ARO
Association for Research in Otolaryngology

2024 ARO MIDWINTER MEETING

Abstract Book

Saturday, February 3, 2024

Presidential Symposium

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

Platinum Salon 6

Presidential Symposium: The Clinician-Scientist Workforce in Otolaryngology: Strategies for the Future

Chair: Yuri Agrawal*, University of Colorado*

Background and Overview of Clinician-Scientists in Otolaryngology

Aaron Remenschneider[1]

[1]*Boston Children's Hospital*

Individual Abstract: Surgeon-scientists bridge the gap between clinical practice and scientific inquiry, driving investigation that can directly impact patient care. Successful translational research efforts come from surgeons across the practice spectrum and career stage; however, a variety of barriers to the success of a surgeon scientist workforce exist. The focus of this presentation is to review the present state of the surgeon-scientist workforce in otolaryngology and discuss areas for synergy between basic scientists and otolaryngologists at the individual, institutional and national level. At the trainee stage, published NRMP data on dual degree applicants to U.S. otolaryngology residencies and trends in NIH-T32 programs and outcomes for in-residency training of surgeon-scientists are examined. At the institutional level, models for funding surgeon-scientists, the economics of K-awards, and alternate compensation models that encourage academic productivity and collaboration with basic scientists are reviewed. At the national level, successful NIH programs that have targeted and improved the surgeon-scientist workforce in other specialties and the role for such programs at NIDCD is discussed. Data from a 2021 surgeon-scientist survey by the American Neurotology Society is reviewed to identify challenges to and opportunities for creating a supportive academic environment for translational research. Identified barriers to success include limitations in protected research time, increasing clinical and teaching demands, student debt burdens and a paucity of diverse role models. Societies such as ARO provide a unique forum for the career development of junior surgeon-scientists, for featuring critical aspects of team based translational research and for the promotion of bidirectional mentorship between otolaryngologists and basic scientists across the entire career arc.

Mentoring and Supporting Clinician-Scientists in Diverse Otolaryngology Departments: A PhD's Perspective

Jennifer Stone[1]

[1]*University of Washington, Virginia Merrill Bloedel Hearing Resource Center*

Individual Abstract: Dr. Stone is the current Program Director for a NIDCD T32 supporting research training for medical students and residents and the Director for Research in Otolaryngology at the University of Washington. For 20 years, she has conducted collaborative projects, offered mentorship to clinical residents and faculty, and provided training and guidance to students and residents with budding research interests. More recently, she has worked to sustain the productive research environment that was established in UW Otolaryngology over 40 years ago. She will share her perspectives on the ways in which clinician scientists and basic scientists can interact in mutually beneficial and enriching ways to build and sustain strong research and training programs in Otolaryngology. She will discuss the importance of establishing and reinforcing goals amongst a diverse faculty of basic scientists and clinician scientists who have varying interest, time, and resources for research. In addition, she will touch upon ways in which having a NIH-funded research training program has enriched her department's culture and empowered residents to continue research throughout their career.

How Should we Train the Next Generation of Physician-Scientists?
Alan Cheng[1]

[1]*Stanford University*

Individual Abstract: Physician scientists are significant contributors to scientific discoveries and innovative therapies (e.g. otoferlin gene therapy). The contribution is unique and can go beyond research investigation, given their medical training and their role as health care providers. They are also essential components of academic departments and institutions (e.g. NIH institute directors, department chairs). Despite their recognized roles and a steady increase in the health care workforce and NIH funding, the number of physician scientists has declined. This decline is concerning as it can limit the recruitment and training of future physician-scientists who are often inspired and mentored by established investigators. It further hinders the generation of a diverse physician-scientist workforce. Many factors contribute to this decline, including but not limited to financial responsibilities and limited research time. I will discuss the numerous national and regional programs established in order to replenish the physician scientist workforce, and share approaches and challenges along the way, with a focus on the training of future otolaryngologist-scientists.

Role of the Department Chair in Supporting Clinician-Scientists
Marlan Hansen[1]

[1]*UIHC*

Individual Abstract: This presentation will attempt to address the responsibilities, opportunities, challenges, strategies, and rewards of department and institutional leadership to recruit, develop and mentor, and promote clinician-scientists.

Developing and Sustaining the Clinician-Scientist Workforce: NIDCD Perspective
Debara Tucci[1]

[1]*National Institute on Deafness and Other Communication Disorders/NIH*

Individual Abstract: A robust clinician-scientist workforce is critical to successful translation of scientific discovery into evidence-based clinical care. After many years of declining numbers, we have recently begun to see an increase in surgeons and other clinicians funded by NIH. One of the challenges to maintaining a vibrant clinician-scientist workforce is the sequential loss of investigators across various stages of training and career development, described as the 'leaky pipeline.' The contributions of all clinician-scientists, inclusive of those with diverse perspectives, such as women and underrepresented and/or marginalized investigators, are critically important to scientific discovery.

To address these challenges, NIDCD has a number of programs in place to support the training and development of the clinician-scientist workforce. This presentation will review the current status of NIH funding of the workforce, mechanisms put in place to address challenges, and future opportunities for progress, including the need for a collaborative approach among NIH, other funders, academic medical centers and professional societies.

Ultimately, NIDCD seeks to support the integration of research into clinical care and the democratization of research to facilitate broad and diverse participation by both practitioners and research participants. Clinician-scientist leadership is critically important for the success of this goal to optimize the health of the population and must be prioritized in our training programs and health systems.

Late Breaking Presidential Symposium

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

Platinum Salon 5

OTOF Gene Therapy Clinical Trial
Chair: Yuri Agrawal*, University of Colorado*
Co-Chair: ZhengYi Chen*, Massachusetts Eye and Ear Infirmary*

SENS-501 Gene Therapy for Autosomal Recessive Non-Syndromic Deafness 9 (DFNB9)
Guillaume Oliver[1], Lise Barrot[1], Julie Dos Reis[1], Pierre Rambaud[1], Sandra Pierredon[1], Audrey Broussy[1], Anne-Gabrielle Harrus[1], Selma Dadak[1], Geraldine Petit[1], Jerome Nevoux[2], Ghizlene Lahlou[2], Natalie Loundon[3], Marie-Jose LeComte[2], Saaid Saffiedine[2], Christine Petit[2], Christine Le Bec[1], Emilie Bousquet[1], Arnaud Giese[1], Laurent Desire*[1]

[1]*Sensorion,* [2]*Institut de l'Audition/ Institut Pasteur,* [3]*Hôpital Necker-Enfants Malades*

Individual Abstract: Congenital sensorineural hearing defects heavily impact patients' life and their ability to communicate. Among this family of disorders, the non-syndromic autosomal recessive deafness 9 (DFNB9) is a common form of congenital deafness. This severe-to-profound auditory neuropathy, which typically presents with absent or reduced auditory brainstem responses (ABR) and present otoacoustic emissions, is caused by biallelic loss-of-function in the OTOF gene. OTOF gene encodes for OTOFERLIN, a calcium sensor protein important for neurotransmitter release at presynaptic level between inner sensory hair cells (IHCs) and spiral ganglion neurons.

In this talk, we present an overview of SENS-501 development strategy for DFNB9. SENS-501 is an adeno-associated virus (AAV)-based dual-vector gene therapy designed to restore the expression of the full-length active human Otoferlin, thereby providing durable and physiological hearing restoration to individuals with hearing loss caused by OTOF gene mutations.

