. Each mouse was tested on beams 5- and 10mm widths three times, totaling six trials per mouse. After the behavioral tests were completed at 24 months, the temporal bones were dissected, decalcified, tissue-cleared and labeled with antibodies to Myosin 6 and Neurofilament heavy chain. Lightsheet microscopy was used for imaging and the reconstructed 3D temporal bones were analyzed for spatial orientation of the cochlear and vestibular organs.
Results: On the rotarod, there was no difference in performance between the WT and Stat1 KO mice nor between males and females at 3 months of age. At older ages, the Stat1 KO males showed the best performance, with males generally performing slightly better than the females. Over time, performance deteriorated in all the groups. On the balance beam test, a statistical difference in performance was observed at 3 months of age on the 10 mm beam, with Stat1 KO males performing the best compared to other groups. Performance declined across all groups by 24 months, with statistically significant differences only seen in WT males and females. Lightsheet microscopy showed no notable morphological differences in hair cells and neurofilaments between the groups in the cochlea and vestibular organs in the inner ear.
Conclusions: The results from our study suggest that both genotype and sex play a role in motor coordination and balance over time in the Stat1 transgenic mice. While all groups had similar performance at younger ages, the Stat1 KO males demonstrated better performance at older ages. The decline in performance across all groups by 24 months highlights the impact of aging on motor abilities. Lightsheet microscopy showed no significant structural differences in vestibular organs. We conclude that the Stat1 KO mice are suitable for vestibular function studies, and that both sexes should be included and compared as individual groups in future research.

T170. Sex Specific Peripheral Vestibular Dysfunction in Two Mouse Models of Autism

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Category: Vestibular: Basic Research & Clinical
Background: Balance and movement difficulties in autism spectrum disorder (ASD) are well documented and are often linked to issues in central neural pathways (Stins and Emck, 2018; Butera et al., 2023). However, recent research has indicated that impaired peripheral sensory inputs, resulting from targeted shank3 KO in peripheral somatosensory receptors, can lead to ASD-like phenotypes and alterations in central pathways (Orefice LL et al., 2019). In this study, we investigate the sensory peripheral vestibular pathway in two ASD models: shank3 KO and cntnap2 KO mice.
Methods: We assessed peripheral vestibular function in male and female mice with knockout mutations for shank3 and cntnap2, two scaffolding proteins. Vestibular sensory evoked potentials (VsEP) measured field potentials resulting from synchronized responses of vestibular

nerve afferents to a 2 ms rapid head movement. We used ‘contact righting reflex’ to evaluate detection of gravity by vestibular organs.
Results: We found no difference in VsEP responses between female KO and WT mice across both ASD models. However, male KO mice exhibited a 20% reduction in VsEP responses compared to females, in line with the greater prevalence and severity of ASD phenotypes in males. This finding is in line of other studies showing females as more likely to be neurotypical even if they carry the Cntnap2 or Shank3 mutations (Dawson et al., 2023; Liu et al., 2024). VsEP relies on potassium-mediated, glutamate-independent synaptic transmission between type I vestibular hair cells and calyx afferent terminals (Pastras et al. 2023). This 'non-quantal' transmission depends on potassium conductances. Disruptions in these channels have been observed in shank3 KO and cntnap2 KO mice (Saint-Marten et. al. 2018).
Shank3 also anchors glutamate receptors, and its absence impacts vesicular (or 'quantal') transmission in various brain regions (e.g., Amal et al., 2020; Duffney et. al. 2013). To investigate whether quantal (Q) transmission is affected in the vestibular periphery of shank3 KO mice, we used the 'contact righting reflex'. Blocking Q transmission by NBQX, a glutamate receptor antagonist, in WT mice significantly increased the time-to-righting (27 +/- 6 s vs. 437 +/- 90 s, n = 6, paired t-test, p = 0.009). Similarly, half of the male shank3 KO mice took over 30 s to right (68 ± 40 s, range: 30–370 s), indicating impaired Q transmission in these KO animals. In contrast, female shank3 KO mice had similar time-to-righting as WT mice (11 ± 3 s, range: 2–20 s).

Conclusions: Our results indicate a sex-specific disruption in quantal and non-quantal synaptic transmissions in the peripheral vestibular sensory pathway in two ASD mouse models. This suggests peripheral vestibular dysfunction could be a factor in ASD subjects, contributing to their balance and movement issues.

T171. Transcriptomic and Epigenomic Characterization of Adult Mouse Vestibular Hair Cells

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Category: Vestibular: Basic Research & Clinical
Background: The sensory organs in the mammalian vestibular system house specialized mechanosensory hair cells that detect head movements. These hair cells are currently classified into two types, I and II, that differ in shape, molecular markers, physiology, and innervation. There is limited knowledge regarding the actual genetic diversity of mature vestibular hair cells and the regulatory mechanisms that control this diversity. Defining gene expression patterns in each distinct hair cell type in vestibular organs will inform on their unique features and functions and on strategies to drive functional hair cell regeneration.

Methods: We are analyzing the transcriptomes and epigenomes of vestibular hair cells in mature mice at 6, 7, 10, 14, and 22 weeks of age. Single nucleus multiome sequencing (RNAseq and ATACseq) was performed on utricles using 10x Genomics. mRNA sequences were analyzed using Seurat Library, creating cell clusters based on transcriptional similarities, and were verified using known marker genes. Chromatin accessibility data were first analyzed using Signac and ArchR to infer enhancer-promoter interactions. Then, pseudo-bulk ATACseq data were derived based on transcriptome clusters to analyze cell type/subtype specific regulatory elements. Genes of interest (GREATER THAN 2x enriched in each subtype) were explored using data from other RNAseq datasets via the gEAR portal, along with gene ontology analysis using DAVID and ShinyGO. Some genes of interest were validated using fluorescent in situ hybridization.
Results: Our data revealed five distinct groups of hair cells. Two groups of type II hair cells (Calb2+, Sox2+) were differentiated by region (Ocm+/-). Three groups of type I hair cells (Spp1+) were differentiated by region (Ocm+/-) and gene expression level. We confirmed the spatial and cell type-specific expression of over 10 newly defined genes. For example, Dlk2 was validated as a pan type II hair cell gene, Bmp2 as a pan type I hair cell gene, and Paqr9 as a striolar type I hair cell gene. Cntnap5b shows specific gene expression in striolar type II hair cells. Mgat4c gene expression is limited to a subpopulation of type I hair cells. We also identified cell type/subtype-specific regulatory elements for those genes, including Dlk2 enhancer, Bmp2 promoter and enhancer, Paqr9 enhancer, and Mgat4c promoter and enhancer.
Conclusions: We identified transcriptionally and epigenetically distinct clusters of mature utricular hair cell that varied based on region and type. We will further study gene regulation in each cluster of mature hair cells to learn how hair cell diversity is maintained.

T172. Age-Related Synaptic Changes in the Vestibular System of an Alzheimer's Mouse Model

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Category: Vestibular: Basic Research & Clinical
Background: The vestibular system plays a fundamental role in sensing acceleration, gravity, and maintaining balance and posture. The decline of vestibular function with advancing age is considered the leading cause of fatal falls among the elderly and has also been associated with increased risk of cognitive impairment. Reports suggest that ~60% of Alzheimer's disease (AD) patients suffer from the loss of spatial orientation. In addition, some studies show improved cognitive function after vestibular stimulation. Thus, we hypothesized that vestibular degeneration occurs at an accelerated rate in AD patients, prior to the onset of cognitive decline. To investigate this question, we used the APPNL-F/NL-F knock-in mouse model in which the amyloid precursor protein (APP) harbors two humanized pathogenic mutations. This mouse line expresses normal levels of APP while producing elevated amounts of amyloid beta, making it a good model for sporadic AD. Our preliminary findings using this mouse model indicated minimal changes to utricular hair cell (HC) density, but abnormal stereocilia morphology with advancing age. Previous studies using wildtype mice suggest vestibular synaptopathy as a mechanism for age-related vestibular dysfunction in rodents. Therefore, we investigated age-related changes in the density of vestibular HC synapses in APPNL-F/NL-F mice.

Methods: Temporal bones were collected from APPNL-F/NL-F and age matched C57Bl6/J controls between 2 and 18 months of age. Micro-dissected utricles were labeled with the HC marker myosin VIIa, and pre- and post-synaptic markers, CtBP2 and Shank1/2/3, respectively. Sox2 labeling was used to distinguish type I and type II HCs. For the analysis of synaptic changes, 3 areas of the utricle were imaged in the striolar, lateral extrastriolar and medial extrastriolar regions using confocal microscopy.
Results: Qualitative analysis of synapse density in the 3 utricular regions suggest a similar density of ribbon synapses between 2 and 18 months of age in wild type C57Bl6/J utricles. However, we found a decreased number of synapses in the utricles of APPNL-F/NL- F mice at 18 months compared to 2 months of age. We plan to quantify the synapse density in both data sets using IMARIS software to confirm these preliminary findings.
Conclusions: Preliminary findings suggest possible degeneration of synapses in APPNL-F/NL-F mice with advancing age. However, further validation with quantification and a larger sample size is needed.

T173. The L9'T Point Mutation in Alpha9 Nicotinic Acetylcholine Receptors Mitigates Concussion-Induced Vestibular Deficits in Mice

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Category: Vestibular: Basic Research & Clinical
Background: Vestibular dysfunction is common following concussion, with 30-80% of affected individuals reporting symptoms such as dizziness, imbalance, and vertigo. Our previous studies have developed a mouse model to investigate concussion-induced vestibular deficits. This model uses the closed head impact model of engineered rotational acceleration (CHIMERA) to induce consistent, repeatable concussions in mice. We observed that multiple impacts reduced the sensitivity of vestibulo-ocular reflexes (VORs) and impaired peripheral vestibular function, as indicated by changes in vestibular afferent activities. The goal of this study is to examine whether the efferent vestibular system, which can modify afferent output of the peripheral vestibular organs, plays a role in these trauma-induced deficits. In particular, we investigated the role of the A9-nicotinic acetylcholine receptor (A9-nAChR), crucial for acetylcholine-driven efferent vestibular function, in concussion-induced vestibular deficits.
Methods: We used Chrna9L9'T knock-in mice, which carry a point mutation in the A9-nAChR subunit, resulting in A9-containing receptors that are hypersensitive to acetylcholine and exhibit slower desensitization kinetics (Taranda et al., 2009). Male Chrna9L9'T knock-in mice (3 months old) were anesthetized with isoflurane and subjected to three repetitive head impacts over three consecutive day at an intensity of 0.65J. VORs were measured before the head impacts, and retested at 1 day, 7 days, 2 weeks and 4 weeks after the third impact. Horizontal eye movement responses to head rotation (rotational VOR or rVOR) and translation (translational VORs or tVOR) were recorded to assess canal and otolith function, respectively. The animals were subjected to sinusoidal head rotation (0.2-4Hz) and translation (0.2-2Hz), while their eye movements were recorded using an ISCAN video-based eye tracking system. Gains and phases

of the rVOR and tVOR were calculated using a fast Fourier transform (FFT) analysis on the de-saccaded eye velocity and head velocity signals.
Results: Preliminary analysis revealed that Chrna9L9'T knock-in mice did not show significant changes in the VORs following repetitive head impacts. These results are in contrast to the findings in the C57Bl6J mice, which exhibit substantial and persistent reductions in the rVOR gains, lasting for over two weeks.
Conclusions: The preliminary findings suggest that the efferent feedback, mediated through α9-nAChR, may protect against concussion-induced vestibular deficits.
DV, WZ, HZ *co-senior authors

T174. Auditory and Vestibular Consequences of High-Intensity Noise Exposure After Mild Traumatic Brain Injury

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Category: Vestibular: Basic Research & Clinical
Background: Traumatic brain injury (TBI) is a significant public health concern, with long-lasting effects for individuals and communities. While the effects of TBI on cognitive and motor functions are well documented, sensory dysfunctions resulting from head injuries are often overlooked due to the complexity of TBI presentations in patients. Among these less-explored but clinically significant outcomes are hearing and balance impairments. In mild TBI (mTBI), these dysfunctions primarily manifest as sensorineural, caused by transient or permanent damage to hair cells and associated neural pathways. CDC reports that individuals involved in occupations and recreational activities with elevated mTBI risk are often exposed to high-intensity noise, which alone can cause irreversible mechanical and physiological damage to both cochlear and vestibular structures. The limited understanding of the temporal dynamics and pathophysiology of auditory and vestibular symptoms in mTBI increases the risk of dual insults in these populations, where noise exposure potentially exacerbates mTBI-related sensory deficits, complicating rehabilitation efforts.
Methods: We investigated short- and long-term changes in auditory and vestibular function in ecologically valid models of mTBI and mTBI combined with acoustic trauma (n=4/group male Brown Norway rats, 14-16 weeks). Closed head injuries were induced using an accelerated weight drop system (450g, 1 meter). To simulate clinically relevant scenarios, rats in the double insult cohort were exposed to 4-16 kHz noise at 110 dB SPL for 1 hour, four days post-TBI—an adequate timeframe for recovery according to cognitive studies. ABR thresholds were evaluated at 2, 4, 8, 16, 24, and 32 kHz prior to trauma and up to 28 days post-the respective injury. Concurrently, we assessed thresholds, amplitudes, and latencies of cVEMPs evoked by 1 and 8 kHz pure tone stimuli.
Results: ABR measurements revealed that mTBI alone causes significant temporary shifts in hearing thresholds, with longer recovery times for low-frequency stimuli (D7) compared to those at higher frequencies (D3). cVEMPs evoked by 1 kHz stimuli show transient changes in threshold and P1-N1 amplitude, temporary P1-N1 amplitude shifts were also noted at 8 kHz. Full recovery for these metrics occurred by D3. No effect of head injury on cVEMP latency was observed. Preliminary data from the double insult cohort, acquired 1 day post noise, reveal that

the occurrence of acoustic trauma before full recovery of mTBI-related auditory symptoms further increases ABR thresholds at high frequencies, but not at low frequencies. A similar pattern emerged in vestibular responses, where acoustic trauma did not exhibit a synergistic effect with mTBI at 1 kHz but significantly increased the cVEMP threshold and amplitude shift at 8 kHz.
Conclusions: These results highlight the importance of considering sensory outcomes alongside cognitive and motor assessments in TBI research and clinical practice. Overlooking these sensory dysfunctions may prolong recovery and result in lasting deficits.

T175. Mapping Genetic Contributors to Vestibular Function: A Gwas Approach With the Hybrid Mouse Diversity Panel

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Category: Vestibular: Basic Research & Clinical
Background: Disequilibrium is a common condition among the elderly, significantly affecting both health and quality of life, with its prevalence increasing with age. Despite its widespread occurrence, the genetic basis of vestibular dysfunction remains unclear.
Methods: This study aimed to identify genes and pathways involved in variations in vestibular function through a genome-wide association study (GWAS) utilizing the Hybrid Mouse Diversity Panel (HMDP). The panel comprised a diverse set of 84 common and recombinant inbred mouse strains (BXD RI sets, derived from C57BL/6J and DBA/2J), alongside 18 common inbred strains obtained from Jackson Laboratory (Bar Harbor, ME). Approximately six mice from each HMDP strain were subjected to vestibular evoked potentials (VsEP) and balance beam tests. Only female mice were included in the analysis. Mice were received at 4 weeks of age and allowed to acclimatize until 6 weeks to ensure uniform environmental exposure. GWAS was conducted as previously described (Pezman et al., 2020). Association testing for vestibular phenotypes in the HMDP strains was performed using genotypes of ~500,000 single nucleotide polymorphisms (SNPs) derived from the Mouse Diversity Array (Yang et al., 2009). FaST-LMM (factored spectrally transformed linear mixed model) was used for the analysis, incorporating 100 sets of permutation tests and parametric bootstrapping of size 1000. The genome-wide significance threshold, set at a family-wise error rate (FWER) of 0.05, corresponded to a p-value of 4.1 x 10-6, similar to previous HMDP studies (Bennett et al., 2010). Further analyses were performed using LocusZoom to visualize linkage disequilibrium (LD), and RefSeq genes were characterized using data from the UCSC Genome Browser (GRCm38/mm10 genome assembly).
Results: Through GWAS, no SNPs reached genome-wide significance; however, marginally significant SNPs were identified on chromosomes 6, 13, and 14, highlighting candidate genes such as Cntnap2, Atxn1, Xkr6, Ccdc126, Gpnmb, and Tmem176a. These genes demonstrated considerable expression in cochlear and vestibular tissues, with Cntnap2 and Atxn1 showing robust expression in hair cells and a key role in cochlear maturation. Additionally, Gpnmb and Tmem176a were highly expressed in the stria vascularis, implicating their involvement in cochlear function.

Conclusions: This study provides novel insights into the molecular mechanisms underlying vestibular function, identifying potential genetic targets for further investigation and therapeutic intervention in vestibular disorders. The identified candidate genes may serve as the foundation for future research aimed at understanding vestibular dysfunction and developing targeted therapies.

T176. The Attempt to Measure Vestibular Evoked Myogenic Potentials Using Amplitude-Modulated Sound Stimuli

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Category: Vestibular: Basic Research & Clinical
Background: Cervical vestibular evoked myogenic potential (cVEMP) is a myogenic response recorded in the ipsilateral sternocleidomastoid muscle in response to auditory stimulation. Although it is a response to auditory stimulation, it is a unique reaction originating from the saccule of the vestibular system. We have previously studied the frequency response of cVEMP and reported on its clinical applications. Recently, cVEMP using amplitude-modulated (AM) sound stimulation (AMcVEMP) has been reported, and its clinical application is expected. In this study, we attempted to record AMcVEMP at our facility and considered the significance of AMcVEMP.
Methods: As with the standard cVEMP measurement, the active electrode was placed on the midpoint between the sternocleidomastoid muscle's sternal head attachment and the mastoid process. The reference electrode was placed on the sternal head attachment of the sternocleidomastoid muscle. The ground electrode was placed on the forehead. To ensure muscle tension in the sternocleidomastoid muscle, subjects lay supine and turned their necks to the side opposite the auditory stimulus. Electromyography was performed using Neuro Pack (Nihon Kohden), with a band-pass filter set from 5 Hz to 1 kHz, and recordings were made by averaging 100 responses.
Results: The amplitude spectrum at each modulation frequency was measured, and the maximum amplitude spectrum was observed at 37 Hz. The signal-to-noise ratio (SNR) at each modulation frequency was determined, and the SNR was found to be highest at 37 Hz.
Conclusions: 1.By attaching a function generator to the existing evoked potential measurement device, it became possible to record potentials with frequency response characteristics.
2.The amplitude spectrum of the FFT and the SNR were maximized at 37 Hz for these potentials. This result is consistent with the characteristics of the previously reported AMcVEMP.

3.Using a function generator and an evoked potential measurement device, AMcVEMP could be recorded.

T177. Structure and Function of Cupulae, Accessory Structures of the Lateral Line Neuromasts and Ampullae of the Inner Ear

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Category: Vestibular: Basic Research & Clinical

Background: Cupulae are present in the vestibular system of the vertebrate inner ear, and also the lateral line organs of fish and amphibia. In the inner ear, the cupulae are found in the ampullae of the semicircular canals. In the lateral line organs, the cupula covers the neuromasts, small groups of sensory hair cells found along the head and body. The cupula is thought to be the most primitive form of hair-cell accessory membrane but, despite this, little was known about its protein composition until relatively recently. ZPLD1 (zona pellucida-like domain 1) is now known to be a major component (Dernedde et al., 2014) and two spontaneous, recessive, missense mutations in the gene cause circling behaviour in mice with variable penetrance (Vijayakumar et al., 2019). In zebrafish, Zpld1 is encoded by two genes, zpld1a and zpld1b. Expression of zpld1a has only been reported in the zebrafish inner ear (Yang et al., 2011, Shi et al., 2023), while that of zpld1b is yet to be investigated. In this study we look at the expression of both genes in the inner ear and lateral line, and examine how gene knock out influences the morphology of the cupula and behaviour of the fish.
Methods: RNAscope in situ hybridisation was used to assess the expression of zpld1a and zpld1b in embryos from 50 hours to 4 days post fertilisation. Single and double zpld1a and zpld1b mutants were generated using CRISPR/Cas9 gene editing technology. Control and mutant zebrafish were recorded swimming for 5 minutes and videos analysed using DeepLabCut. Heads from control and mutant fish were fixed, decalcified, serial cryosectioned and double-labelled with Wheat Germ Agglutinin and phalloidin for morphological analysis.
Results: Expression of zpld1a and zpld1b was only observed in the inner ear and not in the lateral line. Cursory observations of mutant zebrafish identified a subset of zpld1a mutants that showed circling activity while zpld1b mutants showed no circling activity. Detailed analysis showed elevated activity in zpld1a mutants when compared to control zebrafish and zpld1b mutants, but numbers of mutants were low and the difference was not significant. Analysis of the inner ear showed a cupula is present in zpld1b mutants but absent in zpld1a mutants. Absence of zpld1a and zpld1b expression in the lateral line prompted an Ensembl blast search for similar genes. Five were identified and analysis showed all are expressed in the lateral line.
Conclusions: These findings suggest that zpld1a is crucial for the production of the cupula in the fish inner ear, and that zpld1a or zpld1b are not required for genesis of the cupula in lateral line neuromasts. The cupula of the inner ear may therefore be distinct from that of the lateral line.

T178. The Contribution of Vestibular Head Translation Cues to Postural Control

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Category: Vestibular: Basic Research & Clinical

Background: Imbalance and falls pose significant health risks, especially as people age, leading to hospitalizations and serious injuries. While many factors contribute to postural control, a key factor is sensory feedback from the vestibular, visual, and proprioceptive systems. Conventionally, feedback about tilt orientation relative to gravity has been considered the most important vestibular cue for postural control. In fact, postural control models, which have been used to further understanding about sensory contributors, typically only include vestibular feedback about tilt orientation relative to gravity. However, the vestibular system provides crucial information about head tilt, translation, and rotation. In a recent experimental study [1], we measured postural sway and vestibular function using vestibular perceptual thresholds. These vestibular thresholds are robust measures of vestibular function, and measure the smallest motion that can be reliably perceived. We measured thresholds for lateral translation, vertical translation, tilt relative to gravity, and rotation. We found that lateral translation thresholds were correlated with postural sway across subjects, but not with other thresholds. This supports a role for vestibular lateral translation cues in postural control. This finding is further supported because typical tilt of the body during quiet stance is much less than roll-tilt thresholds.
Methods: To further support these findings, we developed a closed-loop postural control model that includes independent feedback channels for vestibular tilt, translation, and roll velocity using a single-link inverted pendulum. As in past models, we included sensory neural noise/variability in each channel. Since thresholds are related to neural variability (i.e., neural noise) via signal detection theory, we made the noise for each pathway equal to the threshold. Noise was filtered white noise. Simulations were performed in Matlab/Simulink.
Results: When both tilt and translation cues are available in the model, the predicted postural sway is significantly reduced compared to using only tilt cues. The predicted postural sway with both tilt and translation cues is similar to experimental postural sway.
Conclusions: This finding strengthens support for the hypothesis that translation cues play a role in postural control. Future modeling will include proprioceptive and visual feedback.
[1] Karmali, Faisal, et al. "The role of vestibular cues in postural sway." Journal of neurophysiology 125.2 (2021): 672-686.

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T179. Evaluating the Effects of Levodopa on Vestibulo-Ocular Reflex in Parkinson's Disease: Preliminary Observations

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Category: Vestibular: Basic Research & Clinical
Background: Parkinson's disease (PD) is a neurodegenerative disorder primarily affecting individuals over 60. The disorder is characterized by motor symptoms due in large part to the loss of dopamine-producing neurons. The vestibular system, crucial for maintaining balance, may be impacted in PD, contributing to balance issues through dysfunction in the vestibulo-

ocular reflex (VOR). This study aims to investigate the effects of Levodopa (L-DOPA), a primary PD treatment, on the VOR using caloric, video head impulse testing, and rotational testing.
Methods: The study evaluated nine PD patients, who underwent videonystagmography (VNG), video head impulse testing (vHIT), and rotational chair testing both on and off their dopaminergic medications.
Results: The results did not show significant differences in vestibular function between the on and off medication states across all tested parameters. Specifically, there were no significant differences in total slow-phase velocity (SPV) during caloric tests, VOR gain from the video head impulse test (vHIT), or VOR gain, phase, or asymmetry in rotational chair testing. However, a substantial number of the tested variables showed effects sizes within the minimum clinically important difference (MCID) range.
Conclusions: These findings suggest that L-DOPA does not significantly alter vestibular function in PD patients supporting current clinical practice to study PD on their medication during vestibular assessments to ensure patient comfort and reduce test artifacts. The study's limitations include the small sample size, variability in medication dosages, and the exclusion of participants with chronic vestibular diseases. Observed effect sizes were within the MCID range warranting future large sized clinical trials.

T180. Molecular Profiling of the Fetal Human Utricle: Insights From Single-Nucleus Multiomics

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Category: Vestibular: Basic Research & Clinical
Background: The utricle is a sensory organ critical for detecting balance. Although the cellular diversity of the developing utricle has been studied in neonatal mice, our understanding of the human utricle is still limited. To address this gap, we employed single-nucleus multiomic sequencing to analyze the transcriptome and epigenome of the fetal human utricle at gestational weeks 15 and 19. This approach offers an integrated view of gene expression and regulatory interactions during these stages.
Methods: We dissected the utricle from the inner ear at gestational weeks 15 and 19 (n=2 each) and enzymatically separated the sensory epithelium from the underlying mesenchyme using thermolysin. Following tissue dissociation and cell lysis, a single-nucleus suspension was generated and applied to the 10x Chromium Single Cell Multiome ATAC + Gene Expression platform to acquire simultaneous RNA- and ATAC-seq data for each cell. For each gestational stage, the multiomic dataset underwent quality control and batch correction, followed by dimension reduction and clustering using integrated transcription and chromatin accessibility information. We also utilized bioinformatic analysis with tools such as CellChat and Pando to infer inter- and intracellular interactions within the fetal utricle.
Results: We identified distinct clusters of hair cells, supporting cells, and transitional epithelial cells in the utricle at both gestational stages, each characterized by differentially expressed genes and accessible chromatin regions. At week 15, we discovered a population of proliferative

supporting cells, which we validated in situ. Comparative analysis between gestational weeks 15 and 19 revealed significant epigenomic changes. By correlating open chromatin regions with gene expression, we further determined the active transcription factors specific to each cell type and sub-type.
Conclusions: Our study produced the first single-nucleus multiomic atlas of the fetal human utricle, revealing cellular heterogeneity and gene regulatory networks. This work provides valuable insights into transcriptomic and epigenomic changes occurring in the utricle between gestational weeks 15 and 19. We anticipate that these findings will enhance our understanding of the human utricle and inform future research on human-specific factors that may drive hair cell regeneration.

T181. Effects of Unilateral Vestibular Loss on Roll Posture and Neural Activity

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Category: Vestibular: Basic Research & Clinical
Background: Maintaining balance is necessary for animal survival. However, the development of postural control remains poorly understood. The larval zebrafish points a way forward with its advantages of its small size, optical transparency, genetic tractability, and conserved vestibular circuitry and reflexes.
Methods: I have adapted both behavioral and optical approaches to study posture in the roll axis after injury. First, I can reliably target the vestibular nerve for unilateral photoablation, disrupting vestibular sensation. Next, I've pioneered analysis of roll posture as fish swim freely after injury.
Results: I found that fish roll to the side after unilateral lesion, but can improve postural stability with a recovery period. Thirdly, I can non-invasively monitor central vestibular neuron activity before and after injury to examine how different nuclei process vestibular signals.
Conclusions: This work reveals how bilateral input is used for postural computations during development.

T182. Human Vestibular Perception on a Curve: What is the Key Factor in Determining Heading Direction?

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Category: Vestibular: Basic Research & Clinical
Background: Previous work on vestibular translation perception has focused on perceptual thresholds and translation in a single direction. We studied curved trajectories in which direction and timing of peak acceleration were varied for both visual and vestibular stimuli to improve understanding of translation perception. Two hypotheses were investigated: 1) acceleration (vs. deceleration) is used to perceive heading; and 2) only supra-threshold acceleration (~1 cm/s/s) is considered.

Methods: Thirty-two healthy human subjects participated (mean age 25 ± 7 years, 25 female). Trials were unisensory and included either a visual stimulus (coherence 100%) or inertial stimulus. Both were a 2 s movement that covered 15 cm of displacement. After each stimulus presentation, subjects reported the direction of each trajectory as left or right of straight ahead in a two-alternative forced choice task. The stimuli were initially offset ±50° with a staircase designed to convert at the point of subjective equality. Three shaped trajectories were interleaved such that the start and end were similar. One of these trajectories was a straight line, while the other two followed the arc of a circle that was offset ±15° then returned to the same point as the straight trajectory. Acceleration trajectories were manipulated to occur in the initial 25% of the stimulus, in the initial 50%, or over the initial 75% of the stimulus. Thus, the peak velocity occurred 0.5, 1.0, or 1.5 s into the trajectory. Afterwards, the point of subjective equality (PSE) was compared between conditions and offsets to determine shifts in the PSE (bias).
Results: Optic flow stimuli were perceived based on the actual end point of the stimulus and were not influenced by curves or changing the timing when the acceleration occurred. However, inertial (vestibular) stimuli perception was significantly biased based on the initial direction of the curved trajectory. The mean bias relative to a straight trajectory was 5.4 ± 0.6° (mean±SD). The trajectory for which acceleration occurred in the initial 25% of the stimulus had a larger bias at 8.7 ± 0.7°. The bias decreased to 3.2 ± 1.0° when the acceleration occurred over the initial 75% of the stimulus . These perceived biases corresponded to the heading direction when the acceleration dropped below 1 cm/s/s which was previously established to be the approximate threshold of human vestibular perception.
Conclusions: Inertial heading was biased towards the initial curve suggesting that acceleration but not deceleration contributed to perceived heading. When timing of peak acceleration was varied, it became clear that heading direction at the time the acceleration passed below the perceptual threshold predicted the bias shift. These findings suggest that the effects of visual and inertial on heading perception are very different which has implications for integration of multisensory stimuli for navigation.

T183. Interactions Among Merlin, Arkadia, and SKOR2 Mediate NF2-Associated Human Schwann Cell Proliferation

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Category: Vestibular: Basic Research & Clinical
Background: NF2-related Schwannomatosis (previously referred to as Neurofibromatosis Type 2, or NF2) is a genetic-associated disease resulting from mutations in the gene, NF2. NF2 encodes the merlin protein, which acts as a tumor suppressor. Bilateral vestibular schwannoma (VS) is a hallmark of NF2. Although the exactly molecular mechanism mediating NF2-driven schwannomatosis is not fully understood, it is known that defective Merlin protein functionality leads to abnormal cell proliferation.
Methods: We utilized a human induced pluripotent stem cell (hiPSC)-based Schwann cell (SC) model to investigate the role of merlin in human Schwann cells (SCs). SCs were derived from hiPSCs carrying a NF2 mutation (c.191 T GREATER THAN C; p. L64P), its isogenic wild-type

control cell line, and a NF2 patient-derived hiPSC line. Phenotypes were determined via immunohistochemistry and various bioassays. Different proteins interacting with merlin in wild-type and NF2 mutation SCs were identified using co-immunoprecipitation followed by mass spectrometry.
Results: SC derived from NF2L64P hiPSCs showed significantly higher proliferation and abnormal morphology compared to NF2WT SCs. Moreover, interactome profiles of merlin (NF2) were different in SCs derived from NF2WT- and NF2L64P- hiPCSs. Among identified proteins, we validated the interactions among merlin, an E3 ubiquitin ligase (Arkadia), and a SKI family co-repressor (SKOR2) and revealed the roles of such interaction in the SC proliferation. Our findings were further validated by SCs derived from the patient-derived hiPSCs carrying a deletion in the chromosome 22 including the NF2 gene.
Conclusions: Our results established a new model in which merlin interacts with Arkadia and SKOR2 and this interaction is required for the proper activation of the SMAD-dependent pathway in TGFβ signaling.