SENS-501 efficacy is supported by multiple proof-of-concept and efficacy studies in congenitally deaf DFNB9 (Otof-/-) mutant mice, in which long-term reversal of the deafness phenotype is demonstrated and is supported by behavioral assessment confirming the ability of corrected DFNB9 mutant mice to integrate sounds.

In mice and non-human primates (NHPs), dose-ranging and early biodistribution studies helped to design 3-month NHP and 6-month mice toxicity and biodistribution studies that were performed under Good Laboratory Practice (GLP) in these two species to support the conduct of a first-in-human clinical trial. NHP studies served to develop and validate the surgical approach and SENS-501 delivery modality to be used in clinics. In the GLP studies, SENS-501 was well tolerated, with no SENS-501-related clinical signs or adverse effects either in the inner ear or outside the inner ear tissue.

Biodistribution data indicated that the vast majority of SENS-501 vector components remained in the inner ear and that SENS-501 shedding in fluids was limited. In NHPs, presence of pre-existing neutralizing anti-AAV8 antibodies was not associated with loss of transgene expression in IHCs or with safety issues, suggesting that pre-existing systemic anti-capsid neutralizing antibodies may be of limited impact on local delivery of SENS-501.

Altogether, our nonclinical pharmacology, safety, biodistribution, and two ongoing natural history studies support the clinical development of SENS-501 and the initiation of our phase I/II clinical trial first half of 2024.

Clinical Development of AK-OTOF Gene Therapy for *OTOF*-Mediated Hearing Loss

Emmanuel J. Simons[*1], John A. Germiller[2], Oliver Haag[3], Chen-Chi Wu[4], Ann E. Hickox[1], Michelle D. Valero[1], Eva Andres-Mateos[1], Michael J. McKenna[1], Brian Lin[1], William A. Sewell[1], Shimon P. Francis[1], Yuan Gao[1], Emily Liu[1], Katie Wachtel[1], Donna Mackey[1], Amy Burke[1], Aaron D. Tward[1], Kathleen Z. Reape[1], Jennifer A. Wellman[1]

[1]*Akouos, Inc.,* [2]*The Children's Hospital of Philadelphia,* [3]*Sant Joan de Déu Barcelona Hospital,* [4]*National Taiwan University Hospital*

Individual Abstract: The otoferlin gene (*OTOF*) encodes otoferlin, a protein critical for signaling at the inner hair cell synapse; individuals with mutations in *OTOF* typically present with congenital, Severe to Profound sensorineural hearing loss. Recent advances in gene therapy and intracochlear delivery support the potential to restore hearing in individuals with *OTOF*-mediated hearing loss using a one-time, local administration of AK-OTOF (AAVAnc80-hOTOF). A phase 1/2 clinical trial (NCT05821959) has been initiated to assess the safety, tolerability, and bioactivity of escalating doses of AKOTOF in children with Profound hearing loss due to *OTOF* mutations.

Nonclinical studies in otoferlin knock-out mice and non-human primates (NHPs) evaluated the delivery of genetic medicines to the inner ear, including the development of a dual adeno-associated viral vector encoding full-length human otoferlin, a delivery device, and an intracochlear administration procedure. These studies informed the design of the clinical investigation of AK-OTOF, including demonstration of biological plausibility, evaluation of intervention window, identification of biologically active dose levels, assessment of onset and durability of functional recovery, and evaluation of safety. Participants in the AK-OTOF-101 Clinical Trial have Profound hearing loss at baseline and receive, via a minimally invasive external auditory canal approach, a single intracochlear administration of AK-OTOF in one ear; participants in the first cohort receive AK-OTOF at a dose of 4.1E11 total vector genomes. Hearing restoration is assessed by behavioral audiometry and auditory brainstem response.

Administration of AK-OTOF to NHPs and otoferlin knock-out mice results in robust expression of full-length otoferlin, with restoration of auditory function observed in the knock-out mice. Safety data demonstrate AK-OTOF was systemically and locally well tolerated, with no adverse effects related to AK-OTOF, including in clinical pathology, otic or systemic histopathology, or cochlear and auditory function. In the AK-OTOF-101 Clinical Trial, the first participant, an 11 year old with Profound congenital hearing loss, experienced restored hearing within 30 days of AK-OTOF administration, achieving thresholds of 65 to 20 dB HL. The surgical administration procedure and the product candidate were well tolerated, and no serious adverse events occurred. Safety and efficacy data from participants receiving AKOTOF prior to January 2024 will be presented.

The AK-OTOF preclinical development strategy, leading to an actively enrolling interventional clinical trial in children with *OTOF*-mediated hearing loss, can serve as an exemplary path to achieving the broader goal of developing precision genetic medicines with the potential to restore, improve, and preserve high-acuity physiologic hearing.

CHORD: A Phase 1/2 Open-Label, Multicenter Trial to Evaluate Intracochlear Administration of Db-Oto Gene Therapy in Pediatric Patients with Profound Sensorineural Hearing Loss Due to Biallelic Otoferlin Mutations

Vassili Valayannopoulos[1]

[1]*Regeneron*

Individual Abstract: DB-OTO is a one-time gene therapy intended to provide hearing function to pediatric patients diagnosed with congenital auditory neuropathy secondary to biallelic mutations of the otoferlin (OTOF) gene. Patients with OTOF-related auditory neuropathy typically present at birth with profound

hearing loss, characterized phenotypically by an absent auditory brainstem response (ABR) contrasting with normal otoacoustic emissions, and have no expected natural recovery. While cochlear implants (CI) have been successfully adopted in these patients, they present notable shortcomings including acute and delayed risks associated with the surgery, limitations associated with the CI device, and limited efficacy compared with natural hearing. DB-OTO is a dual adeno-associated virus 1 gene therapy vector carrying a hair cell–specific promoter, myosin 15 and driving the expression of a complementary DNA encoding the human OTOF isoform 5. DB-OTO is administered by surgical injection into the cochlea and targets the inner hair cells that use otoferlin for proper synaptic transmission to the auditory nerve. Nonclinical studies with DB-OTO in murine models of OTOF-related hearing loss, showed durable, dose-dependent recovery of ABR, which is an objective, surrogate measure of hearing function. Toxicology studies with DB-OTO in murine models have revealed a tolerable local and systemic profile. Further, evaluation of DB-OTO and its surgical delivery in nonhuman primates has supported the foundation for translation to humans. DB-OTO is currently being evaluated in a multicenter, phase 1/2 open-label trial (CHORD, NCT05788536) in children and infants with profound hearing loss secondary to biallelic, loss-of-function OTOF mutations. The trial is composed of two parts. Part A is a single ascending dose trial with DB-OTO injected unilaterally (contralateral ear may or may not have the standard of care CI). Part B is a cohort expansion involving the injection of DB-OTO bilaterally at a dose determined from safety and efficacy from Part A.

A 10-month-old female, with profound OTOF-related hearing loss at baseline, received a single intracochlear injection of DB-OTO (7.2 x 1012 vg). DB-OTO was infused into the right inner ear perilymph via the round window using a standard facial recess approach; a CI was placed in the left ear. At 12 weeks post-injection, the DB-OTO treated ear showed measurable improvements (55 dB HL) in thresholds versus baseline (absent thresholds at 100 dB HL, maximum air conduction intensity tested, 250–4000 Hz). ABR showed hearing recovery via positive wave V response with thresholds of 40–80 dB; no response was elicited at 100 dB at baseline (500–4000 Hz). Through week 12, no dose-limiting toxicities and no DB-OTO related adverse events were reported, including absence of vestibular manifestations tested clinically and by cervical vestibular-evoked myogenic potentials. LittlEARS auditory questionnaire indicated improvement in hearing based on natural vocalizations (without CI) observed by caregivers.

AAV-Mediated Gene Therapy Restores Hearing in Patients with DFNB9 Deafness

Jieyu Qi*[1], Fangzhi Tan[1], Liyan Zhang[1], Ling Lu[2], Shanzhong Zhang[3], Yabo Zhao[1], Yicheng Lu[1], Xiaoyun Qian[2], Wendi Dong[3], Yinyi Zhou[1], Ziyu Zhang[1], Xuehan Yang[1], Lulu Jiang[3], Chaorong Yu[3], Jiancheng Liu[3], Yilai Shu[4], Lei Xu[5], Xia Gao[2], Huawei Li[4], Renjie Chai[1]

[1]*Southeast University,* [2]*The Affiliated Drum Tower Hospital of Nanjing University Medical School, China,* [3]*Otovia Therapeutics Inc,* [4]*ENT Institute, Eye and ENT Hospital, Fudan University,* [5]*Shandong Provincial ENT Hospital*

Individual Abstract: Mutations in OTOFERLIN (OTOF) lead to the autosomal recessive deafness 9 (DFNB9). The efficacy of adeno-associated virus (AAV)-mediated OTOF gene replacement therapy is extensively validated in Otof-deficient mice. However, the clinical safety and efficacy of AAV-OTOF is not reported. Here, AAV-OTOF is generated using good manufacturing practice and validated its efficacy and safety in mouse and non-human primates in order to determine the optimal injection dose, volume, and administration route for clinical trials. Subsequently, AAV-OTOF is delivered into one cochlea of a 5-year-old deaf patient and into the bilateral cochleae of an 8-year-old deaf patient with OTOF mutations. Obvious hearing improvement is detected by the auditory brainstem response (ABR) and the pure-tone audiometry (PTA) in these two patients. Hearing in the injected ear of the 5-year-old patient can be restored to the normal range at 1 month after AAV-OTOF injection, while the 8-year-old
patient can hear the conversational sounds. Most importantly, the 5-year-old patient can hear and recognize speech only through the AAV-OTOF-injected ear. This study is the first to demonstrate the safety and efficacy of AAV-OTOF in patients, expands and optimizes current OTOF-related gene therapy and provides valuable information for further application of gene therapies for deafness.

AAV1-HOTOF Gene Therapy for Children with Autosomal Recessive Deafness 9

Yilai Shu*[1], Jun Lv[1], Hui Wang[1], Xiaoting Cheng[1], Yuxin Chen[1], Daqi Wang[1], Longlong Zhang[1], Qi Cao[1], Honghai Tang[1], Kaiyu Gao[2], Mengzhao Xun[1], Biyun Zhu[1], Chong Cui[1], Luo Guo[1], Luoying Jiang[1], Bing Chen[1], Wuqing Wang[1], Renjie Chai[3], Zheng-Yi Chen[4], Huawei Li[1]

[1]*ENT institute, Eye and ENT Hospital, State Key Laboratory of Medical Neurobiology and MOE Frontiers Center for Brain Science, Fudan University,* [2]*Shanghai Refreshgene Therapeutics Co., Ltd.,* [3]*State Key Laboratory of Digital Medical Engineering, Zhongda Hospital, School of Life Sciences and Technology, Advanced Institute for Life and Health, Jiangsu Province High-Tech Key Laboratory for Bio-Medical Research, Southeast University* [4]*Harvard Medical School, Eaton-Peabody Laboratory, Massachusetts Eye and Ear*

Individual Abstract: Autosomal recessive deafness 9 (DFNB9), caused by mutations of the OTOF gene, is characterized by congenital or prelingual, severe-to-complete bilateral hearing loss. However, no drug treatment is available for hereditary deafness. AAV1-hOTOF, adeno-associated virus (AAV) serotype 1 carrying a human OTOF transgene, was designed to treat patients with DFNB9. Here, we report the safety and efficacy of AAV1-hOTOF gene therapy as the treatment of children with DFNB9 via unilateral or binaural injection.

In this single-arm trial, patients (1–18 years of age) with severe-to-complete hearing loss were eligible. AAV1-hOTOF was administered into one or two cochleas through the round window. The primary outcome was dose-limiting toxicity (DLT). Adverse events (AEs), immune response, auditory function and speech perception were evaluated.

From December 2022, to September, 2023, 9 patients with 95 dB of average auditory brainstem response (ABR) thresholds at 0.5–4 kHz were enrolled. Six patients received unilateral injection, of which patient #1 received 9×1011 vg dose and patients #2–6 received 1.5×1011 vg dose. Subsequently, patients #7-9 received binaural injection at a dose of 1.5×1011 vg. Follow-up visits ranged from 6 to 26 weeks. No DLT was observed. 61 adverse events were observed, with 97% (59/61) being grade 1 or 2, and 3% (2/61) being grade 3. In patient #1, the average ABR threshold was recovered to 68 dB at 4 weeks, 53 dB at 13 weeks, and 45 dB at 26 weeks. In patients #2-5, the average ABR thresholds were greater than 95, 48, 38, and 40 dB at 26 weeks, respectively, while the average ABR threshold was 60 dB in patient #6 at 13 weeks. In patients #7-8, the average ABR thresholds of left (right) ear were reduced to 63 (63), 88 (83) at 13 weeks. In patients #9, the average ABR thresholds of left (right) ear were reduced to 60 (63) at 13 weeks. Speech perception was improved in 5 patients who had hearing recovery. The ability of sound source localization was improved in all patients who received binaural injection.

The AAV1-hOTOF gene therapy is safe and efficacious as a novel treatment for patients with DFNB9.

Poster Session 1

1:00 p.m. - 3:00 p.m.

Marquis Ballroom

S1. Open Board

S2. Neurofeedback Training of Auditory Spatial Attention Changes Oscillatory Activity of Parietal Cortex

Hwan Shim*[1], Akira Takeuchi[1], Inyong Choi[2], Sungyoung Kim[1]

[1]*Rochester Institute of Technology,* [2]*University of Iowa*

Category: Auditory Cortex and Thalamus: Human Studies
Background: Selective attention, which enhances cortical responses to attended sensory inputs while suppressing others, can be beneficial for speech-in-noise (SiN) interpretation. Attentional modulation Yue Ren

describes this cortical process. Our previous work has shown that neurofeedback training paradigms are effective in improving attentional modulation of cortical auditory evoked responses, but we are still in the early stages of understanding how this neurofeedback training improves attentional modulation.
Methods: A modified neurofeedback training paradigm was created to enhance the control of cortical auditory evoked responses by auditory spatial attention. A voice repeating “Up” five times and a voice repeating “Down” four times were played concurrently from the left and right loudspeakers, respectively, in a female or male voice. Participants received a neurofeedback that had been gamified i.e., if the "up" stream was attended, an object would travel upward on the computer screen, and vice versa. The “neurofeedback training" was repeated by the subjects over the course of two occasions which were often two weeks.
Results: Subjects demonstrated stronger alpha oscillation in the right parietal cortex during the after-cue-before-sound period during the second experiment compared to the first, indicating that spatial inhibitory processing to block sound inputs from the left was enhanced. Following the two weeks of training, there was higher attentional modulation of beta oscillation in the right parietal cortex, indicating improved brain activity in predicting the target. Additionally, the strength of attentional modulation on sound-evoked cortical responses improved.
Conclusions: These results show that training with neurofeedback improves top-down processing and auditory grouping in the parietal cortical network for auditory spatial selective attention.