T184. Effect of Ambiguity of Localization for Virtual Sound Source on Semicircular Canal-Ocular Reflex

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Category: Vestibular: Basic Research & Clinical
Background: This study is part of an investigation into multi-modal vestibular rehabilitation, aiming to enhance sensory integration across various modalities to improve vestibular function. Recent research has shown that auditory cues, particularly sound source localization, can influence vestibular responses such as the Semicircular Canal-Ocular Reflex (ScOR). In this study, we focus on how the accuracy of virtual sound source localization affects ScOR, contributing to the development of more effective rehabilitation methods.
Methods: Eleven healthy adults (4 females, mean age 27.0 ± 2.0 years; 7 males, mean age 25.0 ± 2.9 years) participated in two experiments. In Experiment 1, participants' ability to perceive the direction of virtual sound sources was assessed while wearing headphones and identifying sound source locations. In Experiment 2, horizontal eye movements were recorded in complete darkness using video-oculography during chair or sound source rotations. The chair or sound source rotated sinusoidally at 0.32 Hz with a 30-degree amplitude. A single virtual sound source was presented either in front or behind the participants. In the Earth Fixed (EF) condition, the sound source remained stationary relative to the environment during chair rotation. In the Head Fixed (HF) condition, the sound source rotated along with the head. In the Auditory Pursuit (AP) condition, the sound source moved independently of the head. Participants were instructed to focus on the sound location while keeping their eyes open during recordings. As a control, ScOR was measured without auditory input. The gain of eye movements in response to chair and sound source rotations was analyzed.
Results: No significant differences in ScOR gain were observed between the EF and HF conditions, nor between front and rear sound source positions, including in the AP condition. However, in the EF condition, sound localization accuracy was negatively correlated with ScOR

gain when the sound source was behind the participants. A similar negative correlation was found between sound localization accuracy and the gain of eye movements in the AP condition for rear sound sources.
Conclusions: Both front and rear sound sources had a comparable effect on inducing eye movements. Although ScOR gain did not differ significantly between conditions, the virtual sound source tended to increase ScOR, even in the HF condition, where ScOR is typically suppressed by visual targets. Difficulty distinguishing between front and rear sound locations may cause perceptual confusion. When the sound location is ambiguous, a moving sound source synchronized with head rotation could potentially arouse vestibular perception, rather than suppress the ScOR. This suggests that, under certain conditions, moving sound sources, particularly those located behind the participant, may have potential benefits in vestibular rehabilitation by enhancing sensory integration and response.
This work is supported by JSPS KAKENHI JP19K12088 and JP22K12119, Tateisi Science and Technology Foundation S2198001.

T185. Utricular Sensory Cells Exposed to 4-Hydroxynonenal Exhibit Senescence-Like Phenotypes

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Category: Vestibular: Basic Research & Clinical
Background: Probable vestibular migraine (PVM) was defined as partial fulfillment of the diagnostic criteria for vestibular migraine (VM). This means that the diagnostic certainty of VM is higher in the VM group than in the PVM group. We have previously reported that VM is associated with dysfunction of the utriculo-ocular pathway more frequently than PVM by examining ocular vestibular evoked myogenic potentials (Fujimoto C et al., J Neurol, 2020). Given that the VM group better reflects the pathophysiology of VM than the PVM group, it is possible that the pathophysiology of VM involves the function of the utriculo-ocular pathway. In the present study, we investigated the effects of 4-hydroxynonenal (4-HNE), an endogenous agonist of the transient receptor potential ankyrin 1 (Trpa1) channel, which has been proposed as a pathway in the pathophysiology of migraine, on utricular sensory cells.
Methods: Expression of TRPA1 in a vestibular cell line derived from the neonatal mouse utricle (University of Bristol/Utricular Epithelium-1, UB/UE-1) was assessed by immunostaining. 4-HNE administration was followed by cell counting kit-8 assay for viable cell count and trypan blue staining for dead cell count. Senescence phenotype was evaluated by Spider-β-gal activity. In addition, the expression of cell cycle regulators encoded by cyclin-dependent kinase inhibitor 1A (Cdkn1a), cyclin-dependent kinase inhibitor 1B (Cdkn1b), cyclin-dependent kinase inhibitor 2A (Cdkn2a) and transformation related protein 53 (Trp53) was evaluated by quantitative PCR (qPCR). The expression of Interleukin 6 (Il6), the senescence-associated secretory phenotype factor, was also evaluated by qPCR.
Results: Expression of TRPA1 was confirmed in the cytoplasm of UB/UE-1 cells. Spider-β-gal activity increased under 4-HNE-treated conditions, which inhibited cell proliferation without

increasing the number of dead cells. Under the same conditions, the expression of Cdkn1a, Cdkn1b, Cdkn2a, Trp53 and Il6 was increased.
Conclusions: UB/UE-1 cells exposed to 4-HNE exhibit senescence-like phenotypes.

T186. Deafness Progressing to CI Eligibility Is Eight Times More Likely in the Hypoplastic Than the Degenerative Endotype of Meniere's Disease

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Category: Vestibular: Basic Research & Clinical
Background: Meniere's disease (MD) is characterized by progressive sensorineural hearing loss, with varying dynamics and severity among patients. Approximately 10% of MD patients eventually develop profound hearing loss and become eligible for cochlear implantation (CI). In this study, we investigated predisposing factors for profound hearing loss and the likelihood of CI in MD patients. We focused on two previously defined disease endotypes, distinguished radiologically as having either a hypoplastic (MD-hp) or degenerated (MD-dg) endolymphatic sac.
Methods: Retrospective five-center cross-sectional cohort study. Inclusion criteria for "CI cohort": Definite MD (International Classification of Vestibular Disorders [ICVD], 2015) and a history of uni- or bilateral CI between 1996 and 2023. Inclusion criteria for "non-CI cohort": Definite MD and consecutively enrolled from 2010 to 2015. In both cohorts, patients were stratified according to endotype (MD-dg or MD-hp), using an established temporal bone CT imaging criterion, the "angular trajectory of the vestibular aqueduct". The endotype ratios (MD-hp : MD-dg) between the CI and non-CI cohorts were statistically compared.
Results: The CI cohort included 115 adult MD patients, who were identified from electronic medical records and reassessed using current diagnostic criteria of the ICVD. The non-CI cohort included 72 consecutively enrolled MD patients. The CI cohort included significantly more MD-hp patients than the non-CI cohort (72% vs. 24%, p LESS THAN 0.0001). The odds ratio of CI for an MD-hp patient relative to an MD-dg patient was 8.4 (95% confidence interval 4.3 to 16.1).
Conclusions: The MD-hp patient subgroup shows a significantly higher prevalence of CI, being 8.4 times more common compared to the MD-dg patient subgroup. Therefore, endolymphatic sac hypoplasia is strongly associated with severe hearing loss and eligibility for CI within the broader MD population. Since the endotype can be diagnosed at the onset of MD, often years to decades before hearing loss reaches profound levels, endotyping MD patients holds promise for personalized patient counseling, tailored audiological follow-up planning, and informed clinical decision-making regarding CI.

T187. Modulation of Vestibular Perception by Virtual Reality in Sitting and Standing Positions

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Category: Vestibular: Basic Research & Clinical

Background: Vestibular dysfunction is a known cause for falls, a contributor to both disability and death. Physical therapy is the ideal treatment for patients with vestibular dysfunction to improve symptoms and reduce fall risk. Many with vestibular dysfunction have limited mobility and dizziness, thus limiting access to traditional physical therapy. Therefore, identifying new effective vestibular physical therapy methods is crucial.

Virtual reality (VR) is technology that provides a digital simulation or environment. VR is commonly performed with headsets that cover the users' eyes and gives various simulations that users can interact with. This technology has demonstrated effectiveness in vestibular rehabilitation. Our goal was to trial VR in an assisted standing or sitting position of the body, allowing for a different way of controlling movement that could effectively stimulate the vestibular system.

Methods: Healthy subjects (N=30) were recruited for this pilot study. Subjects were randomly assigned to three groups containing 5 males and 5 females each: 1) VR intervention in standing position, 2) VR intervention in sitting position, and 3) mock intervention wearing headset in standing position. Sensory Organization Test on computerized dynamic posturography (Equitest NeuroCom) and the Simulator Sickness Questionnaire (SSQ) were performed before and after VR sessions. VR stimulus was delivered as an interactive "garage" environment through the Meta Quest 2 VR headset (Meta) and the C-Infinity locomotion device (NeuroSync Laboratories). Subjects explored this content for three minutes at two separate sessions several weeks apart. Test results before and after VR and between the first and second sessions were compared. This study was approved by the IRB at UTMB, Protocol 23-0218.

Results: VR sessions during standing evoked more sickness compared to sitting and mock intervention. Composite scores improved after VR sessions while no change was observed without VR. Largest improvement was observed in male participants after VR in the sitting position. Female participants who had lower scores at baseline improved significantly with and without VR.

Visual and vestibular sensory analysis scores improved after VR. Visual scores improvement after VR in the sitting position, whereas vestibular scores revealed improvement after VR in the sitting position. Overall, vestibular sensory analysis scores showed the most improvement.

SSQ showed worse sickness with VR in standing position compared to sitting position.

Conclusions: Our setup is safe and effective for modulating vestibular perception in healthy subjects. SSQ results showed subjective improvement in oculomotor perception with feelings of disorientation. Standing and sitting VR sessions can induce visual and vestibular stimulation. Subjects with poorer balance function at baseline showed greater change in their performance, while subjects with good performance at baseline had minimal change or worsening performance after VR. We plan to investigate with a larger cohort and in patients with vestibular dysfunction.

T188. Transcriptomic Characterization of the Vestibular Otolith Organ During Development and Screening of Key Transcription Factors for Type I Hair Cell Development

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Category: Vestibular: Basic Research & Clinical

Background: Single-cell sequencing enables sequencing of the genome or transcriptome at the level of individual cells, can accurately characterize the unique histology of individual cells, and can be an approach to address cellular heterogeneity of gene expression in tissues. The advantages of single-cell sequencing are all-encompassing and multilayered compared to conventional whole genome sequencing, which not only measures gene expression levels more precisely, but also detects trace amounts of gene expressors or rare non-coding RNAs. The aim of this study was to search for meaningful differential genes between hair cells and non-hair cells in the early mouse embryo, and to more precisely characterize the hair cell transcriptome during early vestibular development.

Methods: Mouse embryos from E13, E14, and E15 were dissected to obtain the vestibular ellipsoidal bursa after obtaining the auditory vesicles, and the stromal cells were sorted and sieved by flow cytometry, and the prepared cell suspensions were wrapped in a droplet of gel beads (bead) with a cell barcode sequence (cell barcode) and cells by using a microfluidic chip. In the droplets, the cells ruptured and the released mRNA was linked to the cell labeling sequences on the gel beads to form single-cell GEMs structures (Gel Bead in Emulsions). The rna-seq data of three hair cells were taken at each stage and compared with three non-hair cells.

Results: There were 406 up-regulated genes and 396 down-regulated genes in hair cells compared to non-hair cells on day E13; 790 up-regulated genes and 422 down-regulated genes in hair cells compared to non-hair cells on day E14; and 1025 up-regulated genes and 360 down-regulated genes in hair cells compared to non-hair cells on day E15. By KEGG analysis, differential gene enrichment was associated with nitrogen metabolism pathway, axon growth orientation, local adhesion, and cAMP signaling pathway. LBX2 transcription factor was specifically highly expressed in OCM-positive cells.

Conclusions: The gene expression difference between hair cells and non-hair cells in early vestibular period is huge, from the gradual increase of the number of down-regulated genes, it can be hypothesized that the vestibular hair cells are rapidly developing on days E13-E15, and the pathway analysis can reveal that these differential genes are closely related to the neurodevelopmental process. It also shows that single-cell sequencing can play an important role in characterizing the transcriptome of early vestibular hair cells.

T189. Fkbp5 May Control Glutamatergic Synaptic Transmission and Gabaergic Synaptic Transmission in the Vestibule

Yuichiro Tominaga*[1], Ryotaro Omichi[2], Yukihide Maeda[3]

[1]*Hiroshima City Hospital,* [2]*Okayama University Hospital,* [3]*Saitama Medical University Faculty of Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences*

Category: Vestibular: Basic Research & Clinical

Background: Steroids (glucocorticoids) are widely used as a therapy for acute vestibular disorders as first-line treatment. It is also known that there is a difference in improvement rates between sudden hearing loss patients with or without vertigo/dizziness. However, the molecular mechanisms of steroids in vestibular end organ remain unclear.
A better understanding of steroid action within the vestibule and the cochlea, particularly in sensory epithelium (hair cells and supporting cells), is crucial for developing targeted therapies for acute inner ear disorders.

Our previous research identified the Fkbp5 as a key player in glucocorticoid receptor signaling in the cytoplasm of the hair cells and supporting cells in the cochlea.

In this study, we analyzed the function of Fkbp5 in the vestibule (the utricle) by RNA-seq in a mouse model of acute sensorineural hearing loss using Fkbp5 knockout (KO) mice.

Methods: We used homozygous mutant female Fkbp5 KO mice at 5 to 7 weeks of age and C57BL/6J wild-type mice as controls. RNA was extracted from dissected tissues of sensory epithelium of the utricle. (RIN value GREATER THAN 7.0).; Differential expression analysis, biological pathway analysis, and protein-protein interaction database analysis were performed on the RNA-seq data.
Results: Differential expression analysis revealed significant changes in 1698 genes (616 up-regulated and 1082 down-regulated) in the Fkbp5 knockout group. Among them, the gene expression of functional pathways of glutamatergic synaptic transmission, endocannabinoid-induced retrograde synaptic transmission and GABAergic synaptic transmission were significantly altered.
Conclusions: GABA is a major inhibitory neurotransmitter in the central nervous system implicated in neurological and psychiatric disorders and plays an important role in neuronal death and survival.
We hypothesized that Fkbp5 may regulate neuronal growth/decay through GABA expression modulation.

Glutamatergic synapses also occur in the inner ear, where glutamate is released from hair cells as a result of sound input to the spiral ganglion neurons (SGN) that connect the cochlear nucleus to the brainstem. The SGN functions as an afferent ganglion in the peripheral auditory pathway, and the majority (approximately 95%) of SGN neurons form a ribbon synapse with the inner hair cells.

Since excessive glutamate production is thought to cause damage to surrounding neural tissue, it is possible that Fkbp5 may act protectively against vestibular damage by preventing the release of glutamate.

Fkbp5 may control glutamatergic synaptic transmission and GABAergic synaptic transmission in the utricular sensory epithelium of the vestibule. This study sheds light on the molecular

mechanisms underlying ASNHL onset, advancing our understanding of its pathophysiology. This may contribute to the development of personalized medicine and novel treatment strategies.

T190. The Cellular and Molecular Architecture of the Mammalian Vestibular System

Meghna Kolluri*[1], Paula Fontanet[1], Charles Petitpre[1], Csaba Adori[1], Prach Techameena[1], Haohao Wu[1], Shih-Hsin Chang[1], Beatriz Del Blanco[2], Angel Barco[2], Saida Hadjab[1], Francois Lallemend[1]

[1]*Karolinska Institutet,* [2]*Universidad Miguel Hernandez*

Category: Vestibular: Basic Research & Clinical
Background: Our sense of balance is governed by the vestibular system located in the inner ear. It consists of three semi-circular canals (SCCs) and two otolith organs i.e. the saccule and the utricle. In each organ, the sensory epithelium harbors the mechanosensory vestibular hair cells (VHCs) that detect head movements. The mechanical stimuli, generated upon head movement, are converted to electrical signals that are relayed to higher processing centers in the hindbrain via vestibular afferent neurons (VANs). Although the diversity of VHCs and VANs, with respect to their morphology and physiology, has been described for decades, their molecular classification, and therefore functional organization, has remained unclear.
Methods: Using single cell RNA-sequencing, we reveal a more complex diversity of neurons and hair cells. To characterize the morphology and distribution of the new VAN and VHC subtypes, we use various immunohistochemical and viral tracing methods, and 3D tissue imaging with light-sheet microscopy. The VANs were further characterized by investigating their electrophysiological properties in vitro.
Results: Distinct patterns of innervation at the periphery and centrally as well as characteristic spiking patterns indicate that individual molecular subtypes of VAN may represent functionally diverse cell types that contribute to different aspects of balance and gaze stabilization. Additionally, we show that specific VHC subtypes may also show distinct patterns of distribution within subregions of the sensory epithelium thus adding another layer of complexity to the perception and processing of vestibular behaviors.
Conclusions: By showing morphologically and functionally distinct VHC and VAN subtypes, our research aims to delineate the cell type-specific network architecture of VHCs and VANs and elucidate the multiple pathways that produce an integrated vestibular motor output and provide a basis for pharmacological research and potential drug targets in vestibular disease conditions.

T191. Comparative Assessment of Imaging Modalities for Cochlear Visualization Through Temporal Bone in an Ex Vivo Porcine Model

Akil Turner*[1], Mikalai Budzevich[2], Savannah Gladd[1], Xiao Xia Zhu[3], Bo Ding[3], Robert D. Frisina[3], Parveen Barzard[3]

[1]*Univeristy of South Florida,* [2]*H Lee Moffitt Cancer Center, SAIL,* [3]*.Global Ctr. for Hearing and Speech Res., University of South Florida*

Category: Other

Background: Imaging the cochlea non-invasively clinically remains a significant challenge due to the dense temporal bone encasing it and the lack of viable delivery methods of contrast agents to the inner ear. Current imaging modalities, including PET, MRI, and near-infrared (NIR) fluorescence, offer distinct advantages but are variably limited by bone penetration, signal attenuation, and spatial resolution. This study aims to compare these three imaging modalities and determine their effectiveness in penetrating/visualizing the temporal bone for cochlear imaging. Porcine temporal bones were selected as a model due to their anatomical similarity to human temporal bones, providing a relevant preclinical platform. By analyzing these modalities, we seek to help inform the selection of optimized imaging techniques for clinical use in diagnosing and monitoring inner ear disorders non-invasively.

Methods: 2 Porcine skulls were ordered from Animal Biotech Industries (Doylestown, PA) and prepared by physically removing most of the soft tissue. They were then fixed in 4% PFA and stored at 4°C. Before imaging, the bone was further dissected to include just the surrounding area around the cochlea. A small hole was drilled through the internal auditory canal (IAC) into the cochlea to allow the injection of various contrast agents. PET was used with radiolabeled agents such as F-18, MRI with contrast agents optimized for low water content such as Isovue, and NIR fluorescence with fluorescent agents such as Indocyanine Green. Signal penetration was measured, and the spatial resolution and signal quality were compared across modalities. The study sample included multiple porcine temporal bones (n=3) with consistent preparation and agent injection to ensure reproducibility.

Results: PET demonstrated high sensitivity in detecting radiolabeled agents, but spatial resolution was limited in visualizing the separation in the cochlear chambers due to their small size. MRI provided clear signal penetration, even with low concentrations of contrast agents, as attenuation is not a factor when providing images. However, the absence of soft tissue limited its application in visualizing detailed structures, which is expected ex vivo. NIR fluorescence shows potential in that it is highly modular to allow for various targeting/sensing mechanisms, but fluorescence scattering through the bone led to diminished accuracy and resolution, indicating the need for further optimization of fluorescent agents and techniques. These findings suggest that PET may be useful for high-sensitivity applications, while MRI provides superior signal clarity in specific scenarios. NIR fluorescence requires further refinement before it can be effectively applied in clinical settings.

Conclusions: These findings may have important implications for otolaryngology, particularly in selecting imaging modalities for future non-invasive cochlear diagnostics and monitoring. Future research will focus on refining imaging parameters, and exploring viable delivery techniques of applicable contrast agents, and the optimal combinations of these techniques for imaging inner ear structural and functional properties.

T192. Exploring the Regulation of Myosin-15 by Whirlin in Mechanosensory Stereocilia

Zane Moreland[1], Matthew Petrides[1], James Heidings[1], Juan Guan[2], Linda Bloom[1], Jonathan Bird*[1]

[1]*University of Florida,* [2]*University of Texas at Austin*

Category: Hair Cells: Anatomy & Physiology

Background: Stereocilia are actin-rich protrusions organized into bundles with ranks of ascending height, forming a staircase-like architecture crucial for auditory mechanotransduction. This precise arrangement depends on the trafficking of the elongation complex (EC), a group of proteins transported to stereocilia tips by the molecular motor myosin-15 (MYO15A). Mutations in MYO15A disrupt this structure, leading to human hereditary hearing loss, DFNB3. EC proteins, including MYO15A, are highly concentrated at stereocilia tips, prompting the question: why do they accumulate there, and what do they do? We recently found that MYO15A can directly nucleate actin filaments, a process requiring high local concentrations of the protein. Recent research has also revealed that EC proteins, including whirlin (WHRN), can undergo liquid-liquid phase separation (LLPS) and concentrate MYO15A into biomolecular condensates. We hypothesize that this LLPS behavior facilitates MYO15A's role in actin nucleation within stereocilia.

Methods: To investigate the role of LLPS in stereocilia biology, we purified recombinant full-length MYO15A and the EC component, whirlin (WHRN). We tested WHRN at concentrations ranging from 1 to 10 μM and salt concentrations ranging from 50 to 200 mM NaCl to assess condensate formation. Confocal imaging was used to observe condensate fusion on glass, and fluorescence recovery after photobleaching (FRAP) was performed to assess condensate dynamics. Additionally, microrheology experiments are being developed to probe condensate viscosity by introducing polystyrene beads within them and tracking their motion in the harmonic potential of an optical trap.

Results: WHRN was observed to robustly form spherical liquid condensates, with phase separation sensitive to protein concentration and ionic strength. At concentrations as low as 1 μM and in 50 mM salt, WHRN still formed condensates, though less frequently than at higher concentrations, and this process was further diminished as salt concentrations increased. These condensates readily fused on glass in confocal experiments and exhibited rapid fluorescence recovery after photobleaching (FRAP); hallmarks of liquid-like condensate behavior. The salt sensitivity, fusion behavior, and rapid FRAP recovery support the conclusion that WHRN condensates are forming a liquid phase.

Conclusions: We have established an experimental system to study the physical properties of condensed EC proteins, focusing on WHRN's ability to form liquid condensates. Our current efforts are centered on adding purified MYO15A to these condensates to investigate how MYO15A integrates into WHRN condensates and whether this interaction influences its role in actin nucleation and enzymatic activity. These studies will provide key insights into how EC protein condensates regulate stereocilia structure and function.

T193. Cognitive Biases Cause an Overestimation of Vestibular Direction-Recognition Perceptual Thresholds

Elena López-Contreras Gonzalez[1], Torin Clark[2], Faisal Karmali*[3]

[1]Massachusetts Eye and Ear, [2]Smead Aerospace Engineering Sciences Department, University of Colorado Boulder, [3]Harvard Medical School
Category: Vestibular: Basic Research & Clinical
Background: There has been tremendous interest in the use of perceptual thresholds to understand the physiology and pathophysiology of the vestibular system, and as a potential clinical tool. For example, these thresholds vary with pathology and age, and are highly correlated with postural performance. These thresholds are typically assayed by a sequence of trials, each consisting of a stimulus (i.e., chair motion) and a perceptual response (e.g., left or right judgment). As with other sensory systems, these vestibular perceptual judgments exhibit serial dependence. That is, the response for each trial is affected by the stimulus of the current trial and responses in preceding trials. Prior work has shown that this serial dependence can broadly affect the psychometric curve fit. More specifically, we recently showed that this serial dependence affects the direction-recognition threshold estimate. We found that it caused an overestimation of threshold in a small group of subjects experiencing yaw motion (Gonzalez, Elena Lopez-Contreras, Susan A. King, and Faisal Karmali. "Your Vestibular Thresholds May Be Lower Than You Think: Cognitive Biases in Vestibular Psychophysics." American Journal of Audiology 32.3S (2023): 730-738.)
Methods: We now report new results about the hypothesis that cognitive biases cause thresholds to be overestimated. We determined the theoretical predictions for overestimation as a function of dependence on preceding responses, finding that overestimation occurs whether subjects respond in the same or opposite direction as the preceding response. We also analyzed additional experimental data from subjects experiencing roll tilt, pitch tilt, interaural (lateral) translation and yaw rotation from multiple open-source datasets. We also analyzed visual and auditory datasets.
Results: We found that experimental results did not differ from theoretical predictions for all motion directions (Chi-squared test, $p>0.05$). Thresholds were overestimated by up to 40%. Results were similar for data collected using both adaptive and non-adaptive stimulus selection.
Conclusions: Since the results indicate that the magnitude of cognitive bias varies across subjects, this enhanced model can reduce measurement variability and potentially improve the efficiency of data collection.

Supported by NIH/NIDCD 1R01DC018287.

Mini-Podium 3: Human Inner Ear Anatomy: Techniques

3:00 p.m. - 4:00 p.m.

Ocean Ballroom 5 - 8

Moderators: Alicia Quesnel and amp; Nathaniel Nowak

Automatic Pre-Operative Scalae Segmentation and Quantification Using Synchrotron Radiation Phase-Contrast Imaging

3:00 p.m. - 3:15 p.m.

Ashley Micuda*[1], Daniel Newsted[1], Sumit Agrawal[1], Hanif Ladak[1]

[1]*Western University*

Background: Variations in cochlear morphology have significant implications for cochlear size, scalar size, and tonotopic distributions. Previous efforts to perform automatic scalar segmentation and quantification in clinical scans, using atlases and statistical shape models, have been limited by the artefacts associated with histology and the lack of soft-tissue contrast in micro-CT scans used for ground truth data. Understanding the relationship between cochlear morphology and cochlear tonotopy can aid in the planning of atraumatic and individualized cochlear implantation. By utilizing synchrotron radiation phase-contrast imaging (SR-PCI), high resolution scans of intact cadaveric cochleae can be obtained which include accurate bone and soft-tissue visualization of relevant cochlear micro-anatomy through the entire length of cochleae.
Methods: One hundred fixed, intact cadaveric cochleae were scanned with various resolutions and scanner types including cone-beam CT reconstructed at 0.1 mm and 0.3 mm isotropic; helical CT reconstructed with bone and soft tissue protocols; and with SR-PCI. In each SR-PCI scan, accurate ground truth segmentations (i.e. delineations) were obtained of the round window, scala tympani, scala vestibuli, and spiral ligament from the cochlear base to the apex. Deep learning algorithms were developed to obtain complete scalae segmentation, cochlear measurements, and tonotopic distributions in clinical scans at each angular location within the cochleae. Automatic segmentation was performed on 50 paired pre- and post-operative cochlear implant recipient scans to examine the location of each contact relative to the scala tympani segmentation.
Results: In the automatic segmentations obtained from the cadaveric clinical scans, cochlear length, cochlear angular length, scalar cross-sectional area, and scalar diameter were assessed at each angular depth from the cochlear hook region to the apical most tip of the helicotrema. When compared to the SR-PCI ground truth segmentations, the automatic segmentations achieved high accuracy (measured using the Dice similarity coefficient) in all scan types and at angular insertion depths associated with long cochlear implant electrodes. In the automatic segmentations obtained from patient scans, translocation was assessed at each electrode contact.
Conclusions: Accurate automatic scalar segmentation and measurement of both cadaveric and patient clinical scans has been performed. The statistics of each measurement obtained have provided insight into cochlear variability and individualization purposes.

Characterizing the Human Membranous Labyrinth Position Within the Lateral Semicircular Canal: A Histologic Analysis and Proposal for Pathology-Guided Classification

3:15 p.m. - 3:30 p.m.

Koffi Lakpa*[1], Rafael da Costa Monsanto[2], Maurizio Falcioni[3], Sabrina Huang[1], Gisselle Garcia[1], Yazeed Qahadad[1], Andrew Fishman[4], Józef Mierzwiński[5], Arnaldo Rivera[6], Sebahattin Cureoglu[2], Michael Puricelli[1]

[1]*University of Wisconsin School of Medicine and Public Health,* [2]*University of Minnesota Otopathology Laboratory,* [3]*Azienda Ospedaliero-Universitaria di Parma,* [4]*University of*

Missouri, ACIBADEM BelMedic Clinical Center, [5]Children's Hospital of Bydgoszcz, Audiology and Phoniatrics, Pediatric Auditory Implant Program, Nicolaus Copernicus University, Collegium Medicum. Ul. Łukasiewicza, [6]School of Medicine, Missouri University

Background: Multiple classifications for labyrinthine fistulas of the lateral semicircular canal have been proposed, however, the position of the membranous labyrinth relative to the endosteum and bony canal differ. No histologic study has clarified the position of the membranous labyrinth within the bony labyrinth. Therefore, the distance between membranous and bony labyrinths within the lateral semicircular canal was measured and a histologic-based classification model of semicircular canal fistula is proposed.
Methods: Histological samples of a total of 60 human temporal bones with and without chronic otitis media were examined microscopically. The distance from the membranous labyrinth to the endosteum of the bony labyrinth was recorded.
Results: The membranous labyrinth abutted the bony labyrinth in 93% of both control samples and chronic otitis media samples. In the control group, the average distance of the membranous portion from the bony portion was 0.10±0.05 mm, while in the chronic otitis media group it was 0.37±0.03 mm. These distances were not statistically significant. Depending on the location of the LSCC fistula, dome or lateral wall, these new anatomical findings may have an important application for surgical decision-making during cholesteatoma surgery with LSCC fistulas.
Conclusions: Our key takeaway messages are that 1) the membranous labyrinth directly abutted the bony labyrinth in most of our samples. Conversely, current classification for semicircular canal fistulas assumed a central location of the membranous labyrinth within the bony labyrinth of the lateral semicircular canal. 2) Therefore, based on our findings, we propose a new LSSC fistula classification system that considers not only our histologic findings but also potential location of fistulas within the LSCC, such as the dome or lateral wall. We propose a histologic classification system for fistulas located at the dome of the LSCC, Grade I: Partial endosteum erosion, intact membranous labyrinth; Grade II: Complete endosteum erosion, ruptured membranous labyrinth. For fistulas located at the lateral wall of the LSCC, Grade I: Partial endosteum erosion, intact membranous labyrinth; Grade II: Complete endosteum erosion, intact membranous labyrinth; Grade III: Complete endosteum erosion, ruptured membranous labyrinth. Lastly, to further determine the surgical implications of our study, we think a potential future direction of this work may be to analyze the inner ear with higher resolution imaging modalities or assess the LSCC structures through dye-based imaging in an animal model.