S3. Self-Vocalizations Evoke Neural Activity in the Auditory Cortex of Neonatal Mice

Didhiti Mukherjee*[1], Chih-Ting Chen[1], Patrick O. Kanold[1]

[1]*Johns Hopkins University*

Category: Development: Cellular/Systems
Background: Early sensory experience from the periphery aids neural development and plasticity in the neonatal sensory cortices. For example, in the developing mouse auditory cortex (ACx), peripheral sound-driven activity is observed during the pre-critical period, even before the ear canals are open, and early sound deprivation alters its functional connectivity. The critical question that must be addressed is what natural environmental sounds activate the infant ACx. Since developing synapses exhibit high rates of adaptation to ongoing stimuli and young neurons cannot sustain high firing rates, rare, intermittent sounds are likely to be the most effective for activating the ACx. Some prominent intermittent sounds are produced by self- and other-produced vocalizations by littermates or the mother. Other-produced vocalizations, however, are likely to be attenuated by the closed ear canals in altricial pups, whereas self-vocalization will be less attenuated. Therefore, we hypothesized that self-vocalizations activate the ACx in newborn pups.
Methods: To test this hypothesis, we performed in vivo widefield imaging of the ACx on both hemispheres in awake mouse pups using a bilateral widefield microscope. We imaged pups expressing calcium indicator GCaMP6 in excitatory neurons from postnatal days (P) 9 (before ear-opening) to P15 (soon after ear opening). We imaged the neural activity of the ACx while simultaneously recording the pups’ spontaneous vocalization.
Results: We found that self-vocalization triggers fluorescence responses in multiple regions of the ACx in both hemispheres at all ages. Our results show that self-vocalizations trigger neural activity in the developing ACx even before the ear canals are open.
Conclusions: These findings suggest a role of early vocalizations in shaping cortical responses and the tonotopic organization of the ACx.

S4. Auditory Cortical Coding of Task-Relevant Variables During Active Listening

Nathan Schneider[1], Michael Malina[2], Rebecca Krall[1], Ross Williamson*[1]

[1]*University of Pittsburgh,* [2]*Carnegie-Mellon University*

Category: Auditory Cortex and Thalamus: Structure and Function

Background: Auditory-guided behavior is a fundamental aspect of our daily lives, as we rely on auditory information to guide our decisions and actions. The primary route for auditory information to propagate from the ACtx is through intratelencephalic (IT) and extratelencephalic (ET) neurons in layer (L) 5. These neurons form the major output of the ACtx and, as a result, are in a privileged position to readily influence auditory-guided behavior.
Methods: To investigate the behavioral role of IT and ET neurons, we devised a head-fixed choice task where mice categorized the rate of sinusoidal amplitude-modulated (sAM) noise bursts as either fast or slow to receive a water reward. To ascertain the necessity of ACtx, we conducted bilateral optogenetic inhibition with GtACR2 and observed a significant decrease in hit rate during inhibition trials. We then used two-photon calcium imaging alongside selective GCaMP8s expression to monitor the activity of L5 IT and ET populations.
Results: Clustering analyses of these populations revealed heterogenous response motifs that correlated with various stimulus and task variables. Of particular interest was a distinct motif primarily present in ET neurons, characterized by "categorical" firing patterns that indicated a preference for either slow or fast sAM rates. This categorical selectivity was not initially present, but was revealed through longitudinal recordings, illustrating dynamic alterations in the responses of ET neurons across learning to align with distinct perceptual categories. Critically, this categorical selectivity in ET neurons did not manifest during passive exposure to identical stimuli. This suggests that learned categorical selectivity is shaped via top-down inputs that act as a flexible, task-dependent filter. Moreover, ET activity reflected behavioral choices independently of stimulus identity or reward outcome, with choice selectivity increasing throughout learning.
In contrast, L5 IT neurons initially exhibited category information which then degraded as mice acquired task proficiency. Furthermore, the ability to decode both stimulus identity and behavioral choice from IT activity decreased across learning. This suggests a tradeoff of information between these two distinct populations within L5, with IT projections playing a role in initial task acquisition, while ET projections are recruited and reinforced throughout the learning process.

Conclusions: Collectively, these findings underscore the differential roles of L5 neurons and contribute to our understanding of how auditory information is processed and utilized to guide decision-making and action.

S5. In-Vivo Holographic Stimulation Reveals Functionally Interacting Subnetworks in the Auditory Cortex

HiJee Kang*[1], Travis Babola[1], Patrick Kanold[1]

[1]*Johns Hopkins University*

Category: Auditory Cortex and Thalamus: Structure and Function
Background: Sensory perception requires fast encoding of relevant input from a mixture of complex signals. The auditory cortex (AC) plays a key role for processing of incoming acoustic signals. A group of co-tuned neurons, rather than all neurons in the AC, is activated together for processing a specific acoustic feature. Thus, even though neurons are tuned for a particular sound feature, they might not respond every time. However, how evoked activities are distributed among co-tuned neurons is unknown. In addition, it is unknown how this population allocation of activity changes with aging, which may be a candidate for degraded central hearing ability for aging population, even with no peripheral hearing loss.
Methods: Here, we test how small groups of neurons in AC coordinate their activity in vivo using holographic optogenetic stimulation targeting. This technique allows the stimulation of a small number of neurons. We combine holographic optogenetic stimulation with in-vivo 2-photon imaging in awake adult mice. We first imaged neural activities on a population of neurons to either 100-ms 16 kHz or 54 kHz pure tones using 2-photon imaging (baseline session). We then add the holographic stimulation of 5 neurons that are responsive to a 16 kHz pure tone (target tone), along with pure tone presentations (experimental session). By doing so, we are increasing the activity in a small subset of co-tuned neurons. We then compared the changes in the sound-evoked responses to 16kHz and 54kHz in neurons co-tuned with the stimulated neurons and non co-tuned neurons.
Results: In young adult mice, stimulated neurons showed increased response amplitude during the experimental session compared to the baseline session, regardless of the presented pure tone frequencies. More importantly, non-stimulated, co-tuned neurons, showed response amplitude decrease only when the target tone

(16 kHz) was played along with stimulation. In contrast, in non-stimulated, non co-tuned neurons, such amplitude decrease was not observed.
Conclusions: Our data suggest that neurons sharing the same functional characteristics adjust their evoked activities for processing target sensory input when activities from a small group of neurons within the subnetwork are manipulated. By doing so, the average activity level among co-tuned neurons is maintained. We speculate that such rapid adjustment of evoked activities across co-tuned neurons deteriorates with aging. Our results suggest that the subnetwork activity can be controlled by only a small subset of neurons, and the network change is highly related to the functional properties of neurons which could contribute to efficient sensory processing.

S6. Unsupervised Analysis of Attention Level-Dependent Temporal Coherence Processing Reveals Auditory Cortical Specializations for Acoustic and Cognitive Coding

Kunpeng Yu*[1], Hemant Kumar Srivastava[1], Justin Fine[1], Ben Hayden[1], Kit Jaspe[1], Nikolas A. Scarcelli[1], Hong Jiang[1], Matthew J. McGinley[1]

[1]*Baylor College of Medicine*

Category: Auditory Cortex and Thalamus: Structure and Function
Background: Temporal coherence (TC) between frequency channels is crucial for differentiating a target sound from its background. This mechanism is especially vital during concentrated and deliberate listening and is believed to be processed within the auditory cortex (ACX). To investigate the neural processing of TC, we executed electrophysiological recording in the ACX of mice engaged in a sustained attention value task. The mice were trained to detect TC within a continuous, random tone cloud to earn a sugar water reward. The task necessitated effortful sustained attention to distinguish TC from the tone cloud, and we modulated this attentional demand by varying the reward size between 6 consecutive blocks of 60 trails in each session (De Gee et al, 2022 for task details).
De Gee, Jan Willem et al. "Mice regulate their attentional intensity and arousal to exploit increases in task utility." bioRxiv (2022).

Methods: To understand neural dynamics of different groups of neurons across multiple timescales, we here utilize tensor component analysis to decompose electrophysiology data as a tensor and factorize each component into three interconnected, low-dimensional descriptions of the data: neuron factors, reflecting neuron assemblies; temporal factors, reflecting rapid neural dynamics within each trial; and trial factors, describing both long-term and trial-to-trial changes in neural dynamics (H. Williams et al, 2018 for algorithmic details).
H. Williams, Alex et al. “Unsupervised Discovery of Demixed, Low Dimensional Neural Dynamics across Multiple Timescales through Tensor Component Analysis.” Neuron (2018).

Results: Our preliminary analysis shows a major component associated with a specific group of neurons that increases their firing rate in response to TC emergence whose activity is correlated with trials with correct response. We also see tensor components associated with fluctuating pupil-indexed arousal.
Conclusions: In ongoing work, we are further analyzing decomposed factors associated with different task events (tone cloud emergence, temporal coherence emergence, animal response) and study how attention effort impacts the neural dynamics of each component.

S7. Auditory Cortex Processing of Vocalizations in Natural Bouts

Estelle in 't Zandt*[1], Dan Sanes[1]

[1]*New York University*

Category: Auditory Cortex and Thalamus: Structure and Function
Background: Animal vocalizations are often produced in sequences, or bouts, comparable to the production of words within the context of a sentence. Studies in mice and some songbirds have demonstrated that animals are highly sensitive to the particular order of vocalizations in a bout. Despite these behavioral findings, the underlying neural mechanisms driving these perceptual responses remain unclear. Vocalization responses in

the auditory cortex (AC) have been primarily investigated as single syllables in anesthetized or head-fixed animals. However, since vocalizations are ethologically relevant stimuli to animals, it is crucial to investigate AC responses to vocalizations in their naturally-produced bouts in an awake, freely-moving state. Here, we investigated how gerbil AC responses differ across complete bouts of vocalizations, and how single-syllable manipulations affect those responses.
Methods: We recorded AC responses to vocalization bouts in awake, freely-moving adolescent (n=4) and sexually mature (n=2) Mongolian gerbils (Meriones unguiculatus). Gerbils are a highly social rodent species with a rich vocal repertoire consisting of vocalizations that span the sonic and ultrasonic hearing range. We used chronically-implanted, high-density silicon probes to wirelessly record single AC neuron responses in the same animals across weeks of adolescence or adulthood. We used multiple types of vocalization bouts that were emitted by gerbils in an undisturbed family context. During recording sessions, we presented syllables from different call types either in isolation, within their original bout, or in a bout following a spectrotemporal manipulation of the previous syllable. For all analyses, we only included neurons that displayed a significant response to the original version of the bout, assessed either by a significant change in firing rate from baseline, or through temporally-reliable responses across trials.
Results: Overall, we found that 28% of all cells recorded displayed significant responses to at least one of the vocalization bouts presented (425/1505 recorded single units). For 6 out of 8 syllables tested, we found a significant difference between the firing rate of the AC population to isolated syllables as compared to those same syllables presented within their natural bout (p less than 0.05, two-tailed paired t-test). However, within that population, only 11-24% of neurons had significant modulation by the bout, depending on the stimulus type.
Conclusions: These results suggest that vocalization encoding in AC is sparse, and most neurons do not integrate across the bout for longer than the length of a single syllable. This suggests that bout integration is likely to occur downstream of AC, thereby supporting discrimination of vocal classes or individual vocalizers. Further analyses using a population classifier will address this question.

S8. Probing AMPA Receptors Activity at Ribbon Synapses in the Mammalian Cochlea by Glutamate Uncaging

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Category: Auditory Nerve
Background: AMPA receptors in the mammalian brain mediate fast neurotransmission. These glutamate receptors are concentrated in specific regions of the synapse called postsynaptic densities (PSDs), in close opposition to presynaptic active zones. Across synapses a great variability of PSDs sizes and number of AMPA receptors have been described. This morphological and molecular heterogeneity is a determinant of functional responses in the postsynaptic neuron. In the mammalian inner ear, glutamatergic synapses are also formed between inner hair cells (IHCs) and spiral ganglion neurons (SGNs). Postsynaptic terminals of SGNs are characterized by large PSDs, 5-10 times bigger than those found in the brain. Presynaptically, the IHC ribbon synapse supports high rates of vesicular exocytosis onto a single SGN PSD. However, the sizes and locations of events of glutamate release relative to the PSD are not well understood. We speculate that PSDs are not saturated by glutamate during low/moderate rates of release and are able to accommodate large amounts of glutamate released by IHCs.
Methods: 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. The expanded beam of a 405 nm diode-laser was projected onto the back aperture of a 60x objective producing a diffraction-limited illumination spot at the objective focal plane. The laser light was flashed upon the tissue previously bathed with a caged-glutamate compound (MNI-glutamate), producing fast transients in glutamate concentration. The lateral width of the laser spot was approximately 0.2microns.
Results: Responses to glutamate uncaging were recorded by patch-clamp directly on SGNs terminals. Both intensity and duration of laser pulses could be modulated to generate glutamate transients of different sizes. The relationship was drawn between amplitudes of responses to glutamate photolysis (Iphotol) and laser intensities, obtaining complete curves with maximal responses for n = 4 SGNs. The average maximal Iphotol was 1,483 ± 261 pA with a holding potential (Vh) of -50 mV, which corresponds to 29.6 ± 5.2 nS (or 2,076 ±

366 pA with Vh = -70 mV). In parallel, spontaneous EPSCs were recorded in n = 5 SGNs (greater than 1000 events each) evoked by the superfusion of a high K+ extracellular solution. In agreement with previous results, the EPSC average amplitude was 381 ± 57 pA (Vh = -70 mV), whereas the largest EPSC in each recording averaged 887 ± 129 pA.
Conclusions: In summary, Iphotol were recorded at SGNs terminals in response to brief (0.05 – 0.5 ms) laser pulses of different intensities. The maximal Iphotol was 2.3X greater than the largest spontaneous EPSC recorded in high-K+ solution and 3.9X larger than the average EPSC, suggesting that AMPA receptors on PSDs at SGNs are not saturated during low/moderate neurotransmission.