Machine Learning Pipeline for Human Spiral Ganglion Neuron Quantification, Demonstrated on an Adult and Infant With Consecutive Serial Sections

3:30 p.m. - 3:45 p.m.

Christopher Giardina*[1], Jennifer O'Malley[2], Anbuselvan Dharmarajan[2], M. Charles Liberman[2], Julie Arenberg[2], Alicia Quesnel[3]

[1]*Harvard Medical School,* [2]*Eaton-Peabody Laboratories, Mass Eye and Ear, Harvard Medical School. Boston,* [3]*Massachusetts Eye and Ear Infirmary, Harvard Medical School. Boston*

Background: The quantity, integrity, and distribution of spiral ganglion neurons (SGNs) throughout the cochlea greatly influence speech and hearing ability. In subjects receiving cochlear implants, SGNs are of particular importance because they are the primary target for electrical stimulation. Many research groups have thus attempted to correlate audiometric outcomes with SGN counts, often using distinct approaches to quantify SGNs.
The purposes of this study were to 1) develop a standardized and automated SGN quantification pipeline that uses machine learning (ML) to identify SGN nuclei on digitized images of temporal bone (TB) sections, 2) present the SGN distribution from an adult and infant TB where every section was stained and counted, and 3) determine the minimum number of sections necessary to accurately predict overall SGN count and frequency distribution in a given TB.

Methods: In one adult and one infant postmortem TB, consecutive serial sections (401 and 397, respectively) of 20 um thickness were stained with H and E and digitized. Three mid-modiolar slices in each case were hand-segmented to label SGN nuclei using DragonFly 3D software, and then used as training data to create UNet and UNet++ models for SGN detection. These models were then applied to all other slices in each TB and compared to counts by three experienced observers. The serial images were then aligned in Matlab, and SGNs were assigned characteristic frequencies by applying a modified Greenwood formula to a spiral representation of Rosenthal's Canal. Equally spaced images were then removed from the stack (leaving every 2nd, 3rd, 5th, 10th, or 20th) to determine the section sampling interval needed to ensure overall accuracy.
Results: Segmentation models to automatically identify SGN nuclei from digital image stacks performed with great accuracy: in the adult SGNs were counted in 191 sections with an accuracy of 98% compared to manual counts, and in the infant SGNs were counted in 187 sections with an accuracy of 95%. When images were removed from the stack, counting only on every 10th section was sufficient to ensure a median inferred count accuracy of 98%, with minimal variance in inferred count depending on specific starting section.
Conclusions: We present for the first time the full 3D visualization of all SGNs in a complete serial-section series from an infant and an adult human cochlea. Down-sampling to just every 10th section was sufficient to infer the total count. We also present a flexible ML pipeline for identifying SGNs within digitized human TBs that can be adapted to sections with varied staining intensity. As the Mass Eye and Ear digital TB collection becomes more readily available among the broader researcher community through the NIDCD Registry, knowledge and tools to utilize these digital images will become increasingly valuable.

Multiplex Immunofluorescence Staining of Human Inner Ear Celloidin Embedded Sections

3:45 p.m. - 4:00 p.m.

Ivan Lopez*[1], Achilles Kanaris[1], Gail Ishiyama[2], Akira Ishiyama[2]

[1]*David Geffen School of Medicine at University of California, Los Angeles,* [2] *University of California, Los Angeles*

Background: Conventional immunohistochemistry and immunofluorescence (IF) staining has been applied successfully to human cochlea celloidin sections allowing the localization of one or two cellular markers. Given the limited availability of inner ear human sections, the application

of multiplexed staining techniques allowed simultaneous detection of multiple markers in a single section to study cell composition, cellular and functional cell-cell interactions. A simple protocol to identify several cellular markers in the same human cochlea section is presented in this study.
Methods: Twenty-micron cochlea celloidin-embedded sections from 8 temporal bones (4 male, 4 female, age 50-70 years old) were used in this study. Post-mortem time for the temporal bone harvesting was between 12 to18 hours. Sections were immersed in heated antigen retrieval solution (antigen unmasking basic solution, Vector Labs). Sections were incubated for 8 minutes in a diluted trypsin solution (1:3, Trypsin Kit, Abcam). Sections were incubated for 1 hour with a blocking solution containing 0.5% bovine serum albumin fraction-V (Sigma) and 0.5% Triton X-100 (Sigma) in phosphate buffered saline solution (PB. Followed by the incubation with the primary antibodies against acetylated tubulin mouse monoclonal antibodies 1:1000, and polyclonal antibodies against S-100B (1:500). After 72 hours of incubation (4ºC), primary antibodies were washed with PBS. Sections were incubated with goat anti-mouse antibody labeled with Alexa 594 and goat anti rabbit labeled with Alexa 647 (both 1:1000 in PBS, Invitrogen). Tissue sections were washed with PBS and coverslip with aqua soluble mounting media containing DAPI (Vectashield, Vector). Digital fluorescent microscopic images (20x objective) were acquired using a Leica (SP8) high-resolution light-sheet laser confocal microscope. For the second round of immunostaining the coverslips and mounting media were removed, and primary and secondary antibodies were eluted from the sections using the VectaPlex antibody removal kit (Vector Labs). Removal of IF staining was verified under the fluorescent microscope. After PBS washes, a blocking solution was applied for 1 hour and the second set of primary antibodies directly labeled with Alexa 488 (mouse monoclonal antibodies against beta-3-tubulin) were incubated for 48 hrs. Digital images were acquired thereafter
Results: Acetylated tubulin allowed the identification of spiral ganglia neurons (SGNs) and fibers, supporting cells in the organ of Corti, and the spiral prominence in the stria vascularis. Beta-3-tubulin was confined to the SGNs and fibers. S-100-beta allows visualization of satellite cells in the spiral ganglia and fibrocytes in the spiral ligament. In spite of the multistep labeling steps, tissue sections were well preserved.
Conclusions: This multiplex method allowed the visualization of several antigens of the different species in the same cochlea section. The application of this methodology has the potential for the identification of cellular pathways in different cellular compartments.

Mini-Podium 4: Auditory Cortex: From Inhibitory Networks and Signal-in-Nose Detection to Categorization and Loudness Perception

3:00 p.m. - 4:00 p.m.

Ocean Ballroom 9 - 12

Moderators: Maria Geffen and Omer Zeliger

Recurrent Inhibitory Networks in Layer 1 of the Mouse Primary Auditory Cortex

3:00 p.m. - 3:15 p.m.

Lucas Vattino*[1], Maryse E. Thomas[1], Cathryn MacGregor[2], Christine Junhui Liu[3], Carolyn G. Sweeney[1], Anne Takesian[1]

[1]*Eaton-Peabody Laboratories, Massachusetts Eye and Ear, Harvard Medical School,* [2]*Eaton-Peabody Laboratories, Massachusetts Eye and Ear,* [3]*Graduate Program in Speech and Hearing Bioscience and Technology, Harvard Medical School; Eaton-Peabody Laboratories, Massachusetts Eye and Ear*

Background: Inhibitory interneurons in layer 1 (L1-INs) of auditory cortex (ACx) regulate cortical plasticity and learning (Pi et al., 2013; Abs et al., 2018; Takesian et al., 2018), but the long-range and local cortical inputs that control the activity of these INs have not been characterized. L1-INs can be subdivided into two major classes by the expression of either neuron-derived neurotrophic factor (NDNF) or vasoactive intestinal peptide (VIP). These INs are thought to integrate sensory and neuromodulatory signals from the diverse long-range axons that populate L1. L1-INs also make inhibitory synaptic contacts with their neighbors and are connected electrically through gap junctions, suggesting that they may form complex networks. Here, we mapped the local circuit organization of specific L1-INs subclasses, together with the thalamic inputs that recruit these L1-INs in mouse ACx.
Methods: In this study we combined rabies virus-based monosynaptic tracing with fluorescent guided in vitro whole-cell electrophysiology and optogenetic stimulation to describe the functional thalamic inputs onto L1-INs. We also performed in vivo two-photon calcium imaging and holographic optogenetic stimulation of neuronal ensembles in the mouse ACx to understand how specific sensory inputs recruit these L1-INs.
Results: Our anatomical tracing shows that the vast majority of auditory thalamic inputs to these L1-INs unexpectedly arise from the ventral subdivision of the medial geniculate body (MGBv), the tonotopically-organized primary auditory thalamus. These L1-INs receive robust functional monosynaptic MGBv inputs that are comparable in strength and short-term plasticity to those in excitatory pyramidal neurons within thalamorecipient cortical layer 4. Activation of these thalamic axons drives robust feed-forward inhibition onto both L1-IN subtypes, but differences in feed-forward inhibition between these subtypes suggests that they receive distinct sources of inhibitory inputs. Accordingly, we found that GABAA-mediated synaptic connections between NDNF-INs were significantly stronger than those between VIP-INs or other L1-INs, suggesting a robust recurrent inhibitory network within the NDNF-IN subpopulation. To understand how the connectivity between neighboring NDNF L1-INs impacts in vivo sound processing, we activated ensembles of NDNF L1-INs in awake mice while recording the activity of the NDNF L1-IN network. Strikingly, activation of small NDNF L1-INs ensembles (LESS THAN 7 INs) can significantly modulate the sound-evoked responses of the NDNF L1-IN population.
Conclusions: Together, our findings show that L1-INs are targeted by tonotopically organized projections within the auditory thalamus, suggesting a novel role in auditory sensory processing for these INs in sound processing. Moreover, these L1-INs are embedded within complex local networks with distinct synaptic connectivity patterns that may powerfully shape their coordinated activity in response to sensory signals.

Enhancing Signal-In-Noise Detection by Learning-Induced Plasticity Mechanisms in the Auditory Cortex

3:15 p.m. - 3:30 p.m.

Nilay Atesyakar*[1], Andrea Shang[1], Kasia Bieszczad[1]

[1]*Rutgers University*

Background: Auditory learning experiences can enhance signal-in-noise detection performance. Moreover, associative learning induces signal-specific physiological changes to receptive fields in auditory cortex (ACx) (Bieszczad and Weinberger, 2010; Weinberger 2015). As such, neurophysiological plasticity induced by associative learning may benefit ACx signal processing in noisy backgrounds relative to novel sounds. Thus, learning-induced changes in receptive fields for specific sound signals may selectively promote ACx processing in noisy backgrounds to facilitate sound-cued adaptive behaviors. Sound segregation from background noise constitutes a major problem especially for older listeners, and particularly for those at risk for age-related cognitive decline. A comparison of ACx function in noisy backgrounds in young vs. aged adult brains was examined to test the hypothesized biological links between learning-induced benefits to auditory system function, and their dysfunction in age-related comorbidities.
Methods: To study learning-induced changes to ACx receptive fields in noisy backgrounds, rats were trained in a sound-reward associative task (5.9 kHz, 60 dB SPL pure tones signaled availability of
rewards) followed by in vivo ACx electrophysiological recordings. Following training, behavioral and ACx responses to the signal vs. non-signal tones presented under different signal-to-noise ratios (SNR) were determined. We examined how responses to sounds presented under different SNRs differed in young vs. aged rats vs. in genetic knock-in rats modified with a human Alzheimer's Disease (AD) gene (the early-onset AD Swedish mutation to Amyloid Precursor Protein).

Results: Computational analyses of sound-evoked ACx responses were used to derive a "neurometric" for decoding success of the signal tone in silence vs. noise. Learning induced a change in sound-evoked ACx activity that enhanced responding to the behaviorally relevant sound. However, in a noisy background, only the best performing rats showed differential ACx and behavioral responses to the signal tone vs. nearby novel tones (paired samples t-test, t(111) = -2.869, **p = 0.005) [Best learners: n(rat) = 7, n(site) = 112; Poor learners: n(rat) = 6, n(site) = 69; Naïve: n(rat) = 7, n(site) = 94); Others p GREATER THAN 0.17]. Comparing ACx responses of naïve young animals to aged animals (i.e. both AD-gene and humanized-genetic control rats) in noise revealed a deficit in the observed learning-induced benefit to signal-in-noise processing.
Conclusions: This work highlights the impact of learning experiences on enhancing signal detection via an auditory cortical decoding mechanism. By comparing the auditory processing capabilities of young naïve rats with those of aged models, we aim to elucidate the biological connections between auditory function and age-related dysfunction including cognitive decline. This research holds potential implications for developing strategies to mitigate the effects of hearing loss on cognitive health in older adults. Ultimately, by exploring these mechanisms, we can better understand the challenges faced by older listeners in noisy environments and pave the way for future interventions informed by basic science.

Neuronal Correlates Underlying Auditory Categorization and Bias in Mice

3:30 p.m. - 3:45 p.m.

Anjali Sinha*[1], Jared Collina[1], Gozde Erdil[1], Maria Geffen[1]

[1]*University of Pennsylvania*

Background: Categorization of sensory stimuli forms the basis for higher order cognitive processing. Categorization typically relies on learning an abstract set of modifiable rules. This processing is essential for various function including learning, perception, and prediction. However, we do not fully understand how different neuronal cell types and different areas of the brain perform auditory categorization.

Categorization is often based on prior information and experience and can be modified by new information. Recent work from our laboratory found that during categorization learning, animals use individual strategies and preferences to categorize new stimuli (Collina et al., 2024, BioRXiv). These strategies affect the animals' perception of stimuli category under uncertain conditions for stimuli that surround the categorization boundary. Additionally, animals modified these strategies based on their training. To further understand neuronal correlates of auditory categorization, we recorded neuronal activity from the auditory cortex of mice while they performed categorization task and analyzed the neuronal population responses.

Methods: We trained mice to discrimination between high and low frequency tones. Once their behavior reached criterion performance, we tested how well they categorized tones around the categorization boundary, and how this boundary was affected by biasing the distribution of the stimuli toward high or low frequency tones. We recorded neuronal activity using chronically implanted high-density Neuropixel silicon probes.

Results: Mice were trained on extreme frequencies to set up the categorization task. Our preliminary data suggests that mice can reach 80% accuracy in 6-10wks. Once they reached the criterion, mice were tested on categorization for tones around the frequency categorization boundary. On subset of sessions, we biased animals towards low category or high category frequencies by over-representing high or low frequency tones. To collect neuronal activity, animals are chronically implanted with Neuropixel probe after they reach 80% accuracy on training trials. For our preliminary data, we were able to collect neuronal activity from hundreds of single units in auditory cortex, while presenting broadband noise stimuli or pure tone frequencies.

Conclusions: We are able to consistently train mice on 2AFC task. Additionally, we are recording from hundreds of single units while mice perform task and track the changes in population activity over time.

Cortical Determinants of Loudness Perception and Loudness Hyperacusis: Mechanisms to Treatments

3:45 p.m. - 4:00 p.m.

Kameron Clayton*[1], Matthew McGill[1], Bshara Awwad[1], Kamryn Stecyk[1], Korey Sudana[1], Caroline Kremer[1], Desislava Skerleva[1], Divya Narayanan[1], Jennifer Zhu[1], Kenneth Hancock[1], Sharon Kujawa[1], Elliott Kozin[1], Daniel Polley[1]

[1]*Eaton-Peabody Laboratories, Massachusetts Eye and Ear*

Background: Where and how central auditory neurons transition from representing acoustic sound level to the perceptual quality of loudness is unknown. In auditory cortex (ACtx), a large fraction of single units exhibit tuning to a narrow range of sound levels sculpted by the strength and timing of intracortical inhibition. Intracortical inhibition in turn arises from a heterogeneous class of GABA-expressing neurons with distinct biophysical properties and post-synaptic connectivity. The most numerous are the Parvalbumin-positive fast-spiking interneurons (PVNs), which regulate excitatory neuron firing rates through powerful perisomatic synapses. Here, we identify a surprisingly simple cortical code for loudness that is regulated by local PVNs. Further, we show that PVNs are both a source and solution for loudness hyperacusis after noise-induced sensorineural hearing loss.

Methods: High-density single-unit recordings were made in mouse ACtx to study population responses as a function of sound level, with and without optogenetic PVN activation or inactivation (N = 8 mice, n = 721 sound-responsive regular spiking units). Next, we trained mice to perform a 2AFC loudness classification task while bidirectionally manipulating ACtx PVN activity bilaterally through the cleared skull (Arch: N = 5, ChR2: N =12). Noise exposure (16-32kHz, 103dB SPL) was used to induce a restricted high-frequency cochlear lesion, following which changes in ACtx sound encoding and loudness classification were quantified.

Results: While ACtx single units showed heterogenous sound level tuning, summing sound-evoked spike rates across the population revealed a simple encoding scheme where increases in level were accompanied by linear increases in population spiking. Linear sound level representations in RS units were suppressed by PVN activation and elevated during PVN inactivation. Behaviorally, mice were desensitized to loudness by ACtx PVN activation and hypersensitized by PVN inactivation, causally linking ACtx PVN activity to loudness perception. Following SNHL, ACtx RS units were hyper-responsive to spared sound frequencies and hypo-responsive to optogenetic PVN activation, which gave rise to behavioral loudness hypersensitivity. PVN activation during sound presentation reversed loudness hypersensitivity, but only transiently. In contrast, a brief 15-minute period of 40Hz PVN stimulation, but not 1Hz stimulation, could chronically reverse loudness hyperacusis for up to one week.

Conclusions: We found that ACtx PVNs functioned like a volume knob, turning neural and behavioral reporting of loudness up or down over a 20dB range. While SNHL induced PVN hypofunction, 40Hz optogenetic stimulation could rekindle PVN-mediated inhibition and reverse behavioral hypersensitivity, despite myriad irreversible changes in the cochlea and subcortical auditory pathway. Our ongoing work seeks to characterize relationship between ACtx population activity levels and loudness perception at the single-trial level by measuring loudness classification during two-photon calcium imaging of ensembles of ACtx pyramidal neurons and further understand the precise conditions needed to potentiate PV-mediated inhibition by stimulating PVNs at additional frequencies or durations.

Special Symposium: Young Investigators from the Cross-Disciplinary Otitis Media Mentoring Network towards Diversity (COMMeND): Bridging Gaps in Otitis Media Research

3:00 p.m. - 5:00 p.m.

Ocean Ballroom 1 - 4

Young Investigators From the Cross-Disciplinary Otitis Media Mentoring Network Towards Diversity (COMMeND): Bridging Gaps in Otitis Media Research

Regie Lyn Santos-Cortez*, University of Colorado Anschutz Medical Campus*

Young Investigators From the Cross-Disciplinary Otitis Media Mentoring Network Towards Diversity (COMMeND): Bridging Gaps in Otitis Media Research

Diego Preciado[1], Diego Preciado[2]

[1]*Children's National Hospital,* [2]*George Washington University School of Medicine*

Diego Preciado, Children's National Hospital

Individual Abstract: Otitis media is an important disease in early childhood and is one of the most common reasons for healthcare visits and antibiotic prescription. It is the leading cause of conductive hearing loss in children, which if not addressed may result in permanent deficits in hearing and auditory processing. Despite the global significance of otitis media as a leading disease of communication, there has been attrition of otitis media researchers over the past decades. This has resulted in less attention to otitis media as a topic of scientific investigation and in the remaining knowledge gaps that could be addressed by basic, translational and clinical research. It also prevents the faster adaptation of new technologies toward improved management of otitis media. To this end, the COMMeND program was created and funded by an NIH R25, to encourage young investigators to get into and stay involved in otitis media research. For the program's first year, we recruited a diverse pool of mentees, matched to a group of highly experienced US and international faculty mentors. As part of our yearly activities, this inaugural COMMeND symposium at ARO will highlight the ongoing work of COMMeND mentees. The otitis media research to be presented spans a remarkably wide breadth of scientific techniques, including but not limited to human epidemiology, animal models, cell cultures, audiology, transcriptomics, immunology, microbiology and bacterial genomics, and novel methods for the prevention and treatment of otitis media. Presentation of the cutting-edge work of these young investigators will allow cross-fertilization of ideas with ARO members and networking that can lead to new scientific collaborations. The attendance of the COMMeND mentees at the ARO meeting will allow them to gain new knowledge on hearing research as well as participate in the many career advancement workshops offered at the annual ARO Midwinter meeting.

Young Investigators From the Cross-Disciplinary Otitis Media Mentoring Network Towards Diversity (COMMeND): Bridging Gaps in Otitis Media Research

Allen F. Ryan[1], Allen F. Ryan[1]

[1]*University of California, San Diego*

Allen F. Ryan*, University of California, San Diego*

Individual Abstract: Otitis media is an important disease in early childhood and is one of the most common reasons for healthcare visits and antibiotic prescription. It is the leading cause of conductive hearing loss in children, which if not addressed may result in permanent deficits in hearing and auditory processing. Despite the global significance of otitis media as a leading disease of communication, there has been attrition of otitis media researchers over the past decades. This has resulted in less attention to otitis media as a topic of scientific investigation and in the remaining knowledge gaps that could be addressed by basic, translational and clinical research. It also prevents the faster adaptation of new technologies toward improved management of otitis media. To this end, the COMMeND program was created and funded by an NIH R25, to encourage young investigators to get into and stay involved in otitis media research. For the program's first year, we recruited a diverse pool of mentees, matched to a group of highly experienced US and international faculty mentors. As part of our yearly activities, this inaugural COMMeND symposium at ARO will highlight the ongoing work of COMMeND mentees. The otitis media research to be presented spans a remarkably wide breadth of scientific techniques, including but not limited to human epidemiology, animal models, cell cultures, audiology, transcriptomics, immunology, microbiology and bacterial genomics, and novel methods for the prevention and treatment of otitis media. Presentation of the cutting-edge work of these young investigators will allow cross-fertilization of ideas with ARO members and networking that can lead to new scientific collaborations. The attendance of the COMMeND mentees at the ARO meeting will allow them to gain new knowledge on hearing research as well as participate in the many career advancement workshops offered at the annual ARO Midwinter meeting.

The Role of Diet in Tympanostomy Tube Otorrhea

Kavita Dedhia[1], Alyssa Tindall[1], Jillian Karpink[1], Ashley Williams[1], Terri Giordano[1], Virginia Stallings[1]

[1]*Children's Hospital of Philadelphia*

Kavita Dedhia*, Children's Hospital of Philadelphia*

Individual Abstract: Background: Tympanostomy tube otorrhea (TTO) occurs in LESS THAN 80% of children who undergo tympanostomy tube placement (TTP). A recent study made light of the potential role of diet in TTO. The objective of this study was to evaluate the role of diet quality in children with tubes, by rigorous recording of diet intake.
Methods: We conducted a three day 24-hour diet recall in children 2-6 years old, with TTP performed 6 months to 2 years prior to enrollment. Those with craniofacial syndromes, Down syndrome, cleft palate, known immunodeficiency, g-tube dependent, or from a non-English speaking family were excluded. Sample size was estimated as 60 subjects required in each group.

The primary outcome variable was TTO within 6 to 24 months of TTP which we dichotomized to either "≤1" or " GREATER THAN 1". The primary predictor variable was total daily caloric intake (kcal/d). The estimated energy requirement percentage (EER%) was the indicator of diet quality and calculated using sex, age, physical activity level, weight and height.

Results: A total of 120 families completed the three day 24-hour diet recall. The median age was 27 months (IQR 7.9 to 68.5), mostly male (57%). Fourteen percent were African-American or Black, 2% were Asian, 79% were White, and 2% reported other race. Majority (94%) were of non-Hispanic ethnicity. Most children reported dietary intake within the recommended percent intake for carbohydrates and fat and less than recommended for percent vitamin D. Within this cohort, 63 (52.5%) participants had GREATER THAN 1 TTO episode and 57 (47.5%) with ≤1 TTO episode. The adjusted multivariable regression identified that children with an EER% that was average or high were at higher odds of GREATER THAN 1 TTO episodes compared to participants with a low EER% with ORs of 4.6 (95% CI 1.4, 15.6) and 5.7 (95% CI 1.5, 22.1), respectively.

Conclusion: This study identifies that children with average or high total daily caloric intake are approximately 5 to 6 times more likely to have multiple episodes of TTO compared to those with low intake. For majority of children in this cohort, the average percent 3-day dietary intake were within recommended levels for carbohydrate and fat, and below for vitamin D. This study adds to the growing body of evidence illustrating the role of diet in children with otitis media, suggesting the utility of addressing diet intake in children with otitis media, specifically those with multiple episodes of TTO.

Hearing Thresholds and Middle Ear Analysis of the A2ml1-Knockout Mouse Model

Matthew Hill[1]

[1]*University of Colorado School of Medicine*

Matthew Hill*, University of Colorado School of Medicine*

Individual Abstract: Background: Otitis media is the most diagnosed disease in children and is known to cause hearing loss resulting in speech and reading difficulties later in life. Evidence continues to grow supporting genetic factors conferring susceptibility to otitis media. A2ML1 and its murine homolog, A2ml1, encode alpha-2-macroglobulin-like-1, a protease inhibitor expressed in ciliated middle ear cells. Loss-of-function variants of A2ML1 have been identified in multiple human cohorts with increased susceptibility to otitis media. The primary aim of our study is to determine if otitis media is associated with hearing loss in A2ml1-knockout (KO) mice.

Methods: Our laboratory recently studied a mouse line with a CRISPR-Cas9-mediated deletion of A2ml1 to mimic pathogenic A2ML1 mutations. Thus far, our studies have been performed in adult mice (GREATER THAN 20 weeks of age) that are known to have age-related hearing loss. To determine if otitis media is associated with hearing loss in A2ml1-KO mice, our aims were twofold. First, we performed auditory brainstem response (ABR) tests in mice less than 20 weeks

of age to establish whether A2ml1 loss-of-function was associated with changes in hearing thresholds. We then performed a gross anatomic and histological analysis of the middle and inner ears of wildtype, heterozygous, and homozygous mutant A2ml1 mice less than 20 weeks of age to assess if hallmarks of inflammation are associated with histologic signs of otitis media in the middle ear.

Results: Homozygous A2ml1-KO mice did not have significantly increased ABR thresholds across multiple frequencies when compared to wildtype A2ml1 mice. Gross anatomic examination of A2ml1-KO middle ears demonstrated increased hallmarks of inflammation when compared to wildtype A2ml1 mice.

Conclusion: A2ml1 dysfunction does not appear to influence upper auditory pathways based on ABR thresholds. Tympanosclerosis and tympanic rupture occur at increased rates in A2ml1-KO mice compared to wildtype mice.

Otitis Media: Single-Cell Transcriptomics and Microfluidic Disease Modeling

Arwa Kurabi[1]

[1]*University of California, San Diego*

Arwa Kurabi*, University of California, San Diego*

Individual Abstract: Background: The middle ear (ME) plays a critical role in hearing and is frequently prone to infections. While its cellular composition has been previously studied, understanding inter-species differences remains crucial for translational research. This study aims to identify and compare ME mucosal cell types in mice and humans using single-cell RNA sequencing (scRNA-Seq) and to develop an in vitro model to study otitis media.
Methods: Single cells from normal ME mucosa of C57 mice and normal human mucosa obtained from patients undergoing labyrinthectomy were loaded onto a Chromium 10X Genomics Controller to generate single-cell transcriptomes. PCA-generated cell clusters were annotated based on marker genes, and the gene profiles of each cluster were evaluated for both species. To study the complete progression of otitis media, we developed a middle-ear-on-a-chip (MEoC) model. This model consists of a two-chamber microfluidic system that can be loaded with human ME mucosal tissue, human endothelial cells, and bacteria.

Results: Most cell types present in human samples were also found in murine ME. However, distinct differences were observed: melanocytes formed clusters in murine samples but were rare in humans, while Schwann cells were prominent in humans but sparse in mice. Significant differences in the proportions of ciliated and basal epithelial cells were noted, with a higher presence in human ME. CellChat analysis suggested that interactions involving EGF receptor signaling between stromal, epithelial, and unexpectedly, monocytic cells may contribute to mucosal hyperplasia. The MEoC model enabled the study of early-stage human otitis media by simulating the ME environment, including interactions with human endothelial cells and leukocytes.

Conclusions: This study highlights the conservation and divergence of ME cell types between species and underscores critical differences that impact the translation of experimental findings to clinical applications. The MEoC model offers a novel platform for studying progression of otitis media, bridging the gap between traditional models and human biology, and enhancing the relevance of preclinical research to patient care.

Type I Interferon Signaling Enhances Streptococcus Pneumoniae Middle Ear Infection

Steven Shaw[1], Gabriela Heslop[1], Sarah Gitomer[1], Sarah Clark[1]

[1]*University of Colorado School of Medicine*

Steven Shaw*, University of Colorado School of Medicine*

Individual Abstract: Background: Streptococcus pneumoniae is one of the most common causes of bacterial otitis media (OM) and viral co-infection is associated with increased rate of bacterial OM. However, the host factors during co-infection that facilitate increased OM remain poorly understood. A consequence of a viral infection is the induction of a type I interferon (IFN) response, which signals through the type I interferon receptor (IFNAR) to activate interferon stimulated genes. Type I IFN signaling following viral infection reduces anti-bacterial immunity in the lungs, but the impact of this signaling pathway on bacterial OM is unknown. We hypothesized that virus-induced type I IFN signaling increases S. pneumoniae invasion to the middle ear, contributing to the burden of bacterial OM.

Methods: Intranasal infection of C57BL/6 mice with S. pneumoniae (Spn) with or without poly I:C or influenza A virus (IAV). Mice are then euthanized at 24 hours post infection (hpi) and then tissues are collected for looking at Spn colony forming units (CFUs) and for immune analysis via ELISA and flow cytometry. Statistics were done with Mann-Whitney U test and Kruskal Wallis test.

Results: We found that intranasal treatment with the synthetic viral analog poly(I:C) was sufficient to increase S. pneumoniae invasion to the middle ear. However, poly(I:C) failed to enhance S. pneumoniae infection in IFNAR mice, suggesting a requirement for type I IFN signaling. While investigating the impact of poly(I:C) induced type I interferon signaling on immune cell recruitment, we found that there was no change in the total numbers of neutrophils recruited to the middle ear during S. pneumoniae infection. However, neutrophil expression of TNF-alpha was significantly lower in mice treated with poly(I:C) compared to both mice not treated with poly(I:C) and IFNAR mice given poly(I:C). Higher TNF-alpha-positive neutrophils correlated with improved S. pneumoniae clearance and increased neutrophil phagocytosis of S. pneumoniae.

Conclusions: These findings indicate that type I interferon signaling is sufficient to increase middle ear infection with S. pneumoniae, and that this response is associated with reduced neutrophil activation. In patients with chronic OM, a similar phenotype of decreased neutrophil phagocytic capacity was observed, relative to circulating neutrophils. Ongoing studies will dissect the importance of type I interferon signaling on neutrophil-mediated clearance during

viral co-infection with the goal of identifying novel immunotherapy approaches for bacterial OM.

Role of Muc5b Overexpression in Middle Ear Pathology and Otitis Media Susceptibility

Helen Gomez[1], David A. Schwartz[1], Ivana V. Yang[1], Arwa Kurabi[2], Allen F. Ryan[2], Regie Lyn P. Santos-Cortez[1]

[1]*University of Colorado Anschutz Medical Campus,* [2]*San Diego School of Medicine and Veterans Affairs Medical Center, University of California*

Helen Gomez*, University of Colorado Anschutz Medical Campus*

Individual Abstract: Background: The MUC5B promoter variant rs35705950 is the strongest risk factor for developing idiopathic pulmonary fibrosis (IPF) among adults over 50 years old. Given its minor allele frequency of 8% across multiple populations, a potential protective effect in childhood is suggested. MUC5B is the predominant mucin in middle ear effusions in chronic or mucoid otitis media. Previous studies have shown impaired mucociliary clearance due to Muc5b overproduction in the distal airways and alveoli. This study aims to investigate the role of increased Muc5b expression in otitis media.

Methods: We used a line of transgenic C57BL/6 mice that overexpress full-length murine Muc5b. We examined the expression of Muc5b in the middle ears of heterozygous Muc5b-overexpressing transgenic mice compared to wild-type. Expression levels were determined via quantitative PCR, and histological and immunohistochemical analyses were performed to observe localized expression and pathological changes. Nontypeable Haemophilus influenzae inoculations were conducted on wild-type mice to assess Muc5b expression changes during infection.

Results: Preliminary findings indicated a significant increase in fold expression of Muc5b in wild-type mice within the first two days of infection (day 1 $p=0.13$ and day 2 $p=0.027$), with specific expression in epithelial cells. Histopathological observations of placebo-inoculated transgenic mice revealed squamous metaplasia and lymphocytic cell infiltration.

Conclusion: These results support a role for MUC5B in otitis media pathology in both humans and mice. Overall, this study highlights the significance of Muc5b in middle ear pathology and its potential role in increasing the risk of otitis media. These findings are crucial for understanding the genetic factors underlying OM and may inform future research on targeted therapies for otitis media.

Exploring Bacterial Dark Matter: The Function of Virulence-Associated Accessory Genes in Chronic Haemophilus Influenzae Infections

Mary Marino[1], Evangeline Williams[1], Jocelyn Hammond[1], Karan Bamb[1], Armoni Mayes[1], Kalisse Horne[1], Bhaswati Sen[1], Danielle Piazza[1], Laura Anastor-Walters[1], Ben Janto[1], Donald Hall[1], Garth Ehrlich[1], Joshua Mell[1]

[1]*Drexel University College of Medicine*

Mary Marino*, Drexel University College of Medicine*

Individual Abstract: Background: Haemophilus influenzae is a human-associated bacterium that colonizes the nasopharynx of healthy individuals but also causes otitis media (OM) in more than 50% of children. While most OM infections are acute and cleared by host immune responses or antibiotics, persistent infections leading to chronic or recurrent OM occur in up to 25% of children. Persistent infections can result from environmental and host factors, but bacterial genetic heterogeneity also contributes to virulence. Previous comparative genomics studies using clinical isolates identified accessory genes that were statistically associated with disease but had no predicted function. We discovered that an accessory gene family containing Sel1-like repeats increased bacterial survival after uptake by macrophages, resulting in higher systemic dissemination in an animal model. We initially hypothesized that this "macrophage survival factor" (msf) was involved in signaling metabolic quiescence and the expression of stress responses.

Methods: To test our hypothesis and characterize additional phenotypes associated with msf, we grew msf± strains in planktonic or biofilm states and carried out RNA sequencing (RNA-seq) analysis, followed by an array of phenotypic assays.

Results: RNA-seq showed that msf+ strains had a biofilm-specific shift in gene expression that paralleled the differences between biofilm and planktonic cultures. In particular, expression was downregulated for translational machinery and upregulated for alternative nutrient utilization pathways. Phenotypic assays showed that msf+ biofilms had ~10-fold higher viable cell density (CFU/mL) with no difference in biofilm biomass and only 2-fold increased metabolic activity. When treated with antibiotics, msf+ biofilms appeared to be ~4-fold less tolerant, suggesting that the presence of msf results in improved nutrient utilization within biofilms rather than increased dormancy. Since H. influenzae is also naturally competent (able to take up and recombine environmental DNA into its chromosome), we performed a competence assay by transferring log-phase cells into starvation medium, adding donor DNA, and plating for recombinant colonies. This assay demonstrated that msf+ cells exhibited higher transformation rates and more quickly recovered from starvation than msf– cells.

Conclusions: Taken together, our data suggest that msf contributes to the overall health of cells in biofilm and other starvation states, allowing replication to continue even under stressful conditions. Current efforts are focused on determining the molecular mechanism through which msf signals these proposed nutritional shifts. Further investigations of msf and other novel virulence genes may lead to new vaccine or drug targets for combating persistent infections caused by H. influenzae.

Pan-Transcriptomic Analysis of Diverse Clinical Haemophilus Influenzae Isolates During Biofilm Development

Evangeline Williams[1], Karan Bamb[1], Ari Gordon[1], Rachel Ehrlich[1], Jocelyn Hammond[1], Bhaswati Sen[1], Azad Ahmed[1], Garth Ehrlich[1], Ravinder Kaur[2], Michael Pichichero[2], Joshua Mell[1]

[1]*Drexel University College of Medicine,* [2]*Rochester General Hospital Research Institute*

Evangeline Williams*, Drexel University College of Medicine*

Individual Abstract: Background: Haemophilus influenzae is the most prevalent cause of chronic and recurrent forms of pediatric ear infections (otitis media, or OM), which lead to delayed language development or permanent hearing loss. H. influenzae cells embedded within metabolically dormant biofilms tolerate antibiotic treatments and evade immune responses, contributing to persistent infections. Because genetically diverse clinical isolates dramatically vary in biofilm formation and other traits related to persistent infection, we sought to define the gene expression patterns of biofilm formation across clinical isolates, to systematically measure natural variation in biofilm development and the accompanying changes in global gene expression.

Methods: To identify strains for pan-transcriptomic analysis, we applied a greedy algorithm to maximize the total number of accessory genes present in a minimal set of strains. Isolates were obtained from both healthy and OM-prone children, and a set of 206 genomes was curated. Core and accessory gene content were determined using a 90% amino acid identity threshold using the Panaroo pan-genome pipeline. Selected strains were cultured in 5 conditions along a biofilm development time course and RNA sequencing was performed to define global gene expression. Phenotypic assays of biofilm biomass, metabolic activity, viable CFU/ml, and antibiotic tolerance were also carried out. To avoid artifacts associated with read alignments to a single reference, we mapped reads to their own genomes using the Salmon pseudoaligner. Subsequent expression analysis focused on core genes, applying DESeq2 to define differential expression among strains and conditions, clusterProfiler to perform gene set enrichment analysis (GSEA), and weighted gene correlated network analysis (WGCNA) to define modules of co-expressed genes. Regression analyses using LASSO were applied to define marker genes that can predict phenotypic measures of biofilm traits across the strains.

Results: Nine strains captured all accessory genes present at GREATER THAN 10% across the population. Phenotypic assays found high variation among strains in biofilm traits. Relative expression across core genes dramatically variable, even for strains grown in the same condition. In biofilms, all strains showed repression of translational machinery and induction of alternative carbon source utilization pathways. WGCNA clustered core genes into 32 modules containing between 5 and 216 genes, and LASSO regression found strong predictive accuracy for module expression and biofilm traits.

Conclusion: We established a new approach for comparative transcriptomic analyses on diverse bacterial strains, defined common signatures of biofilm formation, and benchmarked divergent gene expression changes which may explain phenotypic patterns responsible for chronic infections. Ongoing work is aimed at incorporating accessory gene expression into the pan-transcriptomic pipeline, testing the role of specific genes in biofilm formation, and validating a

biomarker panel aimed at measuring biofilm levels in situ from nasopharyngeal and middle ear effusion specimens.

Cold Microplasma Exposure as a Novel Therapeutic Treatment for Bacterial Acute Otitis Media Demonstrated in a Small Animal Model

Guillermo Monroy[1], Zhenglun Wu[2], Eric J Chaney[1], Darold R Spillman[3], Michael B Jamrozy[4], Kavita Desai Kabelitz[2], Andrey Mironov[4], Alexander Ho[1], Gang Xiao[1], Edita Aksamitiene[1], Marina Marjanovic[3], Daniel A Llano[1], Helen Nguyen[5], J. Gary Eden[4], Stephen A Boppart[6]

[1]*Beckman Institute for Advanced Science and Technology,* [2]*Laboratory for Optical Physics and Engineering*[3]*Beckman Institute for Advanced Science and Technology, NIH/NIBIB P41 Center for Label-free Imaging and Multiscale Biophotonics (CLIMB),* [4]*Laboratory for Optical Physics and Engineering,* [5]*Dept. Civil and Environmental Engineering,* [6]*Beckman Institute for Advanced Science and Technology, NIH/NIBIB P41 Center for Label-free Imaging and Multiscale Biophotonics (CLIMB), Carle Illinois College of Medicine*

Guillermo Monroy*, Beckman Institute for Advanced Science and Technology*

Individual Abstract: Background: Otitis media (OM) is a highly prevalent disease occurring in 90%+ of children before the age of 5. For treatment, a course of broad-spectrum antibiotics is often effective, but may have long-term side effects on the microbiome and increase risks of antibiotic resistance. Plasma medicine harnesses technology used in semiconductor manufacturing to generate reactive gas species that influences oxidative stress and increases immune cell recruitment. This can be applied to reduce symptoms for a range of infectious diseases and cancers, and here as a novel promising therapeutic approach for OM.
Methods: A novel device and treatment platform was developed to deliver cold microplasma therapy (CMP) to and through the tympanic membrane (TM), where the reactive species within a carrier gas safely diffuse into the middle ear. 10 chinchillas were inoculated using a transbullar injection of human-derived non-typeable H. influenzae (or saline) and treated using CMP. Experimental groups included: Control (no exposure), CMP, or CMP + antibiotics (Ceftriaxone, 50 mg/kg, 1x or 3x daily). Over 21 days, optical coherence tomography (OCT), otoscopy, and related metrics longitudinally quantified symptoms and changes in the middle ear to track infection formation and treatment outcomes. Pre- and post-treatment, animals were structurally and functionally assessed to validate safety and effectiveness using advanced gold-standard methods: 9.4T MRI, histopathology, and auditory brainstem response hearing tests.

Results: Data shows that with repeated CMP treatments, an infection can be successfully cleared (N = 3) without antibiotics in approximately 10-12 days once therapy begins. Tissue integrity is maintained after repeated daily treatments as validated with OCT and MRI. CMP combined with low or high dose antibiotics (N = 2) successfully clears the infection with a reduced overall time course compared to CMP alone. Mild inflammation of the ear canal and TM was found in all experimental groups, though temporary and fully clears in 2-3 days with no impact to hearing acuity. Many parameters were optimized for this study, including both CMP device and protocol

design (e.g. frequency, dose, interval), however, reducing the frequency to a single treatment will be ideal for future clinical utility.

Conclusions: This is the first demonstration of CMP to clear bacterial OM in an in vivo model, and to confirm successful clearance using a comprehensive suite of validation tests and metrics. Plasma medicine has the potential to be of immense public health value as a non-pharmacological approach for bacterial OM and stands to significantly impact management.

Mini-Podium 5: Innovative Approaches to Hearing Preservation: From Gene Therapy to Light-Based Therapies

4:15 p.m. - 5:15 p.m.

Ocean Ballroom 5 - 8

Moderators: Philippe Vincent and amp; Donatella Contini

An Antioxidative Gene Therapy for the Prevention of Noise Induced Hearing Loss

4:15 p.m. - 4:30 p.m.

Shrivaishnavi Chandrasekar*[1], Damian Gulbin-Murphy[1], Todd Mowery[1]

[1]*Rutgers University*

Background: Nearly half a billion people suffer from some form of mild to moderate severe hearing loss. Most of this hearing loss occurs through occupational and recreational noise exposure throughout the life. Even with the best hearing protection (earmuffs + earplugs) there is a limit to the amount of sound that can be attenuated (~27 dB SPL). Thus, even when worn properly, long term exposure to very loud noise can result in progressive hearing loss. The etiology of NIHL can be traced to the overproduction of superoxides in the inner and outer hair cells of the cochlea. Thus, we have developed a hair-cell targeted AAV to express the transgene for the protein superoxide dismutase at levels that compensate for noise induced increases in superoxide production.
Methods: Animals (Mongolian Gerbils) received bilateral injections through the cisterna magna with hair cell targeted AAV that expresses the transgene for SOD1 (intracellular), SOD2 (mitochondrial) or SOD3 (extracellular). The transgene expressed for three weeks, after which animals were fitted daily with earplugs (25 dB SPL attenuation) and exposed to 125 dB SPL of broadband noise for five hours a day over five days. ABR/DPOAE recordings were carried out prior to initial noise exposure, three weeks after CM injection, and for 4 weeks following noise exposure. Animals were perfused, and the cochlea were prepared for whole mount dissection, IHC, and imaging. Hair cell counting (Myo7a) and synapse counting (GluA2) was carried out and correlated with final auditory thresholds to create a measure of neuroprotection.
Results: Pilot experiments show that increased SOD1 provides significant protection from moderate NIHL. Compared to controls, SOD transgene expression offered significant reduction in hearing loss. The safety test shows that SOD1 transgene expression does not produce any

measurable change to auditory status over time. The efficacy and robustness test shows that SOD1 AAV gene therapy provides long term neuroprotection from repeated noise exposures.
Conclusions: These data suggest that increasing the bioavailability of superoxide dismutase could reduce noise induced hearing loss by mitigating superoxide damage over time.

1) Cochlear hair cell targeted SOD1 gene therapy provides powerful neuroprotection from moderate persistent and acute traumatic noise exposures when used prophylactically.

2) This therapy could be used prophylactically by the general population to prevent the onset of occupational and recreational NIHL.

3) SOD1 gene therapy could provide an essential triage approach for traumatic noise exposures in military personnel.

Maintain Genomic Stability Could Prevent Calcium Imbalance Induced Hair Cell Degeneration

4:30 p.m. - 4:45 p.m.

Ruijie Cai*[1], Hongchao Liu[2], Yunge Gao[3], Xiaotong Ma[1], Huihui Liu[1], Hao Wu[4]

[1]*Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine,* [2]*Shanghai Jiao Tong University,* [3]*Shanghai Jiao Tong University, School of Medicine,* [4]*Shanghai Ninth People's Hospital, Ear Institute, Shanghai Jiao Tong University School of Medicine; Shanghai Key Laboratory of Translational Medicine on Ear and Nose Diseases*

Background: The loss of hair cells and neurons often begins at the base and gradually spread toward the apex of the cochlea in noise exposure, age and drug toxicity induced hearing loss. Calcium ion is important for sustaining functions of IHCs, and the disruption of intracellular Ca2+ homeostasis could account for noise- and drug-induced hearing loss and presbycusis. A better understand of the mechanisms of hair cell degeneration is of the highest interest and further is critical for identifying effective drugs to prevent deafness.
Methods: Immunofluorescence staining, patch-clamp electrophysiology, western blotting was applied to compare the difference of calcium influx, endogenous Ca2+ buffer, Ca2+ extrusion and calcium channels expressing intensity including PMCA1 in apical and medial turn of basilar membrane. The Pmca1 CKO mouse model was found. Using auditory brainstem response, Immunofluorescence staining and western blotting to observe the hearing threshold alteration, morphological changes and pathological process in Pmca1 CKO mouse. RNA-seq transcriptomic analyses of IHCs from Pmca1 CKO mice was applied to explore the mechanism of IHCs damage and discover effective drug to treat auditory dysfunction.
Results: We found that the IHCs from medial turn of basilar membrane release more synaptic vesicles, and the difference was disappeared with high EGTA concentration or block the calcium clearance channels. However, calcium clearance rate was faster in the IHCs from apical turn of basilar membrane. In addition, the expression level of PMCA1 protein was low in the basilar

membrane characterized with high frequency. Together, these results indicate that PMCA1 may be associated with the differential vulnerability of IHCs. (2) To investigate biology changes, we generated Pmca1 CKO mice. The ABR wave was totally invisible at P24. The loss of IHCs was concentrated at the basal part of the cochlea at P18 and progressed further at P24, extending to the apical part. At P24, the expression levels of Bcl-2, Bax, Cleaved caspase-3, and Cytochrome-c of Pmca1 CKO mice were significantly increased. Transcriptional changes in IHCs of Pmca1 CKO mice imply DNA damage/repair impairment and folic acid metabolism dysfunction. Using folic acid which could increase DNA repair, we successfully prevented IHCs damage in Pmca1 CKO mice and alleviated noise-induced hearing loss which could increase DNA repair.
Conclusions: Our findings implied that the differential vulnerability of IHCs may correlate with the properties of calcium homeostasis maintenance. From the IHCs transcriptomic analysis from Pmca1 CKO mice, we showed that DNA damage may be the main reason account for IHCs damage. Thus, we used folic acid to prove that alleviating DNA damage could protect IHCs against damage in Pmca1 CKO mice, which was further confirmed by noise-induced cochlear injury. Collectively, we first show that DNA damage prevention could be an effective strategy to prevention and cure acquired sensorineural hearing loss.

Photobiomodulation as a Non-Invasive Thermal Therapy for Hearing Preservation

5:00 p.m. - 5:15 p.m.

Jeremy Ryan*[1], Fateme Esmailie[1]

[1]*University of North Texas*

Background: 30% of adults above 65 suffer from hearing difficulty in the United States, which is expected to double by 2060. Hearing aids and cochlea implants are common treatments for hearing loss, but prevention methods such as photobiomodulation therapy (PBMT) could reduce the need for these treatments (J. H. Lee, Kim, et al., 2019). PBMT has been tested in animals (C. K. Rhee et al., 2012), auditory brain stem cells (Strübing et al., 2021), and humans (Goodman et al., 2013). The use of PBMT was shown to reduce hearing loss in animals, but the effectiveness of PBMT in a human cochlea is not confirmed. Human studies have been performed with shorter wavelengths and lower power density. Hypotheses surrounding PBMT suggest the process increases the level of adenosine 5-triphosphate (ATP), promoting the damage-repair process, and increase the expression of heat shock protein 70 (HSP70) which protects cochlear tissue. The expression levels of HSP 70 enhances between 40 ℃ to 42 ℃ (Kayastha et al., 2024). This research aims to determine the thermal aspect of PBMT on hearing preservation.
Methods: A three-dimensional model of heat transfer within the cochlea was developed to evaluate the impact of PBMT on mice and human cochlea. Heat transfer within human tissue is governed by Pennes' equation, accounting for accumulation (Eq 1 left hand side (LHS) first term), conduction (LHS second term), convection (LHS third term), radiation (LHS fourth term), convection due to blood perfusion (LHS fifth term), metabolic heat generation (right hand side (RHS) first term), and other heat sources (RHS second term).
Equation 1: ρc_p ∂T/∂t+∇·(-k∇T)+ρc_p u·∇T+∇·q_r+ρ_bl c_(p_bl) ω_bl (T_bl-T)=q_met+q

The maximum safe temperature for tissues is 43°C (Van Rhoon et al., 2013). For the safety of cochlea tissues, the value of Cumulative Equivalent Minutes (CEM43°C) should be 1.9 minutes or lower.

Results: An input power of 7.5 mW was used over 4 min for PBMT in human testing (Goodman et al., 2013), which didn't lead to hearing preservation. However, a study using mice showed the effectiveness of PBMT using 120 mW for 5 to 40 min, with an exposure time of at least 10 minutes being required to prevent hearing loss (Basta et al., 2020). The input power and exposure duration were both larger in the mouse study than in the human study. As a result, the mouse cochlea achieved temperatures over 40°C, while the human cochlea resulted in a maximum temperature of 37.8°C, suboptimal for HSP70 and ATP release. This may be the reason that PBMT in human studies was ineffective.
Conclusions: The results obtained show that photobiomodulation has a thermal impact on cochlea tissue. Further understanding of this thermal impact is needed in order for PBMT devices to achieve therapeutic impact in clinical settings.

Mini-Podium 6: Auditory Midbrain: Structure and Function

4:15 p.m. - 5:15 p.m.

Ocean Ballroom 9 - 12

Moderators: Audrey Drotos and amp; Ken Henry

Auditory Feature Discrimination in a Rat Model of Fragile X Syndrome

4:15 p.m. - 4:30 p.m.

David Gauthier*[1], Noelle James[1], Benjamin Auerbach[1]

[1]*University of Illinois Urbana-Champaign*

Background: Autism spectrum disorders (ASD) is a set of neurodevelopmental disorders defined by atypical social behavior, communication difficulties, and repetitive/restricted interests. ASD individuals often exhibit altered sound sensitivity and feature discrimination, leading to sensory overload and disrupted language comprehension. Fragile X Syndrome (FXS) is the leading inherited cause of ASD and a majority of FXS individuals present with these auditory processing difficulties. We have shown previously that a Fmr1 KO rat model of FXS exhibits sound hypersensitivity that coincides with abnormal perceptual integration of sound duration and frequency. Here we further characterized auditory processing alterations in FXS by examining sound frequency discrimination behavior and auditory cortical tuning properties in Fmr1 KO rats.
Methods: Male Fmr1 KO and wildtype (WT) littermate rats were trained in an operant Go/No-Go task to discriminate between tones an octave distance apart. Following training, discrimination performance was assessed for intermediate tones varied in 1/12 octave steps. Corresponding in vivo neurophysiological recordings were performed from the auditory cortex (ACx) and inferior colliculus (IC) using multichannel electrodes spanning tonotopic regions.

Tuning characteristics were quantified using various analyses including tuning area, q-values, general linearized models, and Bayesian decoding.
Results: We found that Fmr1 KO rats exhibited poorer frequency discrimination behavior that corresponded with broader tuning in the ACx but not in the IC. Specifically, we found increases to both spontaneous and sound-evoked activity in the ACx of Fmr1 KO animals, which resulted in less discriminable responses between preferred and non-preferred frequencies. Using a general linearized model and a Bayesian based population decoder, we found that cortical tuning properties are influenced more by changes to the noise level of the population than to changes in response gain, suggesting that additive shifts in cortical response properties are the primary driver in frequency discrimination impairments in FXS.
Conclusions: These findings suggest that cortical hyperexcitability may account for a range of auditory behavioral phenotypes in FXS, providing a potential locus for development of novel biomarker and treatment strategies that could extend to other forms of ASD.

Auditory and Tactile Processing in the Mouse Inferior Colliculus

4:30 p.m. - 4:45 p.m.

Blom Kraakman*[1], Aaron Wong[1]

[1]*Erasmus Medical Center*

Background: The inferior colliculus is a crucial auditory midbrain processing center. In addition to auditory inputs, the lateral cortex of the inferior colliculus (ICx) also receives non-auditory input in an organized fashion. Previous literature shows several somatosensory regions form direct input connections with the ICx; and peripheral stimuli such as whisker stimulation have been found to elicit responses in the ICx. Moreover, somatosensory stimuli can modulate sound-evoked responses in both awake and anesthetized animals. Recently, a candidate pathway relaying tactile input originating at the paws to the ICx was discovered, raising the question how the ICx responds to vibrotactile stimuli and what their influence on sound-evoked responses might be.
Methods: To answer these questions, in vivo electrophysiological recordings were done in the mouse ICx using multielectrode silicon probes. Anesthetized animals were presented with a variety of stimuli including auditory stimuli (pure tones, white noise, and amplitude modulated noise) and tactile stimuli (pressure and vibrations delivered to the hind paw), both separately and simultaneously.
Results: Preliminary data show that a subset of units recorded show responses encoding the on- and offset of continuous pressure stimuli. An overlapping group responds to low frequency (10-50Hz) sinusoidal vibrotactile stimuli in a phase specific manner as well as to the on- and offset of higher frequency (100-400Hz) vibrations. When paired with broadband noise, the cyclic response to the vibrotactile stimuli attenuated but the firing rate during this period remained elevated compared to broadband noise alone.
Conclusions: This shows that (vibro)tactile stimuli alone can drive neural activity in the ICx while also modulating ongoing sound-evoked responses. To gain insight into the perceptual relevance of the multisensory integration in the ICx on auditory-vibrotactile interactions, future work will focus on the interaction of tactile stimuli with more complex sounds such as amplitude modulated noise.

Differences in Short-Term Synaptic Plasticity at Cochlear Nucleus Synapses onto Two Classes of Inferior Colliculus Neurons

4:45 p.m. - 5:00 p.m.

Yoani Herrera*[1], Michael Roberts[1]

[1]*University of Michigan*

Background: T-stellate neurons in the anterior ventral cochlear nucleus (AVCN) receive direct input from the cochlear nerve and encode information about sound frequency and intensity, including rapid fluctuations in sound intensity called amplitude modulations. T-stellate neurons are the only neuron class within the AVCN that projects directly to the inferior colliculus (IC), the midbrain hub of auditory processing. However, how T-stellate cells contribute to IC neuron excitability and sound processing in the IC remains unknown.
Methods: Using channelrhodopsin-assisted circuit mapping and whole-cell patch clamp recordings in brain slices, we compared the synaptic strength, prevalence, and short-term synaptic plasticity of T-stellate input onto two classes of IC neurons: GABAergic neuropeptide Y (NPY) neurons and glutamatergic vasoactive intestinal peptide (VIP) neurons.
Results: Our results revealed that T-stellate neurons provide excitatory synaptic input to both NPY and VIP neurons. However, T-stellate input was more commonly observed in NPY neurons than VIP neurons, and EPSCs elicited by T-stellate input exhibited larger amplitudes and faster kinetics in NPY neurons than VIP neurons. We also found that T-stellate input to both cell classes exhibited short-term synaptic depression, but the magnitude of depression increased with increased frequency of synaptic activation in NPY neurons but not VIP neurons.
Conclusions: These data provide insight into how T-stellate input influences individual neuron types within the IC, laying a mechanistic foundation for in vivo experiments to examine how T-stellate input contributes to the auditory receptive fields of IC neurons.

Neural Cues in the Budgerigar Inferior Colliculus for Behavioral Detection of Transient and Sustained Tones in Noise

5:00 p.m. - 5:15 p.m.

Yingxuan Wang*[1], Margaret R. Youngman[2], Kristina S. Abrams[2], Kenneth S. Henry[2]

[1]*University of Rochester,* [2]*University of Rochester Medical Center*

Background: In real life listening environments, signal detection in noise can occur in various forms. Signals can be transient or sustained, and may begin at the same time or after some delay relative to the onset of competing noise. It currently remains unclear whether the auditory system uses similar or different detection strategies for these scenarios.
Methods: We used tone-in-noise detection as a simplified paradigm for signal detection in general. The budgerigar, a parakeet species, was chosen as our animal model due to their human-like sensitivity to many simple and complex sounds. We conducted operant-conditioning experiments to measure behavioral thresholds with two-down, one-up tracking procedures and a single-interval, two-alternative forced-choice task. Neural responses from the inferior colliculus

(IC; midbrain) were recorded in awake passively listening budgerigars to investigate the central mechanisms underlying different types of tone-in-noise detection. Stimuli were tones with 20-ms (transient) or 200-ms (sustained) duration, presented either at the onset or 100 ms after the onset of a 300-ms broadband noise masker. Masking noise was presented at 65 dB overall sound pressure level.
Results: Behavioral detection thresholds for transient tones in budgerigars were ~10 dB higher than longer tones, similar to typical human results. Minimal threshold differences were found when comparing signals presented concurrently with the noise onset versus 100 ms after onset. Post-stimulus time histograms of neural responses showed that, in the presence of noise, transient signals produce brief excitation of IC neurons followed by a decrement in response rate. Response decrements were more prominent when the signal onset was delayed. For longer, sustained tones, response decrements following initial excitation often persisted throughout the full 200-ms signal presentation, resulting in lower average response rate when the tone is present compared with the noise-only condition. Neural thresholds based on different neural cues will be compared to behavioral data to investigate potential neural detection strategies in each scenario.
Conclusions: These results show that neurons in the budgerigar IC exhibit multiple potential neural cues related with excitation and subsequent response decrements that could support behavioral detection of tones in noise. This study will help quantify the specific cues used for behavioral tone-in-noise detection across transient and sustained signals and for different signal onset delays, thereby advancing our understanding of neural mechanisms for real-life hearing in noisy listening environments.

This research was supported by grant R01-DC017519.

Honoring the Contributions of Dr. Brenda Lonsbury-Martin to Physiological Measures of Auditory Function

5:15 p.m. - 7:15 p.m.

Ocean Ballroom 1 - 4

Honoring the Contributions of Dr. Brenda Lonsbury-Martin to Physiological Measures of Auditory Function

Laura Dreisbach*, San Diego State University*
Gayla Poling*, National Institute on Deafness and Other Communication Disorders*
Jonathan Siegel*, Northwestern University*

Forty Years of Otoacoustic Emissions Research in the Lonsbury-Martin and Martin Laboratory: From Mysterious Origins to Widespread Adoption to the Implications of Recent Cochlear Mechanics Discoveries
Barden Stagner*, VA Loma Linda Healthcare System*

Individual Abstract: The Lonsbury-Martin and Martin laboratory was a pioneer in research on Otoacoustic Emissions (OAEs) starting in the mid 1980s and helped lead the way to their widespread use for assessing cochlear condition in both research and clinical settings. Noise exposures and third interference tones were used to reveal large distributed basal components of Distortion Product OAEs (DPOAEs) that remained stubbornly unexplained (although far from unexplored) until the relatively recent startling discovery of large longitudinal Vibration Hotspots (VHSs) centered at the interface between the bases of the outer hair cells (OHCs) and supporting Deiters' cells (Cooper et al. 2018). While the Basilar Membrane (BM) is only nonlinear within ~½ octave basal to the BF place of a single pure tone stimulus, these newly discovered VHSs are nonlinear for many octaves basal to the BF and exhibit similar tuning characteristics and properties to the mysterious basal DPOAE components.

This presentation delves into some of this early OAE research, touches on the groundbreaking clinical workshops led by Dr. Lonsbury-Martin that helped lead to widespread adoption of OAEs and documents basal DPOAE components in various mammalian species. In addition, the use of DPOAEs for ototoxicity and interoperative monitoring, efferent system evaluation and as a predictor of noise damage susceptibility is briefly discussed.

Finally, recent human studies are covered in which DP-grams were collected from normal hearing and hearing loss (HL) subjects at a variety of primary-tone levels. Separate DP-grams for near-f2, reflection and basal components were derived for each primary-level condition. Secondary DPOAEs (DPOAE2rys) in the form of iDP-grams which should reflect the properties of forward traveling intracochlear DPs were also collected. Normative statistical data was calculated to be used for comparison.

Near-f2 components showed reduced fine structure, more linear growth at low frequencies and seemed more sensitive and frequency-specific in certain HL individuals. Higher primary-tone level DP-grams exhibited greater signal to noise ratios (SNRs) than L1,L2=65,55 dB SPL DP-grams allowing for the evaluation of more severe HL patterns. Basal components were frequently as large or larger than corresponding reflection components, and both grew compressively. L1,L2=65,55 dB SPL DP-grams were often dominated by basal components at f2 LESS THAN 3 kHz, confirming the hypothesis that "optimal" lower L2 re L1 can sometimes maximize basal components. DPOAE2ry iDP-grams grew compressively in normal-hearing individuals, were more sensitive to a small punctate notch pattern HL and may form the basis for a new test that noninvasively evaluates the cochlear nonlinearity.