S9. Cx30 (GJB6) is Required for Neural Development and Distribution in the Cochlea

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Category: Auditory Nerve
Background: Cx26 and Cx30 are predominant isoforms in the cochlea. Both Cx26 mutations and Cx30 mutations can cause hearing loss. However, there are no Cx26 and Cx30 expressions in the hair cells and spiral ganglion neurons in the inner ear. Our previous studies reveal that Cx26 deficiency can cause cochlear developmental disorders. In this study, we found that Cx30 deficiency can cause spiral neuron development and distribution disorders.
Methods: Cx30 KO mice and littermate wild-type (WT) mice were used. Hearing function was tested by ABR, DPOAE, and cochlear microphonics (CM) recordings. The spiral neuron development and the ribbon synapses distribution were examined by immunofluorescent staining with confocal microscopy. Animal behavior was also examined by acoustic startle response (ASR). Gene transcriptional changes were analyzed by RNA-Seq technique.
Results: Cx30 KO mice showed hearing loss in comparison with WT mice. In comparison with WT mice, innervations of auditory nerves with inner hair cells (IHCs) in Cx30 KO mice were significantly reduced. Ribbon synapses in Cx30 KO mice were also reduced and demonstrated a "deer-hoof-print" like distribution under IHCs. The behavioral test measured by acoustic startle response (ASR) showed that Cx30 KO mice had the similar ASR as WT mice. However, the peak-time of ASR in Cx30 KO mice had significant delay. In addition, as age increased, the responses of acoustic startle in Cx30 mice was significantly decreased in comparison with WT mice. Consistent with morphological and behavioral changes, RNA-Seq analysis revealed that Cx30 KO caused significant changes in axon and synapse formation and specialization pathways, even spiral ganglion neurons have no Cx30 expression.
Conclusions: These data indicate that gap junction gene Cx30 can modify cochlear neural development and distribution, even though there is no Cx30 expression in the spiral ganglion neurons. These results also reveal that connexin GJs play a critical role not only in the cochlear development but also in the neural development in the inner ear.

Supported by NIH R01 DC019687

S10. Inferred Neural Deficits Associated With Difficult Word-Recognition Tasks are Consistent With a Preferential Loss of Low- And Medium-SR Fibers in Normal-Hearing Subjects

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Category: Auditory Nerve
Background: Difficulty understanding speech in noisy backgrounds is the most common complaint associated with sensorineural hearing loss. Previous studies have shown that measures of cochlear nerve degeneration (CND) in normal-hearing listeners were correlated with performance on difficult word-recognition tasks (Grant et al, 2020; Mepani et al, 2021). Here, we examine in normal-hearing subjects if 1) word scores are correlated with suprathreshold envelope-following responses (EFRs) to rectangular

amplitude-modulated (RAM) stimuli and 2) if deficits in performance are consistent with a preferential loss of low-/mid- spontaneous rate (SR) fibers as inferred by a model of the auditory periphery.
Methods: 80 native speakers of English, 19-72 yrs old, with normal audiometric thresholds (≤ 20 dB HL) at both standard and extended frequencies (9-16 kHz) were enrolled.
Word-recognition performance was assessed by counting the number of correctly repeated words from a list of 50 words from the Northwestern University Auditory corpus (NU-6) presented at 70 dB HL with 65% "time compression" and added 0.3 sec reverberation.

EFRs were recorded using a vertical montage with the ground on Fpz, the inverting electrode over the ipsilateral mastoid bone and the other non-inverting electrode on Fz. EFRs were obtained in response to 600-ms long rectangular amplitude-modulated (RAM) tones at 3 kHz delivered at 70 dB SPL using a 120 Hz modulation frequency with 100% modulation depth and a 25% duty cycle.

The Zilany, Bruce, and Carney (2014) model of the peripheral auditory system was used to simulate the responses of three auditory-nerve fiber types of different spontaneous rates (SR): low (0.1 spikes/s), medium (5 spikes/s) and high (100 spikes/s). A time-domain waveform of individual words extracted from the word lists was inputted to the model. The model's output from each fiber type was compared against the wavelet spectrogram of the stimulus using two-dimensional correlation analysis.

Results: Suprathreshold EFRs to RAM stimuli were correlated with word-recognition scores obtained on CNC words presented with 65%-time compression with added reverberation (r = 0.27, p less than 0.001). Correlation coefficients between speech stimulus spectrograms and the simulated neural response obtained from the model varied as a function of SR and stimulus presentation level: at higher SPLs, words with time compression and added reverberation were better represented for low-SR and mid-SR than for high-SR fibers (t-test at 90 dB SPL: p less than 0.001 for low-SR or mid-SR vs high-SR).
Conclusions: Model simulations suggest that the low-/medium-SR fibers provide a better spectro-temporal representation of CNC words presented with time-compression and reverberation at suprathreshold levels when compared to high-SR fibers. These results support the hypothesis that loss of low-/mid-SR fibers is associated with poorer performance on difficult word-recognition tasks in normal-hearing subjects.

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S11. Peripheral Speech Encoding Mechanisms: Comparative Analysis Between Simulations and Human and Gerbil Empirical Measures

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Category: Auditory Nerve
Background: Phoneme and vowel discrimination in speech perception depend on information derived from spectro-temporal differences. However, how speech is encoded in the cochlea and is impaired by peripheral hearing damage remains unclear. Simulated single-unit auditory nerve (AN) responses as well as compound action potentials (CAP) to phonemes and vowels using models of auditory processing mechanisms, can provide insight into these speech coding mechanisms. Especially when comparing the simulations to direct measures of AN responses elicited by the same stimuli in humans and gerbils.
Methods: Four phonemes: /du/, /bu/, /di/, /bi/ and four vowels: /o/, /u/, /i/ and /y/ were presented at 70 dB SPL and were presented in pairs to assess neural discriminability: /du/-/bu/, /di/-/bi/, /o/-/u/ and /i/-/y/. By shifting the formant locations within a pair, a sequential 9-step morphing was generated between the extremes within a pair. Simulated single-unit and summed AN responses were generated by using the Verhulst et al. (2018) model for humans, along with a new version that was adapted to simulate gerbil hearing. For pairs /du/-/bu/ and /o/-/u/, the spectral region below 1.5kHz differs, primarily targeting temporal fine structure (TFS) coding ability for discrimination. The /i/-/y/ and /di/-/bi/ pairs differed in the spectral region above 1.5 kHz and target the ability to use temporal envelope (TENV) cues. Simulations were performed for normal and

hearing-impaired cochleae (outer-hair-cell or AN damage) and compared to collected human CAP data (Reims) and single-unit gerbil AN data (Montpellier).
Results: Both in simulations and in recordings, the summed AN responses to /i/-/y/ were similar across all steps of morphing. This contrasts the simulations and recordings of the summed /du/-/bu/ and /o/-/u/ responses that show apparent waveform differences across morphing steps with /o/-/u/ showing the most pronounced differences, indicating easy neural discriminability for the provided TFS cues. There was a discrepancy between simulated and recorded human /di/-/bi/ responses, where the recordings showed stronger visual differences than the simulations. Here, neurograms that show the AN fiber activation across characteristic frequencies can help to disentangle the difference between population and single-unit place coding mechanisms.
Conclusions: Comparisons between recordings and simulations indicate some interesting overlap suggesting that the simulated TFS/TENV coding mechanisms can capture the experimentally observed responses. Hearing-impairment simulations can furthermore help elucidate which coding aspects (vowel, consonant, TFS, TENV) are predominantly affected, to steer the future development of hearing-impairment treatments.