Brenda Lonsbury-Martin: Early Milestones in a Distinguished Career

Hongzhe Li*, VA Loma Linda Healthcare System*

Individual Abstract: Much of Brenda Lonsbury-Martin's research focused on the multifaceted cochlear mechanisms of otoacoustic emissions, particularly DPOAEs, and their application as a tool to evaluate the sensory organ's responses to noise exposure, ototoxins, and potential otoprotective interventions. However, her scientific interests extended far beyond DPOAEs alone. Over the course of her 50+ year academic career, Brenda's research spanned everything from single-unit recordings to human clinical trials, utilizing a variety of experimental

approaches and animal models. I first met Brenda in person about 10 years ago during my initial visit to Loma Linda, where she and Glen had already been working for more than a decade. Although we come from different generations, our academic paths converged in both time and place. In addition to Loma Linda, Brenda completed all her predoctoral research at Oregon Health and Science University—the same institution where I later conducted my postdoctoral work and transitioned to an independent investigator, and where I had read the hard copy of her thesis in the library long before I met her in person.

This presentation highlights Brenda's early work, particularly her research into supra-threshold responses of individual auditory nerve fibers to acoustic overexposure. Brenda embarked on her academic journey before I was born, and even before the founding of ARO. She was widely known for her approachable personality, and visiting scholars often remembered her for the generous and encouraging feedback she provided after their presentations. On a personal note, I remain deeply grateful for our many conversations about science and life, and for her unwavering support throughout my tenure at the VA Loma Linda.

An Intracochlear Look at the Generation of Low-Frequency Distortion Product Otoacoustic Emissions

Wei Dong*, VA Loma Linda Healthcare System*

Individual Abstract: Background: Cochlear nonlinearity is fundamental to cochlear amplification and compression, enabling high sensitivity across a large dynamic range and generating byproducts such as distortion product otoacoustic emissions (DPOAEs). With the application of optical coherence tomography (OCT) to cochlear mechanics, accumulating evidence indicates that cochlear nonlinearity is broadly distributed in the outer hair cell (OHC) region. This distribution forms the basis for the generation of DPOAE components from regions located basal to the f2 primary tone, as demonstrated through strong enhancement and/or suppression observed by Lonsbury-Martin and Martin's group.

To determine to what extent the basal distributed cochlear nonlinearity contributes to the generation of DPOAEs, we used OCT to map sound-evoked motions of the organ of Corti complex (OCC) cross-section. The contribution region was manipulated using a third, interference tone (IT) with varying frequency and level.

Methods: Most of the methods have been described previously (Meenderink et al., 2022). An OCT system (Thorlabs Telesto III TEL321C1) was used to accurately measure sound-induced motions in the Organ of Corti (OoC) cross-section at the second turn of the gerbil cochlea. The cochlea was stimulated alternately with two equal-level primary tones, with a frequency ratio of f2/f1=1.25, both in the absence and presence of an IT that varied in frequency or level. 2D vibrometry maps of the OoC cross-section, including both amplitude and phase at the primary and newly generated DP frequencies, were constructed from multiple A-lines with a high spatial resolution of 10 μm. The effects of IT suppression were compared with simultaneously measured DPOAEs in the ear canal using a sensitive microphone (ER-10X).

Results: Our results demonstrated that both OHC- DPs and DPOAEs were significantly suppressed by an interfering tone (IT) presented near the f2-primary tone peak, the best frequency (BF) location. This is consistent with other studies in the literature, which indicate that the primary generation site is located close to the f2-peak. Notably, for low-frequency DPOAEs, similar suppression was observed even when the IT tone was positioned more than one and a half octaves basal to the f2.

Conclusions: Low-frequency DPOAEs were generated over a broad region, particularly when evoked by high-frequency primary tones. This finding is consistent with the broadly distributed cochlear nonlinearity observed in this frequency region.

Multiple Mechanisms, Distributed Sources, and Wave Interference: Toward an Understanding of What Shapes Distortion-Product Otoacoustic Emissions

James Dewey*, University of Southern California*

Individual Abstract: The work of Brenda Lonsbury-Martin and colleagues has contributed vastly to our understanding of the origins and diagnostic utility of otoacoustic emissions, and, particularly, distortion-product otoacoustic emissions (DPOAEs). Through measurements in different species, examinations of the effects of various cochlear insults, and clever acoustic paradigms, she explored the contributions of potentially distinct DPOAE-generating mechanisms, how DPOAE sources are localized and distributed throughout the cochlea, and how these factors influence our interpretation of the signals measured in the ear canal. Key studies have demonstrated the different vulnerability of DPOAEs elicited at low and high stimulus levels, the varying contributions of "distortion" and "reflection" components across species, the spatial extent of the generation regions for different DPOAEs (e.g., 2f1-f2 vs. 2f2-f1), and the influence of wave interference between these distributed generators. In this talk, I will review how these basic findings have informed our current conception of DPOAE generation. Additionally, I will present published and preliminary work that characterizes the various intracochlear factors that shape DPOAEs in the mouse – work that was in large part inspired and informed by Brenda's contributions to the field. Lastly, I will identify some of the outstanding questions regarding DPOAE generation, hopefully prompting future work that builds on this legacy and further unravels the sources and mechanisms underlying DPOAEs.

ARO Short Course

5:15 p.m. - 7:15 p.m.

Ocean Ballroom 5 - 8

Short Course Focused on the Use of Artificial Intelligence for Otolaryngology Research

Brandon Cox*, Southern Illinois University School of Medicine*

Jeffrey Holt*, Harvard Medical School / Boston Children's Hospital*

Deep Dreaming of Hearing Proteins – Everything You Wanted to Know but Were Afraid to Ask about Protein Structure Prediction with Alphafold
Marcos Sotomayor*, University of Chicago*

Novel Deep Learning-Based Tools for Inner Ear Research
Uri Manor*, University of California, San Diego*

Novel Deep Learning-Based Tools for Inner Ear Research
Yasmin Kassim*, Post-doc*

Towards a Clinically Viable Speech Neuroprosthesis
Alexander Silva*, University of California - San Francisco, Department of Neurosurgery*

Wednesday, February 26, 2025

Podium 17: Multisensory Interactions

Moderators: Joel Berger and Samantha Davis

8:00 a.m. - 10:00 a.m.

Ocean Ballroom 1 - 4

Multisensory Integration in the Zebrafish Brain: Hearing Loss Affects the Bimodal Audio–Visual Interactions in the Tectum

Peng Sun*[1], Teresa Nicolson[1]

[1]*Stanford University School of Medicine*

Background: Animals integrate multiple sources of information (such as vision, sound and touch) to form a percept of the world that allows for adaptive behavioral decisions. The zebrafish tectum (analogous to the superior colliculus in mammals) is the primary visual processing center but also receives input from auditory, somatosensory, and other sensory modalities. The sensory maps of the tectum are arranged in laminae with visual input predominantly in the uppermost layers, and other sensory modalities projecting to deeper positions. Integration of multisensory modalities are vital for orienting or evasive movements. How hearing loss affects multisensory integration in the tectum is not fully understood. To address this question, we examined tectal integration in zebrafish tmc1/2a/2b mutants, which lack microphonic potentials and acoustic startle reflexes.
Methods: We imaged neural activity in the ascending auditory pathway and various laminae of the midbrain tectum evoked by visual and auditory cues using pan-neuronal GCaMP7a. Intact zebrafish larvae (5-6 dpf) were exposed to light or dark flashes (1 s, 5.6 klux), tone stimulation (80 ms, 500 Hz, 2.6 g) and bimodal stimulation (Flash: 100 ms, 400 lux; Tone: 80 ms, 500 Hz, 1.25 g; ISI: 400 ms) to assess sensory integration.
Results: Firstly, we confirmed the severe hearing loss in tmc triple mutants by assessing responses to tone stimulation in saccular hair cells, afferent neurons of the statoacoustic ganglion, and the medial octavolateralis region in the hindbrain. As predicted by previous electrophysiological recordings, responses were absent in both peripheral and central components in tmc triple mutants. Next, we tested the response to visual cues and found that light flashes evoked tectal activity in tmc triple mutant that was comparable to wild-type responses. In contrast, tones did not activate the tectum in tmc triple mutants. In wild-type larvae, we observed that bimodal stimulation (combined light flashes and tones) led to an enhancement of tectal responses in deeper layers that was greater that either light or tone stimulation alone. This increase in signal to bimodal stimulation was not evident in tmc triple mutants.
Conclusions: Our results demonstrate that multisensory integration of auditory and visual cues enhances responses in the zebrafish tectum. Complete loss of hearing eliminated this enhancement and is likely to have consequences on perception, such as a lower resolution spatial map or longer response latencies. Future studies will focus on the effects seen with different degrees of auditory dysfunction, such as high frequency hearing loss, which is a common feature in human hearing loss.

Do “Intuitive” Auditory Cues Facilitate Performance of a Reaching Task? Investigating Shared Features of Auditory and Motor Dynamics

Bruno Mesquita*[1], Mehrdad Kashefi[2], Rahul Vij[2], Ingrid Johnsrude[2]

[1]*Western University,* [2]*Brain and Mind, Western University*

Background: Maximizing motor coordination is vital beyond professional sports - for physiotherapists aiding patient recovery or developing strategies for independent living in people with disabilities. Automated auditory feedback can be presented and processed rapidly prior to, and in response to movement, and thus be useful for coaching and rehabilitative settings. These sounds can be designed to encode information about the individual's position in relation to their environment, (e.g. loudness = distance) offering a more immediate approach to altering movement patterns, compared to conventional methods of manual or verbal guidance. However, it has been hypothesized that for concurrent feedback to work well, the auditory signals must map to the movements in as intuitive and unobtrusive a way as possible, maximizing accurate interpretation of signals (Sigrist et al 2013), but no research has evaluated the idea that ‘intuitive’ auditory-spatial mappings facilitate motor learning more than “unintuitive” ones. Indeed, the very existence of intuitive mappings – implying that the dynamics of sound and of motion share common cognitive features – has not been systematically tested.
Methods: Participants (N = 19) performed reaches with their dominant arms on a horizontal plane towards visual targets. Movement in 2 dimensions was measured using a robot system (KINARM, Kingston, Canada). Auditory cues or silence preceded the visual target onset, and the pitch dynamics of sound cues were systematically manipulated to either match reach direction (congruent, i.e., increasing pitch = ‘up’, decreasing = ‘down’), were reversed (incongruent), or random (uninformative). Each type of cue (silence, congruent, incongruent, uninformative) was presented in separate blocks, with order randomized among participants. Movement Reaction Time (RT) was measured from the time at which the hand achieved a speed of 2cm/s until reaching the target.
Results: RTs were markedly slower in the silent condition than in auditory conditions and were faster in the informative (congruent and incongruent) versus uninformative cue blocks. RTs did not differ between congruent and incongruent informative cues averaged over trials. However, we observed a significant contrast between these conditions for the interaction between congruency and trial number, where trials in the congruent condition were comparatively faster earlier in the experimental block.
Conclusions: These results suggest that humans represent linear pitch sweeps and linear movements of the dominant arm using a common reference. Whether this common feature is perceptual, motor, or neither, is unknown. These associations can potentially be exploited to optimize motor behavior in response to auditory cues. However, the effect observed was quite small and decayed rapidly across an experimental block. We hypothesize that this is due to the simplicity of the experimental design, and that in more complex task designs, demanding higher cognitive load, the benefits of congruency in auditory-motor dynamics would be enhanced. Explorations are underway to investigate this hypothesis.

Neural Signatures of Emerging Audio-Motor Mappings

Haiqin Zhang*[1], Giorgia Cantisani[2], Shihab A Shamma[2]

[1]*École Normale Supérieure,* [2]*University of Maryland; École Normale Supérieure*

Background: In both humans and animals, the generation of complex sounds such as vocalisations, speech, and music requires precise acoustic control based on a close association ('mapping') between the executed movement and the resulting sound. The Mirror Network theory suggests that audiomotor learning involves bidirectional interactions between motor and auditory regions. Motor-to-auditory projections predict expected sounds, while auditory-to-motor feedback corrects errors and refines motor commands, allowing sound production skills to be improved over time. Studies of perception and production in both speech and music show robust evidence of communication between the motor and auditory cortices during skilled sound production. However, while there has been extensive study of audiomotor maps in mature sensorimotor systems such as speech production, neural dynamics of sensorimotor map formation during the early learning phases are still being elucidated.
Methods: We characterised early audiomotor map learning using electroencephalogram (EEG) recordings of neural activity. EEG was recorded before and after a 30-minute training session in which participants learned novel audiomotor associations on a custom-programmed piano keyboard. The EEG recording sessions were subdivided into i) a passive listening session, ii) a muted keyboard playing session, and iii) an unmuted keyboard playing session. Event-related potentials (ERPs) locked to key presses were compared before and after training, and across the different session types. Musician and non-musician participants were also compared.
Results: We found a decreased ERP amplitude during passive listening for musicians after training. For both musicians and non-musicians, changes in ERP peak times suggest increased covert motor activity during passive listening after the training session. ERPs locked to muted keystrokes also decreased in amplitude after training. Preliminary analyses also suggest that ERPs of unmuted playing represent the sum of passive listening and mute playing ERPs.
Conclusions: This work shows that newly-learned audiomotor associations can be detected on EEG after a short training session. Our findings may also be applied to improve the usability of brain-machine interfaces by accounting for changes in neural activity present in early audiomotor learning.

Sensory Motor Decoding From Violin Playing

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Background: When a musician is engaged in a concert, the brain continuously processes the surrounding sensory environment to produce coordinated actions. In violin playing, for instance, the sound and movements should act in harmony to produce a cohesive music. How does the brain process multi-sensory information and prioritize only task-relevant movements? The precise neural mechanisms by which the brain integrates multi-sensory information and coordinates motor control remain unclear. Current understanding of sensory-motor interactions is limited, with most research focusing on correlational studies rather than causal relationships (Zatorre et al., 2007; Penhune and Steele, 2012). Additionally, recent findings suggest that task-irrelevant movements can overwhelm cortical activity, potentially disrupting performance (Musal et al., 2019). In this study, we investigate the neural dynamics of sensory-motor processing during violin playing by analyzing EEG, video, and audio

recordings to better understand how motor actions related to violin performance are coordinated and prioritized in real time.
Methods: We asked participants to play an open string violin playing for one minute while we recorded their EEG, video and audio. Two control experiments were also conducted: 1. Participants performed the same task without producing sound by replacing bow with a pencil (miming). 2. Participants listened to the music they had previously produced. In each of these tasks, we simultaneously collected EEG, video and audio recordings to assess sensory-motor processing under different conditions. We used a linear model to reconstruct motor variables of one condition using EEG from other conditions.
Results: : Our findings indicate that the models successfully reconstructed motor variables in both the violin playing and violin mimicking tasks. We then explored whether the model trained on violin playing data could also reconstruct motor variables during the mimicking task. The results were promising, showing a moderate ability to reconstruct motor variables in the mimicking condition. Moving forward, we plan to expand the model by incorporating auditory feedback signals to improve predictions of upcoming motor actions.
Conclusions: In conclusion, our study demonstrates the potential of using linear models to reconstruct motor variables during violin playing and mimicking, with future enhancements aimed at integrating auditory feedback to further improve motor prediction accuracy

Subcortical Responses to Continuous Speech Under Crossmodal Divided Attention

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Background: Speech perception often occurs in multimodal contexts where listeners engage in concurrent nonauditory tasks, such as driving while listening to the radio. Successful speech perception in these scenarios requires attention to be distributed across multiple sensory modalities. Dividing attention between modalities, i.e., crossmodal divided attention, has been shown to slightly reduce cortical tracking of continuous speech. Whether crossmodal divided attention also affects subcortical processing of continuous speech remains unclear.
Methods: We employed an audiovisual dual-task paradigm to manipulate crossmodal divided attention. The primary task involved a visual memory task with either low or high cognitive load, and the secondary task was a comprehension task on an audiobook story in a quiet condition. Sixteen normal-hearing young adults performed the two tasks concurrently, during which EEG signals were recorded. In a separate condition, participants performed only the auditory comprehension task. Subcortical responses to continuous speech (i.e., audiobook stories) were estimated using temporal response functions (TRFs), which predicted EEG responses based on speech predictors derived from auditory nerve models. We compared the prediction accuracy, peak latencies and amplitudes of TRF models across the three conditions: auditory-only, low-load dual-task, and high-load dual-task, to assess the impact of crossmodal divided attention on subcortical responses to continuous speech.
Results: TRFs from all participants and conditions revealed a prominent peak at approximately 8 ms, similar to the wave V peak of auditory brainstem responses, indicating subcortical origins of the responses to continuous speech. There were no significant differences in the latencies or amplitudes of this peak across the three conditions. Additionally, prediction accuracy did not vary significantly across conditions.

Conclusions: We did not find evidence that the division of attention across sensory modalities affected the subcortical processing of continuous speech. Our study, combined with prior work, indicates that the influence of crossmodal divided attention on continuous speech processing might be limited to cortical levels.

Can Audiovisual Integration Training Improve Speech Understanding in Noise for Adults With Cochlear Implants?

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Background: Cochlear implants can successfully restore hearing in adults with sensorineural hearing loss, but speech recognition in noisy environments often remains a significant challenge. Speech understanding is a complex phenomenon that involves the integration of auditory and visual speech cues. Audiovisual temporal acuity, characterized by the temporal binding window (TBW), is critical for this process. The TBW, or the timeframe during which different sensory inputs are likely to be integrated and perceptually bound, can be narrowed through training on a simultaneity judgment task. However, the effects of this training on distal measures, such as speech understanding, remain unknown. We hypothesize that audiovisual integration training will narrow the TBW, resulting in improved speech understanding in noise. Additionally, we aim to characterize the neural correlates of perceptual changes using functional near-infrared spectroscopy (fNIRS).
Methods: Adults with normal hearing were randomized to receive either audiovisual integration training and testing (n=16) or audiovisual integration testing only (n=16) over three consecutive days. All adults with cochlear implants received audiovisual integration training and testing (n=8). TBW size, auditory-only, visual-only, and audiovisual word recognition in noise, and cortical activity were evaluated before and after the intervention. Cortical activation patterns were measured using fNIRS during a word categorization task with auditory, visual, and audiovisual blocks.
Results: Among adults with normal hearing, the trained group demonstrated significant improvements in mean TBW size (403 ms to 345 ms; p=0.030), whereas the control group did not (409 ms to 474 ms; p=0.061). Cochlear implant users demonstrated even greater improvements in TBW size following training (503.3 ms to 411.1 ms; p=0.009). Reaction times on the simultaneity judgment test improved by 112 s in the trained group (p=0.019) and 34 s in the control group (p=0.273). Although individual responses to training varied, mean auditory word recognition improved by 4.7 points in the trained adults with normal hearing, 3.2 points in trained adults with cochlear implants, and 0.2 points in the untrained adults with normal hearing. Reductions in TBW were significantly correlated with improvements in auditory word recognition among normal-hearing adults (R2=0.288; p=0.039). Furthermore, individual differences in responses to multisensory integration training were explained by differences in cortical processing of audiovisual speech at baseline, with decreased audiovisual-evoked activity in the superior and middle temporal gyrus being associated with larger improvements in auditory word recognition in noise (R2=0.866; p=0.007).
Conclusions: Our findings demonstrate that audiovisual integration training enhances auditory word recognition by narrowing the TBW and upregulating multisensory speech processing networks. We also show for the first time the effectiveness of this training in adults with cochlear implants. In conclusion, multisensory integration plays a key role in

speech understanding in noise, and audiovisual integration training presents a promising intervention to improve speech understanding in adults with sensory impairments.

Audiovisual Speech-Evoked Oscillatory Dynamics in Younger and Older Adults

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Background: Older adults with auditory (AO) and visual (VO) speech perception deficits can exhibit a preservation of audiovisual (AV) speech perception, identifying AV speech to a degree of accuracy like that of younger adults. Age-group differences in speech identification and speech-evoked potentials (SEPs) reported in recent studies suggest that older adults benefit from multisensory input more than younger adults, exhibiting an age-related increased reliance on multisensory integration. However, the mechanisms that underlie this age-related reliance on multisensory integration are poorly understood. Studies of the oscillatory dynamics of potentials evoked by simply sensory stimuli in younger normal-hearing adults have found that higher-frequency activity in the gamma band is associated with feature extraction and binding, while lower-frequency activity in the theta and alpha bands has been associated with multisensory integration. For the current investigation, we examined age-group differences in the oscillatory dynamics of potentials evoked by AO, VO and AV speech. We hypothesized that older adults would exhibit lower event-related spectral power (ERSP) and intertrial coherence (ITC) than younger adults when processing unisensory AO and VO speech, but greater ERSP and ITC when processing AV speech.
Methods: A group of 20 younger normal-hearing adults (mean age = 25.7 years, SD=3.0) and 32 older adults (mean age = 65.9 years, SD=8.5) with normal hearing or mild-to-moderate sensorineural hearing loss participated in our study. SEPs were recorded while participants were passively presented consonant-vowel syllables AO, VO, and AV (240 trials per sensory condition). Time-frequency analyses were performed using EEGLAB to compute ERSP and ITC for AO, VO, and AV trials in each participant. ERSP was calculated by using a wavelet decomposition on the EEG data to measure power changes across time and frequency, relative to a pre-stimulus baseline (-200-0 ms prior to stimulus onset). ITC was computed to assess phase consistency (i.e., phase locking) across trials at each time point and frequency. Average ERSP and ITC values were computed for activity in the theta (4-8 kHz), alpha (8-12 kHz), and gamma (30-60 kHz) bands 30-120 ms after stimulus onset.
Results: Older adults exhibited lower gamma ERSP and ITC when presented AO stimuli and lower theta and alpha ERSP when presented VO stimuli. Older adults also exhibited lower gamma ERSP and ITC but higher theta and alpha ERSP and ITC when presented AV Stimuli. Importantly, after accounting for AO and VO oscillatory activity [AV – (AO+VO)], older adults exhibited higher AV theta and alpha ERSP and higher theta, alpha, and gamma ITC than younger adults.
Conclusions: The results suggest that older adults may engage mechanisms for the integration of information available from auditory and visual sources more than younger adults to compensate for age-related deficits in unisensory speech encoding.

Audiovisual Integration in Cochlear Implant Users: A Functional Near-Infrared Spectroscopy (fNIRS) Study Comparing Visual Cues in Speech Perception

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[1]*San José State University,* [2]*University of Nebraska-Lincoln,* [3]*The Ohio State University*

Background: Speech and communication are inherently multimodal, integrating various information sources, particularly in challenging environments. Visual cues, such as seeing a speaker's face, significantly improve speech intelligibility through audiovisual (AV) integration, which is especially relevant for cochlear implant (CI) users. However, AV speech perception benefits vary greatly among CI recipients, and the underlying mechanisms remain unclear. This study investigated brain activity in CI users during speech perception tasks, utilizing functional Near-Infrared Spectroscopy (fNIRS) with two types of visual stimuli: a talking face and an abstract visual analog of the amplitude envelope. The study aimed to determine the specific information visual cues provide and how they are processed alongside auditory signals to facilitate speech perception in CI users.
Methods: To date, ten study participants (age: 68 ± 11 y, six male) underwent fNIRS scanning (NIRScoutX) while performing speech perception tasks designed to assess AV benefits. All subjects were implanted with a Cochlear™ Nucleus® device in the test ear(s). The two speech tasks were presented in four conditions: auditory-only (AO), visual-only (VO), audiovisual (AV), and resting. One task used a bouncing sphere as the visual cue, corresponding to the speech envelope's frequency. The other task used a human face as the visual cue. fNRIS data were preprocessed offline to remove motion and physiological artifacts. The general linear model (GLM) was applied to compute group results for the contrast map of AV versus AO. A paired sample t-test was used to compare AV benefits between the results of the two tasks. The significance level was set to q LESS THAN 0.05, corrected for multiple comparisons using False Discovery Rate (FDR).
Results: Preliminary results showed no significant differences in behavioral performance between the AO and AV conditions, regardless of the presence/absence of the visual cue. However, brain activation differed between the human face and the envelope analog. The AV GREATER THAN AO contrast revealed significantly greater oxygenation changes with the human face in the left dorsolateral prefrontal cortex (dlPFC), left precentral gyrus, left pars opercula of the inferior frontal gyrus (IFG), left postcentral gyrus, and left posterior middle temporal gyrus (pMTG). Increased oxygenation was also observed in the right dlPFC, precentral, and postcentral gyri. In comparison, the envelope analog produced significantly more oxygenation changes in the bilateral primary auditory cortices (PACs), bilateral anterior and posterior middle temporal gyri (aMTG, pMTG), bilateral visual association areas, and the left supramarginal gyrus (SMG).
Conclusions: These findings suggest that both types of visual cues enhanced cortical activity during the AV conditions compared to the AO conditions. However, the human face visual cue provided AV benefits primarily through activation in bilateral sensorimotor regions and the left-lateralized pMTG. In contrast, the envelope analog boosted AV benefits through bilateral speech-related regions, including the PACs, aMTG, and pMTG.

Podium 18: Emerging Gene Therapies for Hearing and Balance Disorders

Moderators: Elisa Martelletti and Gwenaëlle Géléoc

8:00 a.m. - 10:00 a.m.

Ocean Ballroom 5 - 8

A Base Editor for the Long-Term Restoration of Auditory Function in Mice With Recessive Profound Deafness

Chong Cui*[1], Shengyi Wang[1], Daqi Wang[1], Jingjing Zhao[1], Bowei Huang[1], Biyun Zhu[1], Yuxin Chen[1], Honghai Tang[1], Yu Han[1], Cheng Ye[1], Dan Mu[1], Chengdong Zhang[2], Yuan Yang[2], Yihan Bao[3], Jun Lv[1], Shuang Han[1], Geng-Lin Li[1], Huawei Li[1], Yilai Shu[1]

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Background: OTOF is the first gene identified as affecting auditory neuropathy spectrum disorders (ANSD) and accounts for approximately 41-91% of ANSD cases. A prevalent recessive mutation (c.2485C GREATER THAN T, p.Q829X) within the OTOF gene leads to profound prelingual hearing loss. Unfortunately, there are currently no commercially available drugs for treating this deteriorating form of hearing loss in clinical practice. Several early clinical trials focusing on OTOF mutation-induced deafness have shown promising therapeutic outcomes. However, these treatments' long-term efficacy and safety remain challenging to elucidate fully. Adenine base editors (ABEs) can accurately convert A·T to G·C base pairs without inducing double-strand DNA cleavage. Here, we developed an ABE treatment system that efficiently corrects the pathogenic mutations in Otof and improves hearing function long-term in the mouse model.

Methods: Humanized mouse models harboring the homologous mutation (Otof: c.2482C GREATER THAN T; p.Q828X and c.2485C GREATER THAN T, p.Q829X) were generated. Six optimized ABEs, comprising combinations of three Cas9 variants (SpCas9-NG, SpG, and SpRY) and two deaminases (ABE8e and ABE7.10max), were screened. Editing efficiencies were evaluated using next-generation sequencing, while off-target effects were assessed through GUIDE-seq and transcriptome sequencing. A dual AAV-PHP.eB vector system, carrying the N-terminal and C-terminal of NG-ABE7.10max, was microinjected into the inner ear of P1-3 mice via the round-window membrane and P13-14 mice via posterior semicircular canal. Auditory function, otoferlin expression, and inner hair cell synaptic function were evaluated using auditory brainstem response, acoustic startle response, prepulse inhibition, immunohistochemistry, and patch-clamp recording, etc.

Results: We show that in Otof mice harbouring a mutation homozygous to human OTOF that faithfully mimics the hearing-loss phenotype. A base editor (consisting of the deaminase ABE7.10max and the Cas9 variant SpCas9-NG) packaged in AAV and injected into the inner ear of the neonatal mice via the round-window membrane effectively corrected the pathogenic mutation, with no apparent off-target effects. The treatment restored the otoferlin protein levels in 88% of the inner hair cells and stably rescued the auditory function of the mice to near-wild-type levels for over 1.5 years while improving synaptic exocytosis in the inner hair cells. Significant auditory function restoration
was achieved even when the therapeutic system was delivered on P13–14 after the onset of hearing. We also show that an adenine base editor that targets the prevalent human OTOF mutation restored hearing in humanized mice to levels comparable to those of the wild-type counterparts.

Conclusions: In summary, our study suggests that ABEs can be used to rescue hearing function in cases of auditory synaptopathy, thus providing a potentially accurate therapeutic strategy for treating hereditary hearing loss, and will offer insights and prospects for the treatment of other genetic diseases.

AAV-Mediated Yap Inhibition for Treatment of Vestibular Schwannoma

Kevin Biju*[1], Juan Llamas[2], Yeeun Kim[2], Dorothy W. Pan[1], Seiji B. Shibata[1], Joni K. Doherty[1], John S. Oghalai[1], Ksenia Gnedeva[2]

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Background: Vestibular schwannomas characteristic of neurofibromatosis type 2 (NF2) are benign and slow growing. It has been shown that YAP is a major downstream target of the NF2 protein. In complex with TEAD transcription factors, YAP drives expression of several mitogens and, therefore, represents an excellent therapeutic target to slow progression of vestibular schwannoma. Small molecule drugs that target YAP-TEAD interaction may slow tumor growth in preclinical models, but they have adverse effects on rapidly dividing tissue. Furthermore, the established Periostin-Cre mouse model of NF2 disease, via conditional ablation of NF2 in Schwann precursor cells, develops schwannomas by ten months of life, making drug development a lengthy process. This necessitates the need for a more efficient preclinical model.

Methods: To inhibit YAP to slow progression of vestibular schwannoma, we developed adeno-associated virus (AAV) containing tumor suppressors expressed under a glia-specific promoter. These tumor suppressors are YAP-TEAD inhibitor peptide (YTIP), a dominant negative inhibitor of YAP, and p27kip1, a potent cell cycle inhibitor. AAVs containing suppressor or reporter control were tested in both human primary culture and mouse model of vestibular schwannoma. All AAV treatments in mice were conducted through posterior semicircular canal (PSCC) injection. Proliferation was measured through both Ki-67, a protein marker of cell division, and 5-ethynyl-2'-deoxyuridine (EdU), a thymidine analog that monitors DNA synthesis. To develop a more efficient preclinical model for vestibular schwannoma, we utilized AAV containing YAP5SA, a constitutively active form of YAP, which we hypothesized would promote schwannoma growth within the mouse vestibular nerve.

Results: We observed that AAV-mediated gene delivery resulted in 50-60% viral expression within glial cells in the vestibular nerve. In human primary culture of vestibular schwannoma, we observed 45% decrease in Ki-67 index upon treatment with AAV-p27kip1 (4.1% vs. 7.4%, p=0.02). We are currently testing AAV treatments in the Periostin-Cre mouse model. Upon delivery of YAP5SA to wild type mice by posterior semicircular canal (PSCC) injection, we observed vestibular and cochlear nerve tumors, composed of SOX10+EdU+ proliferating Schwann cells, within two weeks of administration (n=25). Mice injected with AAV-YAP5SA exhibited significant vestibular phenotype on balance beam testing compared to control injected mice (n=10) with lower success rate (20% vs. 100%) and slower completion speed (3.19 cm/s vs. 7.79 cm/s; p LESS THAN 0.001). Upon harvest of the vestibular nerve, Schwann cells from the YAP5SA-treated mice exhibited greater EdU incorporation compared to control-treated mice (38.5% vs. 0.6%, p LESS THAN 0.001).

Conclusions: AAV-mediated gene delivery targeting YAP represents a promising treatment paradigm in preclinical models of vestibular schwannoma. Furthermore, targeted activation of YAP5SA in vestibular and cochlear nerves may serve as a more efficient mouse model of NF2 disease. With the growing interest in AAV-mediated treatment in the field of otolaryngology, our work suggests the utility of AAV in vestibular schwannoma treatment.

Lentiviral Gene Therapy Can Effectively Address Recessive Hearing and Balance Disorders

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Background: Adeno-associated viral vectors (AAV) have been widely used to rescue recessive genetic hearing losses but are hampered by limited payload capacity. Although dual vector strategies can address genetic defects beyond the capacity of single AAVs, there are disadvantages including loss of efficiency and additional manufacturing cost when considering translational research. Lentiviral vectors are well established in hematologic disease and have also been used in the eye. Third generation lentiviral vectors are capable of transducing non dividing cells and targeting can be improved by pseudotyping the vector.
Methods: We evaluated the effect of capsid pseudotyping on vector distribution in one month old C57Bl/6 mice and additionally tested a range of promoters to optimize vector distribution. We administered a third-generation lentiviral vector (LV) expressing either the myosin VII or the stereocilin genes to a one-month-old homozygous mutant Shaker 1 or STRC -/- mice. We visualized the posterior semicircular canal (PSSC) on the left side, performed a canalostomy using a microdrill, then delivered 1µL of the vector to that side and covered the defect with a muscle or fat graft. Outcomes measures included hearing and balance evaluations, and fluorescent microscopy.
Results: Pseudotyping with vesicular stomatitis virus G envelope glycoprotein (VSV-G) and use of the CAG promoter provided optimal transgene expression in inner and outer hair cells but also demonstrated transduction of the spiral ganglion and stria vascularis. Rescue of vestibular loss or hearing loss was achieved in adult animals with mutations in myosin VII or stereocilin.
Conclusions: Third generation lentiviral vectors provide an effective system for delivering large genes to the inner ear as a single vector without necessitating a split vector strategy. Further refinement of these vectors should allow rapid translation into clinical trials.

Novel Antisense Therapy to Durably Treat USH2A Patients

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Background: Usher syndrome (USH) is the most common genetic cause of combined deafness and blindness. Mutations in USH2A account for half of USH cases. The size of the USH2A coding sequence (~15.6Kb) prevents the use of classic gene replacement therapy mediated by adeno-associated virus (AAV)-based vectors, because it exceeds the capacity of single and even dual AAVs. To circumvent this limitation, we are developing an antisense oligonucleotide (ASO) strategy to correct hearing and vision loss in patients living with USH2A. We target an out-of-frame founder mutation in USH2A identified in patients at Boston Children's Hospital, and a major cause of USH2A in the French-Canadian population.

To restore the proper reading frame, we developed a strategy leading to dual exon skip in human USH2A. Our hypothesis is that in-frame deletion of two exons in humans will result in expression of slightly shorter, yet still functional USH2A pseudo-protein.
Methods: We designed ASO sequences based on computational analyses of cis-regulatory sequences predicted to impact splicing. Candidate ASOs were synthesized with modified 2'-O-methyl nucleotides and phosphorothioate backbones. ASOs were either (1) transfected into retinoblastoma cells (WERI-RB1) or (2) applied gymnotically to inner ear and retinal organoids derived from human mutant and isogenic iPSCs. We performed RT-PCR to validate ASO-induced USH2A dual-exon skipping. At the protein level, in silico analysis was performed using Alphafold2 to predict the 3D structure of both wild-type and pseudo-USH2A protein.
Results: Our data demonstrated successful single and dual exon skip with different combinations and dosages of ASOs in retinoblastoma cells and in human organoids. Our exon skipping strategy led to an in-frame internal deletion of 105 amino acids out of 5202 amino acids. This deletion excised a single fibronectin domain. In silico analysis demonstrated the preservation of protein folding properties suggesting that protein function would be preserved.
Conclusions: ASOs have great promise as a platform to treat genetic disorders, including Usher syndrome. They are simple to customize to different targets, inexpensive to manufacture, and can be efficiently delivered without special formulation. By demonstrating the high efficiency and safety of this approach, we hope to bring this novel antisense therapy from the bench to the bedside of USH2A patients.

Gene Therapy Versus Cochlear Implantation in Restoring Hearing Function and Speech Perception for Deafness Individuals

Xiaoting Cheng*[1]

[1]*Eye and ENT Hospital, Fudan University*

Background: Gene therapy (GT), a novel therapeutic strategy for congenital deafness, has been shown to improve hearing and speech. It is urgent and important to compare its performance with cochlear implantation (CI), which has been the only gold standard for congenital deafness in the last half-century. We conduct a comprehensive evaluation encompassing auditory and speech perception, music perception, sound source localization, cortex development, and quality of life between gene therapy and cochlear implantation patients with congenital deafness.
Methods: Patients with congenital severe-to-complete hearing loss (aged 1-18 years) who received GT or CI between December 2022 and August 2024. Based on the hearing modality, they were divided into: 1) GT-only patients versus CI patients; 2) Bimodal (unilateral GT + contralateral CI) patients: GT+CI versus bilateral CI, GT (CI-off) versus unilateral CI; and 3) bimodal intraindividual comparison: GT+CI, GT (CI-off), and CI (GT-masked). The primary outcomes were the auditory and speech perception evaluated by questionnaires and behavioral tests at T1 (3 months), T2 (6 months), and T3 (≥12 months), including hearing thresholds, the Meaningful Auditory Integration Scale (MAIS), Categories of Auditory Performance (CAP), Speech Intelligibility Rating (SIR), Meaningful Use of Speech Scale (MUSS), Speech, Spatial, and Other Qualities of Hearing Scale for Parents (SSQ-P), speech tests, and singing analysis. The mixed linear model was used for statistical analysis.
Results: A total of 11 GT patients and 57 CI patients were enrolled. The average auditory brainstem response thresholds were restored from GREATER THAN 95 dB to 51 dB in

seven GT patients at T3. For GT-only versus CI, GT patients scored higher than CI in MAIS, SSQP-speech, SSQP-spatial, SSQP-other, and SSQP noise related questionnaires at T1 (P LESS THAN 0.001, P=0.001, 0.004, 0.008, and 0.004), in all questionnaires at T2 (P=0.007, 0.036, LESS THAN 0.001, LESS THAN 0.001, 0.001, 0.003, LESS THAN 0.001, and LESS THAN 0.001) and in CAP, SIR, SSQP-other, and SSQP noise related at T3 (P=0.019, 0.001, 0.006, and 0.005). GT-only patients showed shorter latency (P=0.016 at 6 months) and higher amplitude (P LESS THAN 0.001, at 12 months) of mismatch negativity than CI patients. For GT (CI off) versus unilateral CI, GT patients performed better in speech tests in noise (monosyllable and disyllable) (P LESS THAN 0.001 and P=0.004) and almost all speech tests in quiet (P=0.007, 0.002, LESS THAN 0.001, LESS THAN 0.001, 0.005, LESS THAN 0.001, LESS THAN 0.001, and 0.001); For GT+CI versus bilateral CI, GT+CI patients performed better in monosyllable in noise, ambient sound, initial, final, and singing in-tune rate in quiet (P=0.009, 0.018, 0.023, 0.003, and LESS THAN 0.001). For bimodal intraindividual comparison, GT+CI tended to be better than GT (CI-off), followed by CI (GT masked).
Conclusions: Within one year after therapy, all GT patients exhibited stable hearing recovery. GT patients performed better than CI patients in most auditory speech perception, especially in noise and music.

AAVR Expression is Essential for AAV Vector Transduction in Sensory Hair Cells

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Background: Adeno-associated virus (AAV) vectors are a leading platform for gene therapy due to their specificity in targeting cells, persistent gene expression, lack of pathogenicity, and low immunogenicity. Recently, AAV-mediated gene therapy in the inner ear has progressed from laboratory use to clinical trials, but the lower transduction rates in outer hair cells (OHCs) in the organ of Corti and in vestibular hair cells in adult mice are still a challenge. In particular, OHCs are highly vulnerable to inner ear insults, for instance noise-induced and age-related hearing loss
Methods: Immunofluorescence and Western blotting were performed to detect AAVR expression. Co-immunoprecipitation (Co-IP) and fluorescence colocalization were utilized to verify the interaction between AAVR and AAV particles. Antibody blocking, gene knockout, and conditional knock-in approaches were employed to assess the role of AAVR during AAV infection in sensory hair cells.
Results: We show here that expression of a key AAV receptor, Kiaa0319l (AAVR), in OHCs and vestibular hair cells decreases significantly in mature mice. We also demonstrate that AAV particles directly interact with AAVR by forming complexes in an inner ear cell line and cultured cochlear explants. Consequently, knockout or antibody blockage of AAVR significantly inhibits AAV transduction in vivo. Finally, conditional overexpression of AAVR in sensory hair cells of adult mice successfully restored AAV transduction efficiency in OHCs and vestibular hair cells
Conclusions: In conclusion, this strong evidence that AAVR is essential for AAV transduction in sensory hair cells will help to increase the efficacy of future gene therapy for acquired hearing loss and vestibular hair cell deficiency.

PAM-Flexible Adenine Base Editing to Rescue Hearing Loss in a Humanized MPZL2 Mouse Model Harboring East Asian Founder Mutation

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Background: CRISPR-based technologies have been harnessed to directly correct disease-causing mutations for fundamental treatment of genetic disorders. CRISPR-based in vivo gene editing shows significant hearing improvements in autosomal dominant mouse models with postnatal progressive hearing loss by disrupting the reading frame of dominant-negative alleles. However, the autosomal recessive form of deafness is the majority of hereditary forms of hearing impairment, about 80% of non-syndromic hereditary deafness is inherited in this manner. Base editors (BEs) possess therapeutic potential for correcting point mutations without generating DNA double-strand breaks. BEs may provide a one-time treatment for hereditary deafness caused by recessive mutations. However, a significant limitation of BE-based gene therapy is the need to design distinct BE-sgRNA therapeutic systems for different mutation sites even in the same causative gene, making the targeting of founder mutations or mutational hotspots a particularly promising and efficient approach.
Methods: In this study, we identified an attractive target for adenine base editor (ABE) to correct a frequently mutated recessive MPZL2 gene, primarily caused by homozygous c.220C GREATER THAN T mutations in East Asia, leading to autosomal recessive nonsyndromic hearing loss (DFNB111), a leading cause of mild-to-moderate hereditary deafness. We developed a humanized knock-in (KI) mouse model by inserting human MPZL2 cDNA harboring the mutation (c.220C GREATER THAN T:p.Q74X) into the mouse Mpzl2 locus, which recapitulates human DFNB111 deafness. The humanized KI mice displayed progressive hearing loss, recapitulating the phenotype of individuals with DFNB111. Conversely, a humanized mouse model with the insertion of human MPZL2 wild-type cDNA exhibited normal hearing function, which confirms the effect of c.220C GREATER THAN T disease-causing mutation on the auditory phenotype. We tested all combinations of ABE variants, four types of Cas9 variants with different PAM, and sgRNAs to correct the c.220C GREATER THAN T founder mutation.
Results: We optimized the SpRY-based ABE system (ABE8eWQ-SpRY:gRNA3) with higher levels of A-to-G editing at the target adenine compared to other ABE:gRNA combinations, and the ABE8eWQ-SpRY:gRNA3 showed high precision by inducing no bystander editing and neglectable genome-wide and transcriptome RNA off-targets. The PAM-flexible ABE variant with reduced bystander effects (ABE8eWQ-SpRY:gRNA3), packaged in dual adeno-associated viruses (AAVs) and injected into the inner ear of the humanized knock-in (KI) mouse model via the round-window membrane, effectively corrected the mutation (~4% DNA editing) with no apparent bystander and off-target effects. The treatment significantly restored long-term hearing, improved histopathologic changes, and reversed gene expression profiles.
Conclusions: We herein identified a promising target for BE-based inner ear gene therapy to rescue the frequently mutated MPZL2 recessive deafness harbored c.220C GREATER THAN T founder mutation, a significant proportion of hereditary deafness. The development of the humanized mouse models and the successful correction of the founder mutation using a

single PAM-flexible ABE may step toward clinical application of BE-based gene therapy for the treatment of hereditary deafness, including most cases of DFNB111 deafness.

Neutralizing Anti-AAV Antibodies in Blood and CSF: Lessons Learned for the Inner Ear

Paul Krumpoeck*[1], Kleopatra Rapti[2], Ellen Wiedtke[2], Erdem Yildiz[1], Aldine Tu[1], Christian Matula[1], Christoph Arnoldner[1], Dirk Grimm[2], Lukas Landegger[1]

[1]*Vienna General Hospital, Medical University of Vienna,* [2]*Section Viral Vector Technologies, Medical Faculty, University of Heidelberg, BioQuant, Heidelberg, Germany,*

Background: Gene therapy using adeno-associated viral vectors (AAVs) is a rapidly advancing field aiming to develop treatments for many different disorders. However, a major challenge is the generation of antibodies by the adaptive immune system, preventing cellular uptake of the vectors and thereby rendering the treatment ineffective. To what extent different antibody titers in the blood correlate to titers in the central nervous system or inner ear and consequently the degree to which local approaches bypass the humoral immune response has yet to be determined in humans.
Methods: In this work, we assessed anti-AAV neutralizing antibody titers against twenty different AAV capsids (including multiple hitherto unstudied capsids) in human serum and cerebrospinal fluid (CSF). Samples were prospectively collected for two biobanks at the Medical University of Vienna and underwent in vitro neutralizing antibody assays with serial dilutions of 1:5 up to 1:320 and Luciferase assays to determine the amount of antibodies.
Results: The overall prevalence of anti-AAV neutralizing antibodies in the serum was 37.2% across 33 samples, with the highest levels found for antibodies directed against AAV6, AAV1, AAV2, AAV3B, AAV13, and engineered capsids. We also observed significantly higher antibody frequencies in females (44.6% vs. 34.4%, $p = 0.0175$) and against synthetically engineered vectors (45.3% vs. 35.4%, $p = 0.0334$). No influence of patients' age or liver function parameters on antibody seroprevalence was observed. Additionally, we found antibodies to be nearly non-existent at a prevalence of 2.6% in 10 samples of human CSF (p LESS THAN 0.0001).
Conclusions: These results are promising both for the translatability of animal studies and for clinical trials using direct AAV delivery to the CSF or, by extension, to the anatomically and conceptually related inner ear, as both compartments are separated from the systemic circulation by the blood-brain- or blood-labyrinth barrier, respectively. Future clinical trials using local approaches could be incentivized to test neutralizing antibody levels in the CSF or perilymph of potential participants whose serum screening is positive for the vector of interest, instead of excluding them from the trial right away.

Podium 19: Psychoacoustics: From Acoustic Startle to Auditory Attention

Moderators: Mahan Azadpour and Ebtesam Sajjadi

8:00 a.m. - 10:00 a.m.

Ocean Ballroom 9 - 12

Acoustic Startle, TTS, and Tinnitus Decrease Sound Localization and Speech-In-Noise Performance

Nathaniel Greene*[1], Carol Sammeth[1], Nick Brunstad[2], Greg Rule[2], Ted Argo[2]

[1]*University of Colorado Anschutz Medical Campus,* [2]*Applied Research Associates, Inc.*

Background: An unexpected, very loud sound can cause an acoustic startle reflex, and can also potentially cause temporary threshold shift (TTS) for hearing and noise-induced tinnitus for a short period of time. This can happen, for example, with the high-amplitude noise burst that occurs along with a blinding light and concussive force when police or military use a "flashbang" grenade to temporarily disable or confuse an adversary. This study evaluated the effects of an unexpected loud acoustic impulse sound on human subject performance in terms of: (1) speech recognition in noise, and (2) sound localization, both examined in a hemi-anechoic free field with a 32-loudspeaker array.
Methods: Eleven male, normal hearing, young adult subjects were tested under conditions of acoustic startle, simulated TTS, simulated tinnitus, and combinations thereof, to help determine the levels of these variables necessary to negatively impact performance without the startle stimulus being loud enough to cause permanent hearing loss. Subjects were fitted with a Polhemus Fastrak electromagnetic head tracking system connected to a semi-rigid headband to measure head position, a pulse sensor to measure stress, and electromyography (EMG) sensors to measure muscle activity of the eyelid, jaw, neck, and bicep. For localization subjects were instructed to orient their head towards the position of a 50 ms duration white noise stimulus (70 dBA) played through one of the speakers in the array, in the presence of a 3 dB noise masker presented from 11 equidistantly spaced loudspeakers in the array. The Speech-In-Noise (SIN) task similarly had subjects seated in the center of the darkened chamber, and repeating target words from a version of NU-6 word list modified to remove the carrier phrase, likewise presented (~70 dBA) in the presence of the same noise masker. Combinations of three adverse listening conditions were presented: a startle eliciting stimulus (105 dBA, 40 ms white noise), when present, was played just prior to the target stimulus. TTS and tinnitus were simulated by remotely adjusting the passthrough gain (0, 15, and 30 dB attenuation) and playback of a high-pass (8 kHz cutoff, -1 and +5 dB SNR) noise audio signal through a custom Bluetooth enabled headset.
Results: Results indicated that performance on these auditory tasks can be impaired if the startle eliciting stimulus is within ~160 ms of the target, and across the three adverse listening conditions, that each condition reduces performance on localization and SIN tasks on their own, and that combinations of these conditions reduce subject performance further, with the worst performance occurring with all three main acoustic effects combined.
Conclusions: These results suggest that the startle reflex may provide a substantial contribution to the disorientation characteristic of a flashbang exposure.
This program funded by the Joint Intermediate Force Capabilities Office (JIFCO), contract N00174-17-D-0032.

Development of Gap Detection During Adolescence

Julia Huyck*[1], Lauren Cammenga[2], Allison Steinbrenner[1], Preston Wise[1], Serena Sereki[1], Jordin Benedict[1]

[1]*Kent State University,* [2]*Northeast Ohio Medical University*

Background: Most late-developing auditory skills involve the perception of time-varying sounds, such as the perception of a temporal gap in an ongoing sound. In humans, auditory skills require that the auditory signals are encoded, the listener engages with the task, and the listener is able to attend to, remember, and make decisions about what they perceived. There is evidence of prolonged development of both auditory cortex and the frontal lobe, which is largely responsible for executive functions such as attention and working memory. Therefore, we investigated the extent to which auditory cortical encoding of temporal gaps, executive functions, and listening effort contribute to performance on a gap detection task during adolescence and young adulthood.
Methods: We examined gap detection abilities in listeners between 10 and 23 years of age. All listeners had typical hearing and did not report any history of learning disabilities, neurological disorders, or auditory processing disorders. The study consisted of four main components: (1) Behavioral testing of gap detection using the method of constant stimuli; (2) Concurrent collection of listening effort data (i.e., pupillometry, blink rate, and saccades); (3) A cognitive testing battery; and (4) The recording of cortical auditory evoked potentials in response to gaps in noise while participants watched a silent movie.
Results: Preliminary analyses showed a non-significant trend towards better gap detection thresholds with increasing age. Regression modeling indicated that a combination of psychometric function slope, cognitive measures, and change in pupil size accounted for some of the variance in gap detection thresholds. Many of the cognitive measures significantly improved with age, suggesting that maturation of executive functions contributes to the development of performance on this listening task. In terms of listening effort, the number of saccades increased with increasing age, but the normalized pupil size, number of blinks, and number of saccades did not change. There were, however, more saccades on incorrect trials than correct trials. The amplitude of the N1-P2 complex to the gap in noise (i.e., the acoustic change complex), normalized to the amplitude of the N1-P2 complex to the onset of the noise, decreased with increasing age due to a larger onset N1-P2 responses in young adults compared to adolescents. The N1 of the acoustic change complex, but not the P1 or P2 latencies, showed a trend towards longer latencies in adolescents than in young adults.
Conclusions: Taken together, these results indicate that auditory cortical encoding, executive functions, and listening effort all play a role in behavioral performance on a gap detection task during adolescence and young adulthood, and that development of these underlying processes may contribute to previously observed maturational improvements in performance on temporal tasks. [Funded by NIDCD.]

Electroacoustic Pitch Matching in Cochlear Implant Users With Single-Sided Deafness

Maya Hatley*[1], Rene Gifford[2], Artur Lorens[3], Jonathan Neukam[2], Annette Lavender[1], Nicole Capach[1], Elad Sagi[1], Ariel Hight[1], Mahan Azadpour[1], Mario Svirsky[1]

[1]*New York University Grossman School of Medicine,* [2]*Vanderbilt University Medical Center,* [3]*Institute of Physiology and Pathology of Hearing*

Background: The efficacy of cochlear implants (CIs) is based on the tonotopic organization of the cochlea, where stimulation of the cochlear base elicits high-pitched percepts and pitch decreases towards the apex. However, CI electrode arrays often do not extend to the most apical cochlear regions, potentially resulting in perceptual frequency mismatch. Percepts resulting from stimulating the most apical electrodes are typically higher in pitch than those

elicited by the corresponding acoustic stimulus in a normal ear. This mismatch can lead to decreased sound quality and speech intelligibility with CIs. CI users with single-sided deafness (SSD) offer a unique opportunity to compare CI-elicited pitch to normal-ear percepts. We employed adaptive psychophysical procedures to examine pitch percepts in SSD-CI users as a function of electrode location and time since initial stimulation, while applying checks to validate electro-acoustic pitch comparisons. We also analyzed electrode pitch matching scores in relation to the spiral ganglion frequency-place function and clinically assigned center frequencies.
Methods: We studied 42 post-lingually deafened CI users with contralateral normal/near-normal hearing. Participants compared perception of CI electrode stimulation to normal-ear pure tones. For each electrode, we used four adaptive runs: 1 up-1 down (two runs with different starting frequencies), 3 up-1 down, and 1 up-3 down. We collected data on time since initial stimulation and electrode insertion angle. A conservative validity check ensured the 1 up-3 down result was highest and 3 up-1 down lowest. Analyses were performed on both the full dataset and the validated subset.
Results: Key findings from our study include:
1. 59% of the data survived the validity test, providing a robust dataset for analysis.

2. Results demonstrated clear tonotopicity, with a strong relationship between pitch perception and electrode location.

3. Unlike some previous findings, we observed no significant effect of time since initial stimulation on pitch perception.

4. Pitch matching scores were consistently higher than programmed central frequencies in the most apical electrodes tested.

5. These scores were also consistently lower than predicted by frequency-place functions commonly used in the literature.

6. Results remained consistent across the full data set and the validated subset, underscoring the robustness of these findings.

Conclusions: Our data corroborates several patterns congruent with prior literature, including the presence of basalward frequency mismatch for the most apical electrodes and the strong tonotopic relationship between electrode location and pitch perception. However, unlike some previous studies, our pitch matching data shows marked stability over time. This discrepancy may be related to our use of psychophysical procedures, which were designed to minimize potential biases. These findings highlight the need for further investigation into the long-term stability of pitch perception in SSD-CI users. Future studies should explore the relationship between pitch perception stability and speech recognition outcomes to optimize CI performance.

Pupil-linked Arousal Tracks Adaptive Auditory Belief Updating in Spatially and Temporally Dynamic Environments

Roman Fleischmann*[1], David Meijer[2], Ulrich Pomper[3], Michelle Spierings[3], Robert Baumgartner[2]

[1]*Austrian Academy of Sciences,* [2]*Austrian Academy of Sciences, Acoustics Research Institute, Vienna,* [3]*University of Vienna*

Background: Auditory perception is subject to sensory noise and rapidly changing environments. To deal with ambiguous input, the auditory system needs to find the correct balance between flexibility and robustness, integrating sensory input with prior beliefs. Bayesian perceptual inference determines the statistically optimal solution. The locus coeruleus (LC) arousal system is suggested to mediate belief updating by amplifying the weight of sensory input through the release of noradrenaline (NA) at the cost of prior beliefs.
Methods: We presented 49 participants (split in two groups for two experiments) with auditory sequences of random lengths, inducing either temporal movement (acceleration or deceleration, via manipulation of stimulus onset asynchronies, SOAs) or spatial movement (clockwise or counterclockwise motion, via manipulation of the stimulus's horizontal localization), while keeping the other dimension constant. Participants were tasked with discriminating the last direction of spatial or temporal movement in a two-alternative forced-choice design. Sporadic change-points (CPs) within the sequences forced strong belief updating to maintain precise perception in the face of change. To increase ecological validity compared to common CP paradigms, we varied the level of sensory evidence continuously and at a rapid pace to elicit a broad spectrum of continuous, fast, online belief updates. We designed the experiment as a low-level perception task, asking participants only for discrimination, not for explicit predictions. We recorded task-evoked pupil sizes via pupillometry as a proxy for LC-NA activation. A Bayesian CP model was fitted to the behavioral responses to estimate momentary surprisal, a precision-weighted quantification of the prediction error.
Results: We showed surprisal to predict task-evoked pupil size on a rapid stimulus-to-stimulus level. Importantly, the relationship between surprisal and pupil size proved to be independent of the tested domain.
Conclusions: The results support the notion of a modulation of auditory belief updating by the LC-NA arousal system, independently of perceptual domain. The result further supports the notion of rapid online adaptation to volatile environments in auditory perception.

Auditory Attention Decoding for Selective Hearing: Bridging Metrics and User Experience

Vishal Choudhari*[1], Kiki Van der Heijden[2], Xiaomin He[1], Nima Mesgarani[1]

[1]*Columbia University,* [2]*Radboud University*

Background: People who are hard of hearing often struggle to follow a single speaker in environments with multiple talkers. Current hearing aids are unable to interpret the wearer's intent and, as a result, cannot selectively amplify the desired speaker. Future smart hearing devices are envisioned to continuously interpret the wearer's intent, allowing them to enhance the voice of the person they are focusing on in such environments.
Several methods have been proposed to decode a listener's focus, known as auditory attention decoding (AAD). The most studied approach involves using brain activity to determine the wearer's focus. However, fundamental questions about the requirements for this technology remain unanswered. Specifically, it is unclear how objective measures like decoding accuracy and speed relate to the user's subjective experience. Additionally, how these requirements vary across different listening contexts and between normal hearing (NH) and hearing impaired (HI) listeners is poorly understood.

Methods: To address these gaps, we conducted three online psychoacoustic experiments. Participants were presented with complex auditory scenes containing two simultaneous

conversations, and they were instructed to follow one conversation. The selected conversation was enhanced using various parameters that were hidden from the participants. Across the experiments, we examined how different parameters influenced both objective user experience (such as word detection and speech intelligibility) and subjective user experience (ratings).

Experiment 1 focused on determining the optimal level of suppression of the unattended speaker (measured as target-to-masker ratio, TMR) to maximize speech intelligibility of the attended talker, while maintaining sufficient audibility of the unattended talker to allow attention shifts.

Experiment 2 investigated the relationship between intent decoding accuracy and both objective and subjective user experience.

Experiment 3 explored how the combination of decoding accuracy and speed affects user experience in contexts demanding frequent and infrequent attention shifts.

Results: We show that the preference for selective hearing technology increases as listening conditions become more challenging and decoding accuracy improves. Interestingly, normal-hearing individuals value this technology just as much as those with hearing impairments. Additionally, decoding speed became more important when decoding accuracy was high, particularly in scenarios involving multiple attention shifts.
Conclusions: Our findings demonstrate how various parameters influence both objective and subjective user experience differently for NH and HI listeners. Moreover, we observed that the impact of these parameters depends on the characteristics of the listening environment and the specific context. Crucially, our findings highlight the importance of identifying the requirements of this technology for different user groups and use cases in order to develop effective real-world applications.

How Working Memory Capacity and Cognitive Load Influence Spoken Word Processing: Evidence From Eye-Tracking and Pupillometry

Gal Nitsan*[1], Boaz M. Ben-David[2], Karen Banai[1]

[1]*University of Haifa,* [2]*Reichman University (IDC) Herzliya*

Background: Difficulties understanding speech are a significant concern among older adults. World Health Organization report (2021) highlights maintaining functional abilities, such as forming relationships, learning and decision-making, is crucial for healthy aging. Successful speech perception plays a vital role in preserving these abilities.
The literature shows an association between individual differences in cognitive factors, particularly working memory, and speech perception, even among young adults with normal hearing. These differences are pronounced mainly when using complex testing materials (i.e., sentences). In contrast to the consensus regarding the association between cognitive factors and spoken sentence processing, there is limited and mixed evidence regarding this association at the single-word level. By focusing on single-word perception, we aim to provide clearer insights into the mental effort involved in speech processing under challenging conditions. The current study aims to examine the effect of working memory capacity and working memory load on spoken word processing in noise. Specifically, we investigate how working memory influences spoken word processing and listening effort in

adverse listening conditions among young and old listeners with varying working memory capacities.

Methods: Using the Visual World eye-tracking paradigm combined with pupillometry, 36 young listeners were instructed to press on objects displayed on a monitor in response to a spoken word presented at a -4dB SNR. Concurrently, listeners performed a working memory load task, retaining either a low (single spoken digit) or high (four digits) memory load for later recall. The combination of eye-tracking and pupillometry has been employed in relatively few studies. This method provides fine-grained information about the time course of spoken word processing and an objective assessment of listening effort. Data collection is currently ongoing with a group of older listeners.
Results: Behavioral accuracy results indicated that, under high memory load, working memory capacity had a positive effect on task performance, with higher capacity linked to higher accuracy. A similar trend was observed under low memory load though it was not statistically significant. Pupil response analysis revealed a distinct relationship between working memory capacity and pupil dilation, varying across load levels. Two opposite trends were observed: under high load, a positive slope was found, with pupil size increasing as working memory capacity increased, indicating greater effort. In contrast, under low load, a negative slope was observed, with pupil size decreasing as working memory capacity increased, reflecting either reduced effort or the task being overly challenging.
Conclusions: These findings suggest that individuals with higher working memory capacity are more resilient to cognitive load than those with lower capacity.

Hidden in Plain Sight: Facial Signatures of Auditory Cognition and Hearing Disorders

Samuel Smith*[1], Jenna Sugai[1], Daniel Polley[1]

[1]*Eaton-Peabody Laboratories, Massachusetts Eye and Ear*

Background: Charles Darwin's 1872 publication "The Expression of the Emotions in Man and Animals" founded a second, albeit less well known, scientific field of research. Facial expressions have a long history in behavioral psychology where rapid, involuntary facial movements such as the curvature of the mouth or a furrowing of the brow have been elicited with visual stimuli to reveal underlying affective states. Even for those with an unflinching poker face, autonomic changes related to respiration and blood flow can still be identified from the human face to indicate affective processing. Here we asked whether sound elicits facial movements, developed a data analysis pipeline to quantify these measures, and identified new signatures of tinnitus, hyperacusis, and misophonia that were uniquely identifiable through analyses of facial movements.
Methods: 181 adults with varying degrees of hearing loss were recruited from neurotypical and disordered hearing (i.e., tinnitus sufferers, sound sensitivities) populations. Participants performed two tasks: (i) listen to and rate a bank of emotionally evocative sounds drawn from the IADS database, and (ii) a digits-in-noise task. Simultaneously, we performed high-speed videography of the subjects' face (Genie Nano-M2020 camera, 16mm IP/CCTV lens). We developed a custom pipeline that extracts facial coordinates, localized movement, ocular activity and blood flow. We also performed separate eye-tracking and heart rate measurements with specialized equipment (EyeLink 1000 Plus, BioSemi ActiveTwo) serving as ground-truth.