Work supported by ERA-NET Neuron grant CoSySpeech (ANR R21034FF and FWO G0H6420N).

S12. Encoding of Vowels in Gerbil Auditory Nerve

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Category: Auditory Nerve
Background: Speech perception relies on the detection of a variety of subtle acoustic spectro-temporal contrasts, but little is known about the speech encoding in cochlea. Here, we performed single-fiber recordings from gerbil auditory nerve in response to vowels featuring specific spectro-temporal contrasts.
Methods: Four synthetic vowels were prepared: /o/, /u/, /i/, and /y/. For each of the /o/–/u/ and /i/–/y/ vowel pairs a sequential 5-step morphing was generated between the two elements of the pair by shifting formant locations. Specifically, the vowels of the pair /o/–/u/ differ in the spectral region below 1.5 kHz which primarily concerns temporal fine structure coding. The vowels of the pair /i/–/y/ differ in the spectral region above 1.5 kHz and are thus thought to primarily rely on envelope coding for distinction. Single-fiber recordings from the auditory nerve were performed in anesthetized gerbils (2-3 months of age, males). Sounds were delivered in closed field, and extracellular action potentials from single auditory nerve fibers (ANFs) were recorded with a glass microelectrode.
Results: Single-unit recordings were performed in 270 ANFs. The distribution of spontaneous discharge rate (SR), characteristic frequency (CF), and threshold of fibers was similar to that reported in previous studies. The response of individual fibers to each morphed pairs of stimuli was recorded at 70 dB SPL (same polarity, 30 presentations per stimulus). In response to vowels /o/, /i/, and /y/, most of the fibers' firing patterns were phase-locked to the temporal envelope of the vowel. In response to /u/, some fibers also fired in synchrony with the phase of the fine structure of the sound. For the vowels generated by morphing /i/ to /y/, the response of most the fibers remained unchanged across the five steps of the morph. In contrast, morphing between vowels /o/ to /u/ evoked gradual changes of firing patterns in a fraction of fibers, thus suggesting a better ability of ANFs to encode in a discriminatory manner the pair of vowels /o/-/u/ rather than the pair /i/-/y/.
Conclusions: These preliminary results suggest presence of distinct neural coding of different vowels (notably /o/-/u/) in individual ANFs that relies largely on the envelope coding. Further analyses using other stimuli (e.g. syllables) and population fibers responses (neurograms) are ongoing.
Work supported by ERA-NET Neuron grant CoSySpeech (ANR R21034FF and FWO G0H6420N).

S13. Differential Proteomics Analyses of the Young and Aging Auditory and Vestibular Neurons

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Category: Auditory Nerve

Background: The spiral and vestibular ganglia contain diverse neuronal subtypes with distinct functions. Among the signatures of age-dependent plasticity are degeneration of the ganglionic neurons. Understanding the mechanisms of age-related hearing loss and vestibular hypofunction may require the identification of relevant molecular markers of aging. Single-cell analysis provides insights for understanding unique features of neuronal populations, cellular lineage, function, differentiation, impacts of microenvironments, and discovery of rare cell types in a complex system such as the auditory and vestibular systems.

Methods: Single-cell sequencing-based transcriptomic profiling (scRNA-Seq) is now commercially available and broadly accessible. However, single-cell transcriptomics only captures part of the molecular state of a cell. Cellular structures and functions are determined mainly by the proteome.

Results: We generated unbiased and in-depth single-cell protein profiles of auditory and vestibular neurons in 2-3, 12, and 24-28 most old CBA mice. We used the nanoPOTS and LC-MS-based proteomics platforms for sensitive and high-throughput single-cell proteomics. The unbiased profiling of auditory and vestibular neurons provides a depth of coverage of greater than 1000-3000 proteins/cell, thus providing a capability for direct protein measurement analogous to single-cell RNA-seq. We will analyze the proteome, provide a complete picture, and identify protein markers associated with ARHL and vestibular hypofunction.

Conclusions: We generated unbiased and in-depth single-cell protein profiles of auditory and vestibular neurons in 2-3, 12, and 24-28 most old CBA mice. We used the nanoPOTS and LC-MS-based proteomics platforms for sensitive and high-throughput single-cell proteomics. The unbiased profiling of auditory and vestibular neurons provides a depth of coverage of greater than 1000-3000 proteins/cell, thus providing a capability for direct protein measurement analogous to single-cell RNA-seq. We will analyze the proteome, provide a complete picture, and identify protein markers associated with ARHL and vestibular hypofunction.

S14. Hyperpolarization-Activated Cation Channels Confer Tonotopic Specializations in the Cochlear Nucleus

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Category: Brainstem: Structure and Function

Background: The intrinsic properties of neurons within auditory circuitry are crucial for accurate signal processing. In the avian model, the brainstem neurons of the nucleus magnocellularis (NM) receive primary auditory input from nVIII fibers and are distributed systematically according to characteristic frequency tuning, an arrangement known as tonotopy. Although NM cells are relatively homogeneous in structure and function, they display several prominent physiological properties that vary systematically along the tonotopic axis. One such property is the tonotopic expression of voltage-gated ion channels. One such channel, HCN, mediates hyperpolarization-activated cation conductances (Ih). We investigated the distribution and functional role of Ih channels in shaping response properties of NM cells to simulated synaptic input.

Methods: We used whole-cell current and voltage clamp methods to assess the function and contribution of HCN channels in the cochlear nucleus magnocellularis cells. Immunohistochemistry was used to evaluate the expression of the HCN1 channel subunit.

Results: We previously established two measures of membrane excitability that vary systematically along the tonotopic axis: slope threshold and integration period. The slope threshold decreased and the integration period increased in NM cells across the tonotopic axis following the application of the HCN1 channel blocker ZD-7288 (40uM). Additionally, the block of Ih channels influenced NM response thresholds and entrainment to pulse stimuli that simulated phase-locked synaptic input over a range of input frequencies.

Conclusions: Together, these results show a novel tonotopic expression gradient of hyperpolarization-activated cation channels (HCN) and their conductances (Ih). Further, they suggest that Ih regulates

excitability and response fidelity during sustained stimulus periods primarily by influencing the input resistance of NM cells.

S15. Characteristics of Suprathreshold Parallel Auditory Brainstem Responses (pABR)

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Category: Other, Auditory Brainstem: Human

Background: Auditory brainstem responses (ABR) are used to objectively evaluate hearing status in both research and the clinic, but obtaining complete tests often requires a lot of time. Time is a commodity when testing infants, toddlers, and adults who cannot complete behavioral testing or when multiple conditions are needed in the same session. The parallel ABR (pABR) speeds up testing by simultaneously presenting all frequencies in both ears. To date, only wave V of the pABR has been characterized because it is the prominent component that reliably predicts hearing threshold – the most common application of ABR testing. However, the pABR at suprathreshold levels may add clinical and research value by evaluating different parts of the early auditory system pathway at different frequencies. This study aimed to assess the proportion of participants with identifiable ABR waves I-V and middle latency responses (MLR) and then characterize these components of the suprathreshold pABR.