Results: We found that emotionally evocative sounds elicited rapid and subtle facial movements that scaled with self-reported valence. Movement patterns were differentiable when participants were grouped by age, degree of hearing loss, and disorder severity. Video-derived estimates of pupil size and heart rate matched ground-truth recordings and were modulated by difficulty on the digits-in-noise task. Fitting of temporal response functions revealed how the acoustic properties of sound stimuli correspond to distinct spatiotemporal features of facial movements.
Conclusions: Recent advances in computer vision have enabled the quantitative assessment of subtle movement (and other) signals hidden within the human face. Here, we spotlight how the human face can assay auditory processing and cognition. These findings identify a cost-effective tool for auditory measurements implementable in the research lab, the clinic, but also in the home. Notably, facial videography captures emotional and social qualities of hearing that are tricky to otherwise measure but distinguish clinical subpopulations, such as those with misophonia or bothersome tinnitus.

Measuring and Modeling Multi-Source Environmental Sound Recognition

Sagarika Alavilli*[1], Josh McDermott[2]

[1]*Harvard University,* [2]*MIT*

Background: Was that my phone ringing? Humans perform environmental sound recognition throughout their daily lives. Despite being a routine task, environmental sound recognition remains poorly understood, partly due to limited research on human sound recognition in natural scenes with multiple sources. We aimed to better characterize this aspect of human perception with large-scale experiments measuring sound recognition in naturalistic multi-source scenes. In parallel, we built models of environmental sound recognition by combining machine learning with biologically inspired models of peripheral auditory processing. The models were then compared to the human behavioral results.
Methods: Human participants performed a sound recognition task. Participants listened to auditory scenes composed of between 1 and 5 natural sounds drawn from 51 different sound classes (applause, shattering, laughter, etc.). Following each scene presentation, participants judged whether a specific queried sound class was present in the scene. To build candidate models of environmental sound recognition, we trained deep neural network models on a sound classification task using the same 51 sound classes. Sound input was passed through a fixed model of the cochlea (bandpass filters, half-wave rectification, and lowpass filtering) followed by a neural network whose output was interpreted as the probability that each sound class was present in the scene.
Results: On average, human listeners performed well above chance, but performance decreased as the scene size increased. The models replicated both overall human performance and the dependence on scene size. Humans also consistently recognized some classes of sounds better than others. The model's performance for individual sound classes captured much of the variance in human performance across classes.
Conclusions: We collected human environmental sound recognition judgments that will be part of a benchmark with which to evaluate models in this domain. The results illustrate that models optimized for environmental sound recognition capture aspects of human perception. These results set the stage for future explorations of auditory scene perception involving salience, attention, and memory.

Podium 20: Frontiers in Auditory Prostheses

Moderators: Thomas Talavage and Athanasia Warnecke

10:30 a.m. - 12:30 p.m.

Ocean Ballroom 1 - 4

A Computational Model of the Electrically or Acoustically Evoked Compound Action Potential and Electrocochleography in Cochlear Implant Users With Residual Hearing

Waldo Nogueira*[1], Yixuan Zhang[1], Daniel Kipping[1]

[1]*Hannover Medical School*

Background: In cochlear implant users with residual acoustic hearing, compound action potentials (CAPs) of the auditory nerve can be evoked by both transient acoustic (aCAP) and electric (eCAP) stimulation, and recorded via the implant's electrodes. Additionally, electrocochleography (ECochG) responses from inner and outer hair cells can be elicited by acoustic tone bursts. The contributions from hair cells and the auditory nerve are superposed, complicating their separation. Furthermore, simultaneous electric and acoustic stimulation leads to interaction effects, thereby adding to the complexity of the observed signals.
Methods: We present a computational model to simulate aCAP, eCAP, and ECochG responses in human cochlear implant users. The model accounts for the contributions of both hair cells and the auditory nerve, as well as the interaction between combined electric-acoustic stimulation in the auditory nerve. It comprises three main elements: a 3D finite element method model of the implanted cochlea, a phenomenological single-neuron spiking model for electric-acoustic stimulation, and a physiological multi-compartment neuron model. The model simulates the membrane currents in inner and outer hair cells, as well as in the spiral ganglion neurons, to predict the contribution of these components to the CAP and ECochG responses. The recorded voltage on the cochlear implant electrodes is then predicted using the 3D model.
Results: The simulated waveforms of eCAP, aCAP, and ECochG were consistent with those observed in human recordings. Furthermore, the amplitude growth functions predicted by the model corresponded well with empirical data. The model also replicated suppressive interaction observed during simultaneous electric and acoustic stimulation, demonstrating its capacity to simulate the combined effects of both stimulus modalities.
Conclusions: The proposed model provides a framework for simulating CAP and ECochG responses to electric, acoustic, and combined electric-acoustic stimulation. It incorporates the spatial dependence of stimulation and recording sites in the cochlea and captures the interaction between electric and acoustic stimulation within the auditory nerve. This model may contribute to the refinement of objective assessment methods involving CAPs and ECochG in cochlear implant users.

Cochlear Implantation Outcomes in Genotyped Subjects With Sensorineural Hearing Loss

Cris Lanting*[1], Mirthe Fehrman[1], Wendy Huinck[1], Emmanuel Mylanus[1], Helger Yntema[1], Lonneke Haer-Wigman[1], Hannie Kremer[1], Ronald Pennings[1]

[1]*Radboud University Medical Center,*

Background: Cochlear implantation (CI) is an effective rehabilitation for individuals with severe-to-profound sensorineural hearing loss (SNHL). Although genetic factors contribute significantly to SNHL, the variability in CI outcomes is not fully understood. This study aimed to assess CI outcomes in a large, genotyped cohort, investigating the relationship between specific genetic causes and cochlear site-of-lesion.
Methods: This retrospective study included 220 genotyped individuals with hereditary SNHL who underwent CI at Radboud University Medical Center from 2002 to 2021. Audiological outcomes were measured pre- and post-implantation, and cochlear site-of-lesion was categorized as pre-synaptic, post-synaptic, or mitochondrial based on gene function or expression level. Multiple regression analysis assessed factors influencing CI outcomes, including age of implantation, duration of SNHL, and cochlear site-of-lesion.
Results: CI outcomes were generally excellent, with a median phoneme score of 90%. Early implantation (≤6 years) led to significantly better performance compared to late implantation. Variability in outcomes was not associated with the cochlear site-of-lesion but with subject-specific factors such as age at implantation and CI experience. Poor performers (phoneme scores LESS THAN 70%) were often those with prolonged auditory deprivation before implantation or older age at implantation.
Conclusions: Genotyped CI recipients showed overall favorable outcomes, with the variability largely explained by non-genetic factors such as age at implantation and duration of hearing loss. While no correlation was found between cochlear site-of-lesion and CI outcomes, future research is needed to explore genetic influences on CI performance. Findings emphasize the importance of early implantation for optimal outcomes.

Cochlear Implants With Dexamethasone-Eluting Electrode Arrays Reduce Foreign Body Response in a Murine Model of Cochlear Implantation and Human Subjects

Muhammad Rahman*[1], Brian Mostaert[1], Peter Eckard[1], Shakila Mahmuda Fatima[1], Rachel Scheperle[1], Md Ibrahim Razu[1], Bryce Hunger[1], Rafal Olszewski[2], Shoujun Gu[2], Cristina Garcia[1], Nashwaan Ali Khan[1], Douglas M Bennion[1], Jacob Oleson[1], Ya Lang Enke[3], Jonathon Kirk[3], Robert Gay[3], Robert Morell[4], Keiko Hirose[5], Michael Hoa[2], Alexander Claussen[1], Marlan Hansen[1]

[1]*The University of Iowa,* [2]*Auditory Development and Restoration Program, Neurotology Branch, National Institute on Deafness and Other Communication Disorders,* [3]*Cochlear Limited, Sydney, Australia,* [4]*Genomics and Computational Biology Core, National Institutes on Deafness and Other Communication Disorders,* [5]*Washington University School of Medicine*

Background: The inflammatory foreign body response (FBR) following cochlear implantation (CI) can negatively impact CI outcome. This study aims to investigate the long-term efficacy of dexamethasone eluting cochlear implant and locally delivered dexamethasone, a potent anti-inflammatory glucocorticoid on the intracochlear FBR and electrode impedance post-implantation in a murine model and human subjects.
Methods: The left ears of 10-12-week-old CX3CR1+/GFP Thy1+/YFP (macrophage-neuron dual reporter) mice on B6-background were implanted with dexamethasone-eluting cochlear implants (Dex-CI) or standard implant (Standard-CI) while the right ear served as unoperated

control. Another group of dual reporter mice was implanted with a standard CI electrode array followed by injection of dexamethasone (10mg/kg) in the middle ear to mimic current clinical practice (Dex-local). Mice electrodes were stimulated between 7- and 28 days post-CI. Following the euthanasia of mice at 10, 28, 56, or 112 days post-operatively, harvested cochleae fixed with 4% PFA were processed and cryosectioned at 30μm parallel to the mid-modiolar plane. Sections labeled with anti-α-Smooth Muscle Actin (α-SMA) and anti-MHCII antibodies were used to quantify intracochlear fibrotic response and MHCII-mediated antigen presentation, respectively. Manually traced outlines of the scala tympani, Rosenthal canal, and lateral wall for each turn were used to measure the volume of each area. The density of nuclei (labeled with Hoechst 33342), CX3CR1+ macrophages, Thy1+ spiral ganglion neurons (SGNs), and CX3CR1+MHCII+ antigen-presenting cells were calculated. The fibrotic response was calculated by measuring the ratio of the volume of α-SMA-positive fibrotic tissue and the volume of scala tympani. Human subjects were implanted with either standard (n=7) or Dex-CI (n=15) followed by serial impedance measurements.
Results: In the murine model, a reduction in electrode impedance was observed in the dexamethasone-eluting group. Dex-local does not significantly reduce electrode impedance. Infiltration of cells, macrophages, and fibrotic tissue into the cochlea implanted with standard CI was associated with no appreciable SGN degeneration. Dex-CI significantly reduced inflammatory FBR compared to Standard-CI until 112 days post-CI. Dex-local is partially effective for mitigating FBR post-CI for the first 10 days post-CI. In human studies, Dex-CI significantly reduced electrode impedance corroborating murine model findings.
Conclusions: Dex-CI reduced electrical impedance in the murine model and human subjects and inflammatory FBR in the murine model for an extended period. Dex-local in the murine model is ineffective for long-term reduction of FBR and electrode impedance. Our data suggests that Dex-CI can potentially be more effective than the current clinical practice of dex-local in reducing FBR and electrical impedance.

Four-Dimensional Computed Tomography of Cochlear Implantation: A Synchrotron-Based Feasibility Study

Seyed Alireza Rohani*[1], Franziska Niemann[2], Ashley Micuda[1], Ning Zhu[3], Sergey Gasilov[3], Masoud Zoka Assadi[2], Sumit K. Agrawal[1], Hanif M. Ladak[1]

[1]*Western University,* [2]*MED-EL,* [3]*Canadian Light Source Inc.*

Background: Atraumatic insertion of the electrode array is crucial for optimal performance of cochlear implants (CIs). Recently, the trajectory of CI electrode insertion has become a topic of interest. However, studies are often limited to computed tomography (CT) imaging of the final insertion location. Two-dimensional fluoroscopy has been reported to continuously monitor the insertion, while lacking three-dimensional (3D) information of the cochlear internal structure. Time-resolved four-dimensional CT (4DCT) imaging of cochlear implantation is challenging due to anatomical complexities and the presence of the metallic electrode array. Our group previously reported the application of synchrotron-based imaging techniques on implanted samples under static conditions. The objective of this study was to investigate the feasibility of using synchrotron-based techniques for 4DCT of the electrode array insertion.
Methods: A custom-built apparatus was designed to insert an electrode array while mounted on a rotating stage, which is used for synchrotron-based imaging. The imaging was conducted at the BMIT-BM beamline at The Canadian Light Source Inc. Multiple combinations of insertion speed and imaging protocols were tested on a phantom to

determine the optimal setup for imaging the CI electrode trajectory. The optimized setup was then tested on a fixed cadaveric cochlea.
Results: Key milestones, challenges, and optimized imaging protocols for imaging cochlear implantation were investigated. Insertion of an electrode array in one cadaveric cochlea was visualized at 10 overlapping CT scans per second using high-speed 4DCT. The precise location of each contact plate, in relation to critical anatomical features of the cochlea, was visualized during insertion at the pixel size of 6 μm, with the high contrast provided by synchrotron-based imaging.
Conclusions: To the best of the authors' knowledge, this is the first study to report cochlear electrode insertion in such detail, providing unprecedented insights about the movement of an electrode array inside the cochlea. This technique could be further utilized to investigate the impact of various factors on the quality of cochlear implantation, such as implant length, insertion speed, and insertion angle.

Audio Recordings With a Fully-Implanted Microphone for Cochlear Implants

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Background: Achieving a fully-implanted cochlear implant relies on the invention of a high-sensitivity and low-noise implanted microphone. We present a microphone implanted in the middle ear and demonstrate its excellent performance in human cadaveric ears. The microphone, called the UmboMic, is a piezoelectric sensor that detects motion of the umbo. We choose to target the umbo because its large unidirectional motion produces a well-represented sound signal at most auditory frequencies. We have previously reported the UmboMic's high sensitivity, low noise, and good linearity. In this work, we present extensive audio recordings taken with the UmboMic in a cadaveric ear.
Methods: We use photolithography and thin-film deposition to fabricate the UmboMic sensors in a nano-microfabrication facility at the Massachusetts Institute of Technology. Audio recordings are performed on an air table in an electrically- and acoustically- isolated sound chamber at Mass Eye and Ear. Human cadaveric temporal bone specimens are prepared via a mastoidectomy to access the middle-ear cavity and the UmboMic sensor is held in the middle-ear using a stable clamp. The middle ear is almost closed off with putty. A funnel is attached to the bony ear canal to reproduce the pressure gain of the outer ear. Speakers and musicians stand in the sound chamber and produce sound from about a meter away.
Results: Previous measurements demonstrate that the UmboMic has a low noise floor of approximately 62e-18 C from 200 Hz to 20 kHz. At 2 kHz the UmboMic has a sensitivity of 230e-15 C/Pa. Given these results, the UmboMic has an equivalent input noise comparable to high end commercial hearing aid microphones. This performance is reflected in a diverse range of audio recordings. The recordings include samples of 18 different people speaking in their native tongue, live violin and guitar music, singing, and concurrent speaking. Audio recordings are clear and speakers and instruments are recognizable. Opinions among evaluators are that the recording with our system seems indistinguishable from recordings with a high-end reference microphone.

Conclusions: Collection of audio recordings aids the interpretation of the UmboMic's performance as an implanted microphone. Sound produced by voice and non-electric instruments cannot suffer electrical coupling to the recordings. Given the quality of audio recordings from the UmboMic's system and its objectively high sensitivity and low noise floor, we believe the UmboMic to be a viable microphone for use with fully-implanted cochlear implants.

"First in Man" Optoacoustic Stimulation of the Peripheral Hearing Organ

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Background: The activation of the peripheral hearing organ using optoacoustic generated sounds is a novel method that has been considered in past years as an alternative to the mechanical and electrical stimulation to compensate for hearing loss. Our work focuses on the principle of optoacoustic as the strategy to induce auditory perception in the peripheral hearing organ using light energy. In previous animal studies, we demonstrated that it is possible to elicit auditory evoked potentials (AEP) in mice using an amplitude modulated laser pulse train. AEP amplitudes reached ones elicited by 70dB SPL clicks. Furthermore, we have performed biocompatibility studies, resulting in the power limits for our first-in-man study. At this point, the question arises if this technique performed in the animal model can be translated to humans. We therefore herein present our first data regarding the optoacoustic stimulation of the peripheral hearing organ in humans.
Methods: 3 healthy adults between 23 and 28 years, with normal hearing volunteered to enter our "first-in-man" study that has been approved by the "Ethic committee of Saarland". We had 2 female and one male participants. All experiments have been performed in a sound proof room. Pure tone audiometry has been performed pretesting. 10 sets of 3 different frequencies (440, 523 and 659 Hz) translated in laser pulses have been presented at a constant level of about 35dB SPL. Additionally, ten sets of 3 different intensities (27 dB SPL, 35 dB SPL and 40 dB SPL) at constant frequency of 500 Hz, translated in laser pulses have been presented to the participants. The third test was constructed out of applying 3 different melodies translated into laser pulse trains. The stimulation has been performed using a 365 µm laser fiber (THORLABS) and the laser pulses have been applied onto an absorbing film positioned in the cavum conchae.
Results: The different frequencies have been distinguished in an average of 90 %, the different sound intensities in an average of 93 % and all participants recognized all three melodies. The testing was very well tolerated from all participants and no side effects including burning, heating or further later onset complications could be observed.
Conclusions: We demonstrated for the first time in humans that the activation of the auditory system using optoacoustic stimulation is possible. Our data demonstrates as well through basic psychoacoustic tests that the central auditory cortex perceives theses activation as intelligible signals. The results demonstrate a good distinguishability between different frequencies and intensities as well as understanding of more complex signals like music. This novel stimulation strategy demonstrated therefore to be as a very potent candidate for a new generation of auditory protheses for which however, further R and D work is still needed.

Auditory Nerve Penetrating Electrode Device Stability and Nerve Function following Chronic Implantation

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Background: Cochlear implants are a transformative treatment for individuals with sensorineural hearing loss. Difficult listening tasks such as hearing in noise, however, have proven challenging due to poor neural selectivity of intracochlear electrical stimulation. We are developing an intraneural auditory nerve implant (ANI) device for direct microelectrode stimulation to improve selectivity, decrease power requirements, and ultimately improve hearing outcomes. In this study, we evaluate the safety and performance of the ANI device after chronic implantation and electrical stimulation in a feline model. This custom ANI device has three key features: a fifteen slanted electrode array, wire bundle, and pedestal. A final device for chronic human use will not terminate in a pedestal, but will instead be integrated with a MED-EL Synchrony 2 stimulator.
Methods: The feline ANI device was surgically implanted in the right auditory nerve of three felines at the University of Utah. Immediately post-implant measurements of impedances and electrically elicited auditory brainstem responses (eABRs) confirmed the devices were successfully implanted: impedances were low (1-100kΩ with Tucker-Davis Technologies hardware) for 44/45 electrodes, and eABRs were measured across 42/45 electrodes across three felines. After implantation, recording and stimulation sessions in each feline occurred every two weeks for a total of fourteen weeks (three months). During each session, electrode impedances and eABRs were recorded, followed by a two-hour block of consistent electrical stimulation (20 nC/phase, 100 µA x 200 µs per phase, 166 pps) across all electrodes. These stimulation parameters were on the maximum end of what was tested to be safe from previous studies. The two hours of electrical stimulation was delivered with a MED-EL Pulsar 2 stimulator in custom housing.
Results: Impedances were stable throughout the study for most electrodes (42/45 below 100kΩ). EABRs were elicited on nearly all electrodes for two of the three felines (28/30). One feline showed a steep decrease in the number of eABRs elicited between implantation (14/15 eABRs) and the first session (5/15 eABRs). The pattern of non-functional electrodes suggests device migration rather than device failure or nerve damage.
Conclusions: These results suggest the ANI device is capable of stimulation for three months in a feline model. This study provides a scientific foundation for future translational human studies.

A Stable and Broad Frequency-Selective Cochlear Nucleus Implant Using Penetrating Ultra-Flexible Electrode Arrays

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Medicine; Shanghai Key Laboratory of Translational Medicine on Ear and Nose Diseases, [3]*Institute of Neuroscience, Center for Excellence in Brain Science and Intelligence Technology, Chinese Academy of Sciences*

Background: Electrical prostheses in the central auditory pathway hold promise for restoring hearing in patients with profound hearing loss who are ineligible for cochlear implants. Current brainstem implants need to improve spatial resolution of electrical stimulation and access to the tonotopic organization of the cochlear nucleus (CN).
Methods: Here we used UltraFlexible Implantable Neural Electrode arrays (uFINE) to construct a flexible penetrating Auditory Brainstem Implant (uFINE-ABI) that enabled stimulation with broad-spectrum coverage, precise frequency tuning, and long-term stability. The efficacy for tonotopic stimulation of uFINE-ABI in CN was shown in rats with normal hearing by implanting another uFINE in the inferior colliculus (IC) downstream of CN.
Results: Each pure-tone stimulus evoked specific response patterns in CN and IC, with characteristic frequencies (CFs) ranging from 0.5 to 32 kHz. Furthermore, tonotopic CN stimulation evoked tonotopic IC responses that largely mimicked those evoked by pure tones and remained stable for at least 136 days. The average frequency width in IC at 5 dB above the stimulation threshold was 7.1 ± 4.7 octaves for CN stimulation and 1.7 ± 1.9 octaves for acoustic stimuli. Notably, tonotopic IC responses evoked by CN stimulation could be sharpened by 30 min daily sequential burst stimulation of CN and IC neurons (at 10 msec) for a week at corresponding electrode sites, consistent with spike-timing-dependent plasticity of CN-IC synapses.
Conclusions: These findings support the feasibility of uFINE-ABI for tonotopic stimulation with high-frequency resolution over a broad spectrum, and synaptic plasticity of central auditory pathways could allow further use-dependent consolidation of ABI-imposed tonotopic map for hearing restoration.

Podium 21: Transcription, Metabolomic, and Cellular Dynamics in Inner Ear Development: Mice and Human Organoids

Moderators: Alain Dabdoub and Benkafadar Nesrine

10:30 a.m. - 12:30 p.m.

Ocean Ballroom 5 - 8

Constraint-Based Modelling and Functional Imaging Reveal that Metabolic Reprogramming Drives Tonotopic Development in the Murine Organ of Corti

James O'Sullivan*[1], Claire Scott[1], Daniel J. Jagger[2], Anwen Bullen[2], Zoe Mann[1]

[1]*King's College London,* [2]*UCL Ear Institute*

Background: In the mammalian inner ear, the frequency selectivity of sensory hair cells (HCs) is correlated with their spatial position along the cochlea's apico-basal long axis (tonotopy). Despite an increased understanding of the molecular cues that establish positional identity, precisely how these cues are "interpreted" to coordinate the variable characteristics of mature HCs along the tonotopic axis remains unclear. Multiple transcriptomic data across taxa and functional evidence from the avian auditory epithelium implicate metabolic

reprogramming as a key driver of cell differentiation and maturation. Therefore, we aimed to characterise the metabolic phenotypes of developing HCs and their surrounding glial-like supporting cells (SCs) in the mammalian cochlea. Having characterised metabolism along the tonotopic axis we next investigated the effects of disrupting specific metabolic pathways on normal HC development and patterning.
Methods: We utilised Metabolic Reaction Enrichment Analysis (MaREA), a computational approach which utilises transcriptomic data to perform constraint-based modelling of metabolism to analyse singe cells in the developing mouse cochlea. We filtered single cell transcriptomes of HCs and SCs between E16 and P7 from previously published data (Kolla et al (2022)) and leveraged MaREA to formulate predictions of metabolic reprogramming in developing HCs and SCs. We carried out functional characterisation of mitochondrial activity and respiratory capacity in live cochlear explants using tetramethyl rhodamine methyl ester (TMRM) and used quantitative immunofluorescence of IDH3A to infer TCA cycle activity. Fluorescence of the mitochondrial outer membrane in the transgenic mito::mKate2 reporter mouse line was used as a control for mitochondrial mass. Mitochondrial metabolism was modulated pharmacologically in ex vivo explant culture of the developing cochlea by administration of the uncoupling agent CCCP, as well as the addition of α-ketoglutarate.
Results: MaREA analysis predicted a metabolic trajectory in HCs between E16 and P7, consistent with an increased metabolic flux in the TCA cycle early in development, followed by recruitment of the mitochondrial electron transport chain by P7. In addition, folate and fatty acid metabolism were consistently upregulated in HCs compared to SCs. Quantitative immunofluorescence of IDH3A, as well as fluorescence of TMRM and measurements of respiratory capacity in live tissue explants verified our modelling predictions using MaREA. These findings reveal a dependence of TCA cycle activity between E16 and P1 followed by a progressive switch to mitochondrial metabolism between P1 and P7. Elevated fluorescence intensity of mito::mKATE2 suggested this increase in mitochondrial activity is attributable to elevated mitochondrial biogenesis in basal cochlear HCs. Disturbing this metabolic switch with CCCP and conversely encouraging it with supplementary fatty acids and TCA cycle intermediates resulted in modulation of HC morphology along the tonotopic axis.
Conclusions: Transcriptomic modelling, functional imaging and experimental perturbations suggest that metabolic reprogramming in essential for correct tonotopic patterning in the developing mouse cochlea.

Targeted Cell Interconversions Reveal Inner Hair Cell Induction of Supporting Cell Identity and Distribution in the Organ of Corti

Ignacio Garcia-Gomez*[1], Jemma L. Webber[2], Berta Soria-Izquierdo[1], John C. Clancy[1], Yingjie Zhou[1], Trevor D.M. Harriman[1], Charles P. Murphey[1], Anne Duggan[1], Mary Ann Cheatham[1], Jaime García-Añoveros[1]

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Background: The organ of Corti is an intricate mosaic of hair and supporting cells divided into two compartments, each with distinct cell subtypes. In the inner compartment (ic), IHCs are surrounded by inner border (IBC) and phalangeal (IPhC) supporting cells (SCs). In the outer compartment (oc), OHCs are supported by Deiters (DC) and outer pillar (OPC) cells. Inner pillar (IPC) cells separate both compartments. Two transcription factors, TBX2 and INSM1, are involved in determining the identity of IHCs vs OHCs. We wondered how the corresponding SCs are specified and distributed and whether IHCs and OHCs play instructive

roles. To that end, we examined the effects on SCs of converting OHCs into IHCs and vice versa.
Methods: We examined Insm1 cKO mice (Atoh1-Cre;Insm1(F/F)), in which many OHCs convert embryonically into IHCs (termed oc-IHCs), such that the outer compartment contains a mixture of OHCs and oc-IHCs. Conversely, we examined Tbx2 cKOs in which OHCs convert into IHCs (ic-OHCs) either embryonically (Atoh1-Cre;Tbx2(F/F), Gfi1-Cre;Tbx2(F/F), and Fgf8-CreER;Tbx2(F/F) with tamoxifen at E14.5) or postnatally (Fgf8-CreER;Tbx2(F/F) with tamoxifen after birth). Finally, to determine which effects on SCs induced by hair cell swapping were due to FGF8, which is secreted by IHCs, we examined SCs of Pax2-Cre;Fgf8(F/F) and Emx2-Cre;Fgf8(F/F) mice
Results: In Insm1 cKOs, oc-IHCs were surrounded by oc-SCs, whereas in Tbx2 cKOs ic-OHCs were surrounded by ic-SCs. However, in oc-IHC-containing Insm1 cKOs, DCs converted into OPCs. Conversely, embryonic (but not postnatal) replacement of IHCs by ic-OHCs in Tbx2 cKOs caused conversion of OPCs into DCs. A continuous row of IPCs was generated in both animal models, but IPC number was reduced by embryonic (not postnatal) conversion of IHCs into ic-OHCs in Tbx2 cKOs.
IPhCs in Insm1 cKOs extended cytoplasmic projections throughout life that crossed the IPC row and contacted the oc-IHCs. Unexpectedly, in embryonically (but not postnatally) generated Tbx2 cKOs, ic-OHCs were wrapped by cells in the position of IBCs, but those in the IPhCs position were missing.

DC to OPC conversions in Insm1 cKOs were prevented by blocking FGF signaling. Conversely, embryonic FGF8 removal mimicked the effects of embryonic Tbx2 cKOs on OPC to DC conversion but had no effect on IPhCs.

Conclusions: Hair cell type does not determine the inner and outer identity of their surrounding SCs. However, IHCs exert three effects on SCs: (1) forming OPCs at the expense of DCs by embryonic FGF8 signaling; (2) determining abundance, but not identity, of IPCs; and (3) forming presumed IPhCs, but not IBCs, during embryogenesis and subsequently attracting cytoplasmic extensions from IPhC to wrap around them. In these ways, developing IHCs help orchestrate the assembly of the organ of Corti.

Investigating the Molecular Properties of the Stria Vascularis

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Background: Developing biological solutions for inner ear degeneration is essential to advancing treatments for hearing loss. Our study focuses on the stria vascularis (SV), a highly vascularized tissue that lines the lateral wall of the cochlea. The SV plays a critical role in generating the endocochlear potential, which is vital for hearing, and maintaining the blood-labyrinth barrier, which prevents pathogenic infiltration into the cochlea. Degeneration of the SV, caused by aging, ototoxic drugs, and genetic diseases, disrupts cochlear homeostasis, leading to progressive and irreversible hearing loss. Despite its importance, there are currently no treatments for SV-associated hearing loss and research on SV regeneration remains limited. To address this gap and advance the development of regenerative therapies for SV-related hearing loss, we conducted single cell RNA sequencing to uncover the molecular mechanisms governing the SV. Additionally, we developed a new in vitro platform to study the SV in young and mature mice. Lastly, we performed single nucleus multiome sequencing on the human fetal SV to gain insight into developing translational therapies for SV-associated hearing loss.
Methods: We performed single cell RNA sequencing using SV from four P1 and four P30 CBA/J mice. We performed single nucleus multiome sequencing using SV from one gestational week (W) 18-20 human fetus. We conducted sequencing using 10X Genomics protocols, and we conducted downstream analysis using R. We developed an organotypic explant technique to isolate and culture whole SV and associated vasculature. We used n = 10 male and female CD1 mice from three independent litters at postnatal day (P) 0-36. We cultured the explants on Matrigel coated plates for 72 hours in the presence of BrdU at 3.5 µg/mL. To investigate Wnt/beta-catenin signaling, we developed a dose-response curve of the TCF/LEF inhibitor, FH535, to determine its effects on SV proliferation.
Results: Single cell RNA sequencing identified proliferative cells, developmental trajectories, and key genes, transcription factors, and pathways unique to P1 and P30 SV, which may be involved in SV proliferation, development, and maintenance. To further investigate and validate these molecular properties, we developed an in vitro culture system that accurately models the SV. This system successfully recapitulates the proliferative behaviour of the SV found in vivo and as revealed by single cell RNA sequencing, while also proving robust to pharmacological intervention. Finally, we uncovered transcriptomic differences between the mouse and human SV, offering critical insights for translational research.
Conclusions: Our single cell RNA sequencing data in mice, combined with our novel experimental platform for culturing the whole SV, and our single nucleus multiome data in human fetal SV, provides valuable new insights into the molecular mechanisms of the SV. These findings lay the groundwork for developing biological solutions to address SV-associated hearing loss.

Vestibular Ganglion Neuron Organization and Peripheral Targeting Are Regulated by Their Birthdates

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Background: The vestibular ganglion (VG) is a key component in mediating the sense of balance. This ganglion is comprised of bipolar afferent neurons, which innervate the five vestibular end organs of the inner ear in the periphery and the brainstem/cerebellum centrally. Neural-fated cells delaminate from the otic epithelium, adopt either a vestibular or auditory fate, and eventually form two anatomically distinct ganglia. This process is tightly regulated

by a cascade of proneural transcription factors (TF). Much work has been done to tease apart the principles underlying auditory circuit assembly. However, the mechanisms regulating VG organization are not clear. Evidence from chick and zebrafish studies suggest that there is a temporal sequence to the cochleovestibular/statoacoustic ganglion organization. The organization of the VG in mammals, however, is not known, and it does not appear to be segregated according to the sensory organs that are being targeted. To address the underlying principles of VG organization, we investigated whether the birthdate of individual VGNs determines its peripheral innervation target and position within the VG.
Methods: We utilized Neurog1-creERT2 transgenic mice to lineage-trace the neuronal-fated cells and EdU labeling to determine neuronal birthdates within the VG. 4-Hydroxytamoxifen (4-OHT) was administered to pregnant Rosa-tdTomato or Snap25-GFP females crossed to Neurog1-creERT2 and EdU was administered to wildtype pregnant females; both injections were conducted at a single time point between E8.5 to E11.5. Then, embryos were harvested at E16.5 and analyzed. Additionally, to identify transcriptionally distinct VGN populations, we conducted scRNA-seq of VG at E16.5 collected from Neurod1-cre; Rosa-tdTomato and Neurog1-creERT2; Rosa-tdTomato embryos.
Results: Both the birthdating results using EdU and lineage results using Neurog1-creERT2 demonstrated that VGN birthdates determine their final positions within the VG. Specifically, the earliest born VGNs localized at dorsal and ventral poles of the VG and as subsequent neurons were born, they were progressively being added towards the center. Moreover, by tracing peripheral targets of early, middle and late-born VGNs using Snap25-GFP, we found that early and late born neurons innervated defined and separate regions within each end-organ, suggesting that timing of neuronal production also regulates peripheral targeting. To address whether VGN produced at different developmental times are molecularly distinct from each other, we are currently conducting scRNA-seq of the VG at E16.5.
Conclusions: Collectively, these results showed that the VG is molecularly and temporally organized by E16.5. Organ-specific VGNs are born in sequential waves between E9.5-E11.5 that ultimately determine their peripheral innervation targets.

Single Cell Profiling of the Developing Utricle Reveals Transcriptional Diversity of Hair Cells

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Background: The mouse utricle, responsible for detecting linear acceleration, contains two basic types of hair cells (HCs), Type I and Type II. These types differ in cellular morphology, bundle morphology, innervation, physiology, and gene expression. These types are further divided into subtypes according to their position inside (striolar, Str) or outside (extrastriolar, ES) of the central striolar region. Previous work showed that the mouse utricle undergoes significant growth in the first two weeks after birth, during which it adds many new HCs. However, little is known about how utricular HC transcriptomes change over the course of development. Therefore, we sought to elucidate the development of utricular HCs using single-cell RNA sequencing at four time points: postnatal 0/1 (P0/1), P5, P11/12, and P28/30.
Methods: Utricles were dissected from Pou4f3+/- mice, which have been reported to be phenotypically normal. Samples were dissociated and processed according to the 10X Chromium pipeline. The resulting libraries were normalized, integrated, and clustered at high resolution to identify and remove low-quality HCs and non-sensory cells. Differential

expression testing and pseudobulk analysis was performed to identify clusters. Slingshot was used to generate developmental trajectories.
Results: Our combined analysis of 5,459 HCs from all time points indicated a total of ten HC clusters, all of which were present at each time point. ES Type I HCs exhibited the highest change in representation over time (+32% between P0 and P28/30). ES Type II HCs, on the other hand, slightly declined (-6%). The proportion of Str HC types appeared stable. Additionally, we found five novel types and one new subtype. These included two types that corresponded to early stages in HC development, as evidenced by high Atoh1 expression and low Gfi1 expression. We also found a HC type that appeared intermediate between ES Type Is and IIs, which decreased over time, suggesting that it may serve as a transitional state for developing ES Type I HCs. This hypothesis was supported by the Slingshot trajectory analysis and co-expression of specific markers for ES Type I (Spp1) and Type II (Mapt) in the same cells. Similarly, we found two types that may represent transitional stages between ES and Str types. Finally, we found that Str Type I HCs could be further characterized into two subtypes.
Conclusions: These data have revealed a previously unprecedented degree of diversity in the murine utricle. Furthermore, they suggest that the proportion of most HC types changes throughout development, illustrating that postnatal utricular HCs are a highly dynamic population. Future work will examine transcriptional changes along developmental trajectories using TradeSeq, and lineage tracing will be used to assess whether ES Type II HCs serve as a transitional state in the course of ES Type I development.

Coordinated Regulation of Immature and Mature Hair Cell Genes in Thyroid Hormone-Treated Human Cochlear Organoids

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Background: Thyroid hormones regulate growth and development of various organs in the body. In the mouse cochlea, the thyroid hormone thyroxine promotes Prestin expression in outer hair cells. Consistent with this, we recently found that thyroxine promotes Prestin expression, while suppressing the immature cochlear hair cell marker SOX2, in human cochlear organoids. These results suggest that thyroxine promotes maturation of cochlear hair cells. In the present study, we tested whether thyroxine accelerates global transcriptomic profiles of mature hair cells in human cochlear organoids using single cell RNA-sequencing (scRNA-seq).
Methods: PAX2-nGFP/POU4F3-ntdTomato human embryonic stem cells were differentiated into cochlear organoids based on our established protocol (Moore et al., Cell Stem Cell 2023). From d90 to 110, cultures were grown in the presence or absence (control) of thyroxine at 300 ng/mL. On d110, aggregates were dissected, dissociated into single cells, after which POU4F3+ cells were sorted to enrich hair cell populations. The 10x Genomics Chromium Platform was used for cDNA library construction and sequencing. The sequencing data were then mapped onto the human genome and analyzed using Seurat. Additionally, scRNA-seq data from thyroxine-treated and untreated organoids were merged and differentially expressed genes between the samples were identified using DESeq2 and zinger. The results of the DESeq2 analysis were passed to iDEA, a platform for gene set enrichment analysis. For some of the organoid samples, immunofluorescence was performed to assess spatial expression of immature and mature hair cell markers.

Results: In both control and thyroxine treated samples, we identified dorsal root ganglion, transitional cell and immature cochlear hair cell clusters. We also detected an outer hair cell cluster expressing SLC26A5 and this cluster was mostly comprised of thyroxine treated hair cells. The differential gene expression analysis between immature cochlear hair cells and outer hair cells showed that SOX2, ATOH1 and INSM1 were highly expressed in the immature cochlear hair cell clusters, whereas SLC26A5, IKZF2, OCM and LMOD3 were highly expressed in the outer hair cell cluster. In support of our scRNA-seq data, we observed IKZF2 and OCM protein expression in thyroxine-treated hair cells, but not in untreated hair cells. Additional genes associated with hair cell mechanotransduction and human sensorineural hearing loss were also highly expressed in the outer hair cell populations. These data Indicate that the thyroid hormone directly or indirectly regulates expression of a battery of hair cell genes in organoid outer hair cells.
Conclusions: Our results demonstrate concomitant upregulation of mature outer hair cell genes and downregulation of immature cochlear hair cell genes in thyroxine-treated human cochlear organoids. These results suggest that thyroxine accelerates overall maturation of cochlear hair cells, rather than just promotes Prestin expression.

Everted Inner Ear Organoids: Generating Surface Hair Cell Sensory Epithelia to Better Investigate Human Development and Disease

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Background: Stem-cell derived inner ear organoids (IEOs) generated from human embryonic or induced pluripotent stem cells recapitulate the cellular diversity of the human inner ear (van der Valk et al., 2023) and are an attractive platform for therapeutic evaluation. One overarching limitation of all current 3D IEO models is that the sensory receptors of the inner ear, the hair cells, are buried beneath the surface of the larger cellular aggregate. Inspired by work altering apical-basal cell polarity in intestinal organoids (Co et al., 2019) and cerebral organoids (Suong et al., 2021), we hypothesized that targeted disruption of extracellular matrix (ECM) proteins in immature IEO aggregates would rearrange the IEO cytoarchitecture and produce sensory epithelia with exposed hair cells and apical stereocilia bundles.
Methods: We produced IEOs from two cell lines: a human embryonic stem cell line driving a fluorescent hair cell reporter and an induced pluripotent stem cell line driving constitutive expression of the calcium indicator GCaMP6f. To study the immediate effect of disrupting the ECM, we performed live imaging for three days following our treatment, investigated early transcriptomic changes, and performed ECM protein laminin immunohistochemistry. To study the development of the human hair bundle and sensory epithelium, we performed immunohistochemistry for early and mature otic lineage markers, calcium imaging, live actin staining, late-stage single-cell transcriptomic profiling, and high-resolution scanning and transmission electron microscopy.
Results: Our new procedure produces developing human hair cells on the surface of 3D IEOs with remarkable efficiency in approximately 90% of IEOs. Individual organoids contain approximately 1000-3000 surface hair cells with sensory epithelia comparable in size to the developing human utricle. This preparation allows for assessment of the developing human hair cell stereociliary bundle in both live and super resolution fixed preparations. We also

observe a transient phase of electrical activity in the form of calcium waves, a phenomenon described in the developing mouse sensory epithelia. One line of ongoing work includes generation of hair cells from a human stem cell line bearing a severe Usher Syndrome Type 1 mutation and preliminary transduction studies in an effort to delay the onset of hair cell pathology.
Conclusions: This method opens new avenues of research, from molecular mechanisms to therapeutic applications. These "everted" IEOs may allow for protein enrichment of components involved in the human mechanotransduction complex, modeling ototoxic exposure, and studying mechanisms of genetic hearing loss and vestibular dysfunction. Additionally, their accessibility makes them an ideal platform for regenerative studies and drug testing. This procedure is likely to accelerate both the understanding of inner ear development and the validation of therapies targeting hearing and balance disorders.

Progenitor Cell Dynamics in mESC-Derived Inner Ear Organoids: Insights From Single-Cell RNA Sequencing

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Background: In 2014, a method was introduced to guide mouse embryonic stem cells (mESCs) toward vestibular-like sensory epithelia and mechanosensory hair cell fates in vitro. These inner ear organoids represent a potentially limitless source of inner ear tissues for investigating the development, function, and responses of sensory hair cells to ototoxic and regenerative treatments. Recently, we employed 10x Genomics single-cell RNA sequencing (scRNA-seq) to profile all cells from dissociated inner ear organoids at six developmental stages, spanning from definitive ectoderm formation to the emergence of sensory epithelia. Utilizing this atlas, we have performed a developmental trajectory analysis of DIV11, DIV16, and DIV21 inner ear lineage cells to identify the genes that dynamically change as DIV11 otic vesicle-like cells differentiate into vestibular-like supporting cells and hair cells at DIV16/DIV21.
Methods: For this study, inner ear organoids were generated using Fbxo2-Venus-Hygromycin-Cre (Fbxo2-VHC) and Atoh1/nGFP mESCs, following established protocols with minor modifications. Fbxo2-VHC organoids were dissociated in biological duplicates at six developmental stages: days in vitro (DIV) 3, DIV4, DIV8, DIV11, DIV16, and DIV21. Cells were processed for scRNA-seq using 10x Genomics Chromium Single Cell 3' Reagents v3.1. After sequencing, Cell Ranger was used for read mapping and quantification, followed by further analysis with Seurat, Slingshot, and tradeSeq.
Results: Our trajectory analysis of DIV11, DIV16, and DIV21 otic lineage cells revealed that developing organoid-derived hair cells follow a general hair cell transcriptional program, characterized by the expression of well-known hair cell transcription factors such as ATOH1, POU4F3, and GFI1. Additionally, we identified a population of cells most abundant in DIV11 inner ear organoid otic vesicle-like structures that express high levels of proliferative genes, likely representing pro-sensory progenitor cells. This population became less prominent as sensory epithelia development progressed to DIV16 and DIV21. To validate this, we quantified the expression of the cell cycle marker Ki67 within DIV11, DIV16, and DIV21 otic vesicle-like and sensory epithelia-like structures. We observed a rapid downregulation of Ki67 between DIV16 and DIV21 in sensory epithelial cells. We hypothesized that this downregulation could be due to the upregulation of p27Kip1, a cell

cycle inhibitor responsible for cell cycle exit in inner ear progenitors during development. Staining for p27Kip1 showed an increase in expression between DIV16 and DIV21.
Conclusions: Our analyses suggest that organoids between DIV11 and DIV21 are valuable models for studying and manipulating early vestibular-like hair cell development. Additionally, they reveal a coordinated transition of organoid-derived sensory epithelia from a proliferative progenitor state to terminal differentiation between DIV16 and DIV21, mirroring the in vivo process. Therefore, inner ear organoids also represent a compelling model for investigating the mechanisms underlying supporting cell quiescence.

Podium 22: Brainstem: Structure and Function

Moderators: Ross Maddox and Ruili Xie

10:30 a.m. - 12:30 p.m.

Ocean Ballroom 9 - 12

Rapid, Accurate Prediction of Hearing Thresholds Using the Parallel Auditory Brainstem Response (pABR) in Adults With Hearing Loss

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Background: The parallel auditory brainstem response (pABR) is a stimulus presentation paradigm that provides diagnostic frequency-specific ABR measurements faster than traditional methods. Its speed comes from presenting tonebursts at all test frequencies in both ears all at once, rather than one at a time, which is how current ABR systems work. Previous studies have demonstrated the pABR's speed advantages in adults with normal hearing and have also shown that the pABR's responses have better place specificity than traditional methods. The pABR's accuracy and speed, however, have not yet been tested in people with hearing loss. Here we tested both.
Methods: Behavioral pure-tone audiometry is the gold standard clinical test of hearing loss. We assessed the pABR's accuracy by recruiting over 60 adult subjects with widely varying hearing loss configurations. For all subjects we ran a pABR threshold search using our custom real-time user interface. We also measured pure-tone thresholds behaviorally. We determined the pABR's accuracy by computing the correlation coefficients between the two, as well as the threshold error distribution.
To determine the pABR's speed advantage we also estimated thresholds using a standard clinical serial ABR system. We compared the times to estimate all thresholds in the pABR to the serial ABR. The pABR provides ten thresholds (500-8000 Hz in each ear), while the clinical system provided eight (500-4000 Hz in each ear).

Results: The correlation between pABR and behavioral thresholds was high, with r = 0.90 (0.88–0.92 confidence interval). Absolute error was low, with 79% of errors within the 10 dB step size, and 90% of all errors under 14 dB. The pABR was also fast. In the ten subjects where we compared the speed of the ABR paradigms, the mean ± standard deviation recording time for the pABR was 28 ± 8 minutes. For the serial ABR it was 70 ± 20 minutes. The pABR was faster than the standard clinical paradigm in every subject, by an average of 42 ± 19 minutes. Expressed as a ratio, the average pABR exam was 2.5× faster than the standard exam, even while measuring thresholds up to 8 kHz instead of the typical 4 kHz.

Conclusions: The pABR provides highly accurate threshold estimates in adults with hearing loss. It is substantially faster in clinical use than the current standard of care. It is therefore a viable method for objective threshold estimation in adults and holds great promise for accurately diagnosing infant hearing loss while addressing the primary drawback of current methods: speed.

Tonotopically Patterned Expression of HCN Channels Contributes to the Precision of Temporal Encoding in Cochlear Nucleus Neurons

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[1]*Lehigh University*

Background: In birds, a major functional role of the cochlear Nucleus Magnocellularis (NM) is to encode temporal information from acoustic input via phase-locked discharge output to the Nucleus Laminaris, the site of binaural coincidence detection. Neurons of NM receive primary input from auditory nerve fibers (ANFs) that are topographically distributed based on their characteristic frequency tuning, an arrangement known as tonotopy. In order to preserve and improve phase-locking precision with respect to that observed among ANFs across the tonotopy, NM neurons express physiological properties that are correspondingly tonotopically distributed. One such attribute is the patterned expression of voltage-gated channel conductances. Voltage responses to hyperpolarizing current injections feature a prominent depolarizing membrane potential sag that is itself differentiated tonotopically. Voltage sags are indicative of an inward current activated at hyperpolarized membrane potentials known as IH conductances, or hyperpolarization activated inward current.
Methods: To further investigate these properties, we performed whole-cell patch clamp recordings with pharmacological isolation of IH conductances in both current-clamp and voltage-clamp modes. In addition, we performed immunohistochemistry to document protein expression of HCN channels, the channel that underlies IH current.
Results: In order to investigate the functional contribution of HCN expression with respect to phase-locking, we first applied a depolarizing ramp injection protocol to NM neurons to measure the impact of pharmacological blocking IH on two measures of neural excitability, slope threshold and integration period (Oline, Ashida, and Burger 2016; McGinley and Oertel 2006). We show that IH conductance contributed to membrane excitability along the tonotopic axis. Next, we investigated whether this tonotopic patterning of HCN facilitates phase-locking across the tonotopic axis. We injected depolarizing current pulse trains at 100Hz or 200 Hz in order to quantify stimulus encoding behavior in NM neurons before and during HCN pharmacological block. In the presence of 40 μM ZD-7288, a specific HCN antagonist, there was a reduction of spike entrainment in response to both pulse train frequencies indicating that IH conductances are essential for NM cells to reliably follow a sustained synaptic input. Immunostaining supported the hypothesis that HCN expression is expressed in a low-to-high frequency gradient along NM's tonotopic axis.
Conclusions: Overall, our results show that there is a tonotopic distribution of HCN channels in NM, and this patterned HCN expression provides an additional mechanism that enables NM neurons to follow high frequency excitatory input.

DNLL Neurons Improve on Poor MSO IPD-Tuning at Very Low Frequencies through an Iceberg Effect

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Background: The dorsal nucleus of the lateral lemniscus (DNLL) is a major but little studied source of input to the inferior colliculus. It receives binaural inputs from the medial and lateral superior olive (LSO and MSO).
Methods: We recorded from axons and cells in the lateral lemniscus (LL) and its associated nuclei (NLL) and characterized sensitivity to interaural phase differences (IPDs) using binaural beating tones, and examined the ipsi-, contra-, and binaural phase of spike times. Tri-phasic dot rasters, in conjunction with additional data (spike shape, frequency tuning) allowed us to categorize the majority of responses into LSO, MSO, and NLL categories.
Results: We found that IPD-sensitivity, as quantified by vector strength (VS) at the beat frequency, was higher in NLL than in MSO or LSO. Especially below ~ 300 Hz, where IPD-sensitivity in MSO and LSO strongly decreases with decreasing frequency, VS was largely LESS THAN 0.5 in MSO and LSO but GREATER THAN 0.5 in the NLL. We were able to intracellularly record from and label NLL neurons. These recordings revealed prominent tonic inhibition in DNLL neurons to both ipsi- and contralateral stimulation. Responses to binaural beats showed an alternation of depolarization and inhibition, whose balance affected spiking threshold. By increasing spike threshold, inhibition reduces the IPD range over which the neuron responds, transforming poorly tuned inputs into sharply tuned output.
Conclusions: We conclude that IPD-sensitivity is sharpened by a generic mechanism (referred to as the iceberg effect) which is not contingent on monaural temporal information.

Noise-Induced Hearing Loss Enhances Ca2+-Dependent Spontaneous Bursting Activity in Lateral Cochlear Efferents

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Background: Lateral olivocochlear (LOC) efferent neurons may protect hearing sensitivity. Previously we showed that LOC neurons from juvenile mice exhibit an infra-slow (~0.1 Hz) burst firing pattern, whose genesis is dependent on L-type Ca2+ channels. However, it remains unclear what sets burst duration, or how burst firing and the ion channels that control bursts change with noise-induced hearing loss.
Methods: The auditory brainstem response (ABR) of transgenic mice (ChAT-Cre × tdTomato) was tested before and ~6 days after a broad-spectrum noise exposure at 110 dB SPL for 2 hours. Brain slices from deaf mice were harvested for electrophysiological recordings: cell-attached and whole-cell patch-clamp to examine burst firing pattern and Ca2+ current, respectively. A model LOC neuron was constructed to assess the roles of different ionic currents in regulating the firing pattern.
Results: We first examined the role of K+ channels in terminating the spike burst. Spontaneous burst firing in LOC neurons persisted despite the application of Ca2+-activated or voltage-gated K+ channel antagonists, indicating these channels are not involved in burst pattern. Instead, Ba2+-sensitive K+ currents, unaffected by these antagonists, had profound effects on firing activity, pointing to two-pore domain K+ (K2P) channels as likely drivers. This inference is supported by two pieces of evidence: first, immunostaining confirmed the expression of TREK-1 channels in the LOC; and second, reducing K2P conductance in the

model neuron prolonged burst duration until the neuron exhibited tonic firing when K2P conductance was reduced to zero. Given that K2P currents are voltage insensitive, the rhythm of neuronal activity likely depends on alternating cycles of Ca2+ channel activation and inactivation. Ca2+ inactivation kinetics were slowed when intracellular Ca2+ chelation power was enhanced or when extracellular Ca2+ was replaced with Ba2+, indicating that Ca2+-dependent Ca2+ channel inactivation (CDI) may contribute to burst termination. Indeed, in the model, slowing Ca2+ inactivation to simulate CDI attenuation resulted in prolonged burst duration. Noise-induced hearing loss significantly increased burst duration and reduced burst frequency. It differentially affected Ca2+ channel subtypes, increasing high-voltage-activated Ca2+ currents without affecting low-voltage-activated ones. In the model, increasing Ca2+ conductance led to longer bursts, supporting a causal relationship. Additionally, noise exposure compromised CDI, as neither enhanced chelation nor Ba2+ could prolong Ca2+ channel inactivation as in control neurons.
Conclusions: K2P and CDI work synergistically to terminate bursts in LOC neurons. Noise-induced hearing loss caused increased Ca2+ channel conductances with altered inactivation properties. Such changes might be a result of upregulated Ca2+ channel expression and altered Ca2+ signaling molecules like calmodulin. The changes in firing pattern of LOC neurons might play an important role in amplifying neuropeptide release in the cochlea following noise exposure.

SK Channel Dysfunction Underlies Excessive Neurotransmission in Fragile X Syndrome Mouse

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Background: Fragile X syndrome (FXS) is the most prevalent inherited cause of intellectual disability and the most common monogenic cause of autism spectrum disorder (ASD), resulting from silence of FMR1 gene and loss of Fragile X Messenger Ribonucleoprotein (FMRP). Among other deficits, FXS patients and animal models demonstrate auditory hypersensitivity and impaired sound localization. FMRP is extensively expressed in the auditory brainstem, including the medial nucleus of the trapezoid body (MNTB), a nucleus critical for sound localization. Fmr1 KO animals display changes in cell morphology and excitability; however, how FXS affects neurotransmission in MNTB is largely unknown.
Methods: Brain slices from wildtype (WT) mouse and Fmr1 KO mouse model of FXS were harvested for electrophysiological recordings. The auditory brainstem response (ABR) of WT and Fmr1 KO mice was tested before and after drug application.
Results: A broad frequency range of spiking signals of globular bushy cells reliably transmit to a contralateral target of MNTB principal neuron through the calyx of Held; we found that the reliable neurotransmission is disrupted in Fmr1 KO mice. Compared to WT, the function of small-conductance calcium-activated potassium (SK) channels in Fmr1 KO was largely reduced, likely due to a decrease in SK's calcium sensitivity. SK channels were activated by Ca2+ influx during spike firing and contributed to the spike afterhyperpolarization. The SK dysfunction in Fmr1 KO disrupted the reliable one-to-one neurotransmission and each presynaptic stimulation could trigger more than one postsynaptic spike. Blocking SK channels with apamin did not affect neurotransmitter release, while mimicking the excessive postsynaptic firing observed in Fmr1 KO. SK channel opener 1-EBIO rescued SK channel function and restored reliable neurotransmission. Moreover, the amplitude of ABR wave IV, which represents synchronous activity of the superior olivary complex including the MNTB,

is increased in Fmr1 KO mice; we found that intraperitoneal injection of 1-EBIO, which can across blood-brain barrier, restored ABR wave IV in Fmr1 KO mice to the wildtype levels.
Conclusions: Our results showed that the reliable neurotransmission from globular bushy cells to MMTB neurons is disrupted in Fmr1-KO mice and that SK channel dysfunction contributes to the neurotransmission dysfunction, providing a potential therapeutic target for treating hyperexcitability defects in FXS.

In Utero Exposure to Valproic Acid Abolishes the MNTB Projection to the Medial Geniculate

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Background: The vast majority of individuals with autism spectrum disorder (ASD) have some degree of auditory dysfunction. This frequently includes difficulty listening in the presence of background noise and ranges from deafness to hypersensitivity. In utero exposure to the antiepileptic valproic acid (VPA) is associated with elevated risk of an ASD diagnosis in humans and timed in utero exposure to VPA is a validated and biologically relevant animal model of ASD. VPA-exposed rats have significantly fewer neurons in their auditory brainstem, thalamus and cortex, reduced ascending projections to the inferior colliculus (IC) and medial geniculate (MG) from the cochlear nuclei (CN) and superior olive (SOC) and reduced descending projections from the cortex to the IC. Consistent with these changes, VPA-exposed animals have abnormal auditory brainstem responses. We have recently described a significant ascending projection from calbindin-positive neurons in the medial nucleus of the trapezoid body (MNTB) to the ventral division of the medial geniculate (vMG) in rats. Since we found that axonal projections to the vMG from the CN and SOC in VPA-exposed rats are reduced beyond what is predicted from neuron loss alone, we hypothesize that VPA exposure would result in a significant reduction in the MNTB projection to the vMG.
Methods: We examined this hypothesis using control and VPA-exposed animals. After timed mating, dams were exposed to vehicle (control) or VPA (vehicle + 800 mg/kg VPA) on embryonic days 10 and 12. Stereotaxic craniotomy was used to make deposits (2 x 100nl) of Fluorogold (FG) in the MG. After a 6 day survival, animals were perfused, brains were sectioned on a freezing-stage microtome and tissue sections counterstained with Neurotrace. The number of FG+ and NT counterstained neurons were counted in the MNTB and averaged for each animal (6 control and 6 VPA-exposed).
Results: Our results indicate that in controls, the MNTB forms the largest projection from the SOC to the MG. In fact, nearly 40% of MNTB neurons project to the MG in controls. After VPA exposure this is reduced to only 2%. Further, in VPA-exposed animals the largest projection from the SOC to the MG is from the dorsal medial wedge.
Conclusions: Our results indicate that VPA exposure nearly abolishes the glycinergic projection from the MNTB to the MG and reorganizes the olivogeniculate projection. Together, these results provide evidence for altered circuitry in the MG and this likely impacts temporal and spectral coding.

Origins of the Auditory Brainstem Response (ABR) in Mice: Source Localization With Multichannel Topographic EEG

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Background: The auditory brainstem response (ABR) is widely used to assess auditory brainstem function in mice. However, despite its widespread use, the origins of ABR waves beyond wave I specific to mice remain poorly validated. Remarkably, only two studies in the past 40 years have addressed this issue, which limits the reliability of ABR waves as markers for specific auditory brainstem structures in mice.
Methods: We used a novel approach to localize ABR wave sources specific to mice by using multichannel topographic EEG recordings. We recorded the topography of binaural click-evoked ABRs using a 30-channel thin-film EEG array from the skull of 15 adult mice combined with 32-channel multielectrode recordings from the auditory cortex. We applied sensor-level analysis and source reconstruction to identify the anatomical origins of ABR waves specific to mice.
Results: The thin-film recorded ABR waves showed a series of distinct spatial topographies. Wave I was strongly lateralized supporting its auditory nerve origin. Waves II/III showed a different lateralized more frontal distribution, supporting a cochlear nucleus and olivary structure origin. This was followed by a distinct wave IV topography at ~4.5 ms with a focused local activity only at electrodes above the inferior colliculus (IC). This IC origin of wave IV was also supported by dipole source reconstruction. Wide-band filtering additionally revealed a late IC wave P0 as a second marker of IC activity at ~9.5 ms, whose latency overlapped with evoked far-field activity in the auditory cortex.
Conclusions: The results show that wave IV of the mouse ABR originates from IC activity, providing a validated marker to distinguish pre-IC from post-IC and cortical activity. These findings enhance the understanding of mouse-specific ABR wave origins. Given its widespread use in mouse models, testing higher density EEG to further refine the localization of mouse ABR components is desirable.

Gene Expression Changes in the Inferior Colliculus After Sound Exposure

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[1]*University of Oklahoma,* [2]*Yale University,* [3]*University of Connecticut Health Center*

Background: The inferior colliculus (IC) is a major hub in the central auditory system. It has been the focus of decades of research, however much of its local circuitry remains incompletely understood. One reason is the lack of specific molecular markers for different neuron population present in the IC. While neuron types have been differentiated by morphology, neurotransmitter or intrinsic membrane properties most studies only combine two of these categories to describe a cell type, e.g. categorization of GABA cells into small and large (Ito et al., 2009). Few studies have been able to consolidate several aspects of neuron classification and identified specific molecular markers for this neuron type (e.g. VIP neurons (Goyer et al., 2019), NPY neurons (Silveira et al., 2020)).
Methods: We examined the transcriptomic profiles of IC cell types and compared gene expression variations between sound-exposed and control CBA/CaJ mice of both sexes. We tested the hearing thresholds and temporal processing abilities of 4 animals per group (sex x sound-exposed/control) before and after sound exposure. Control animals were age-and sex matched and re-assessed at the same interval as sound-exposed animals. Mice were awake

during their 1 h bilateral sound exposure (2 kHz wide narrow-band noise, centered at 16 kHz, 113 dB SPL). Hearing tests were performed using auditory brainstem response (ABR) and amplitude modulation following response (AMFR) measurements. Threshold shifts were assessed 8 weeks after the sound exposure. Sound exposed animals showed a threshold shift of 35 dB across all frequencies while the age and sex matched control animals had LESS THAN 2 dB difference compared to their first hearing test.
After re-testing their hearing, animals were sacrificed, and IC samples were dissociated into single-nuclei solution and processed using 10X chromium. To obtain sufficient nuclei per sample we pooled up to 4 IC from different mice in one sample but processed the left and right IC of each mouse separately; thus, we had a total of two samples per sex and treatment condition.

Results: We obtained gene expression profiles of ~7200 nuclei of which ~6600 nuclei remained after quality control and identified 15 different cell clusters based on initial analysis in SC1 (Moussa and Măndoiu,2021). These clusters include several clusters of excitatory (i.e. glutamatergic) and two clusters of inhibitory (GABAergic) neurons. We also identified several non-neuronal cell types such as microglia, oligodendrocytes and oligodendrocyte precursor cells, astrocytes and endothelial cells.
After sound exposure we found an upregulated expression of genes involved in programmed cell death and apoptotic processes especially in GABAergic neurons.

Conclusions: This study identifies the different cell types in the IC for the first time via transcriptomic methods and shows an upregulation of genes involved in cell death specifically in GABAergic neurons nine weeks after sound exposure.