Methods: Data were downloaded from an open-set repository on Dryad. This dataset included 20 normal hearing adult participants (13 females, 6 males, 1 non-identifying, ages 18–35 years) who listened to pABR stimuli (0.5, 1, 2, 4, 8 kHz in both ears) at six stimulation rates (20–120 Hz) and two intensities of 51 and 81 dB peSPL (~30–40 and 60–70 dB nHL respectively). Only the higher intensity responses were analyzed for this study. ABR and MLR waves were visually chosen and analyzed for their presence, latency, and amplitude. Peak picking consistency across two markers was analyzed using the intraclass correlation type III coefficient. Peak amplitude and latencies were analyzed using linear mixed-effects modeling with participant and peak marker as random effects.

Results: Chosen peaks were reliable across two markers and showed a prominent wave V for all participants. Preliminary results indicate identifiable waves I and III for mid/high frequency responses and at low/mid stimulation rates. Furthermore, latencies and amplitudes differed for each ABR peak, frequency, and stimulation rate. MLR responses were consistently visible and will be further characterized. The ability to identify and characterize ABR and MLR components is significant given the moderate intensity of 60–70 dB nHL of the multi-frequency pABR stimuli compared to the high-intensity 80–90 dB nHL broadband click stimuli typically used in suprathreshold ABR testing.

Conclusions: Suprathreshold testing using the pABR provides information about multiple early stages of auditory processing while using a moderate intensity. Using such a tolerable level to gain this information may facilitate further insights into hearing function beyond sensitivity in future research and clinical practice, particularly for those populations from which it is challenging to obtain behavioral responses.

S16. Neuropeptide Y mRNA Has Altered Expression With Age in the Inferior Colliculus in Fischer Brown Norway Rats

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Category: Brainstem: Structure and Function

Background: Neuropeptide Y (NPY) is one of the most abundant peptides in the brain, it is primarily expressed by a subset of GABAergic neurons and its neuromodulatory effects shape neuronal activity. In the inferior colliculus (IC), the central hub of the auditory pathway, NPY signaling broadly dampens neural excitability, making it a potential influencer of the excitation/inhibition balance that is disrupted in age-related hearing loss. The aim of the present study was to investigate regional changes of NPY mRNA expression in the aging IC.

Methods: We used 3- and 28-month-old Fischer Brown Norway (FBN) rats, which develop low frequency hearing loss at ~24 months of age. Unfixed brains were flash frozen in liquid nitrogen and sectioned coronally at 12 µm. Single molecule fluorescent in situ hybridization assays were performed using the RNAscope Multiplex Fluorescent Reagent Kit v2 according to the manufacturer's instructions (ACDBio) to express NPY mRNA and GAD1 mRNA. Sections were counterstained with NeuroTrace and DAPI to reveal profile areas and nuclei, respectively. The Paxinos and Watson ('98) rat brain atlas and Neurolucida (MBF Bioscience) were used to contour the IC sections and subdivide into central nucleus (ICc), dorsal cortex (ICd), and lateral cortex (IClc). We manually quantified NPY mRNA and measured profile areas of each cell across the IC subdivisions using Neurolucida. Cells were classified as small, medium, or large, as determined by our previous studies.
Results: Nearly all (99.3%) NPY cells also co-expressed GAD1 mRNA. However, the number of GAD1 positive cells that were also NPY positive ranged from 69% in ICd to 43% in ICc to 38% in IClc. While each IC subdivision had a large population of NPY cells, ICd has the greatest density. Most (~80%) cells expressing NPY mRNA had medium cell profile areas with an average profile of 237.4 µm2 at young age and 232.7 µm2 at old age. The average number of NPY mRNA in medium young cells was 81.8 in the ICd and 76.5 in the IClc. In medium old cells, the average number of NPY mRNA was 54.1 in the ICd and 49.8 in the IClc, a reduction of 33.9% and 34.9%, respectively. The number of NPY mRNA did not change in medium cells in the ICc (62 in young and 61.2 in old) with age. Reductions in small and large cells were not as robust.
Conclusions: Our findings demonstrate that NPY-mediated inhibition is likely reduced at old age, in particular the non-lemniscal IC. As NPY dampens neural excitability in the IC, the loss of NPY with age may contribute to an imbalance of inhibitory/excitatory neurotransmission. In turn, the poor temporal precision routinely present in elderly populations with hearing loss may be driven by the downregulation of NPY in the auditory brainstem.

S17. Effects of Noise-Induced Hearing Loss on Dorsal Cochlear Nucleus Neurons in Late Adolescent NF107:Ai32 Mice

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Category: Brainstem: Structure and Function
Background: Noise exposure causes multiple changes in cellular physiology of central auditory neurons and in their sensory responses. However, many physiological studies at the cellular level have used young animals, and it remains unknown whether the reported changes in electrical excitability and synaptic function are the same when noise exposure occurs later in life. Here, we investigated intrinsic excitability and synaptic responses to stimulation of type 1-c spiral ganglion cell (SGC) terminals in the dorsal cochlear nucleus (DCN) of late adolescent mice.
Methods: NF107 mice (FVB background) were crossed with the Ai32 ChR2-EYFP mice, to express ChR2-EYFP in the type 1-c SGCs. There were 4 experimental groups: control mice with no expsoure, mice exposed to 115 dB SPL octave-band (8-16 kHz) at P42D and recorded ~2 weeks (2W, P56D) later, mice exposed to 115 dB SPL octave-band noise at P53D and recorded at ~P56D (3D), and mice exposed to 96-106 dB SPL octave-band noise at P42D. ABRs were performed on all subjects prior to slice preparation. All recordings were performed blinded to treatment. Brain slices were prepared following transcardial perfusion with an NMDG-based solution, and intracellular recordings made with K-gluconate electrodes containing tetramethylrhodamine-biocytin for cell identification. Responses to current injection were collected to assess intrinsic excitability and spike shape. Responses to laser-scanning photostimulation were collected to evaluate synaptic input from type 1c SGCs. Results from all groups were analyzed with custom programs. Treatment effects were analyzed using omnibus tests followed by multiple-comparison corrected post-hoc tests.
Results: Recordings revealed few changes in intrinsic excitability. Three days after 115 dB SPL exposure, firing rate adaptation in pyramidal cells slightly increased. All 3 noise exposure conditions reduced the maximum rising slope of pyramidal cell APs. Tuberculoventral cells showed an increased firing rate gain with current injection 2W after exposure at either level; increased gain was not apparent 3D after noise exposure. Cartwheel cells showed few changes. There were no changes in the time course of spontaneous EPSCs or

auditory-nerve evoked EPSCs, or in the amplitude of spontaneous EPSCs. The rate of spontaneous EPSCs in pyramidal cells significantly increased 2W after exposure at both sound levels.
Conclusions: As compared to previous studies in which juvenile mice or rats were challenged with noise exposures resulting in multiple changes in neuron excitability and synaptic transmission, we found that in older mice there were fewer central consequences that could be detected. Surprisingly, the tuberculoventral cells, a class of glycinergic interneuron in the DCN that projects throughout the cochlear nucleus, showed increased firing gain. Given there was very little functional recovery from the higher-level noise exposures, these results suggest that there is limited plasticity of intrinsic excitability in DCN neurons after hearing loss.

S18. Glutamatergic Pathway-Specific Loss-Of-Function of mGluR5 Disrupts the Development of Synaptic Excitatory Transmission Onto Mouse MNTB Neurons

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Category: Brainstem: Structure and Function
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 P14 (range 14-15) and P30 (range 30-35). 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.
Results: Elimination of mGluR5 in the glutamatergic pathways compromised the synaptic properties required for precise temporal processing in MNTB, especially at P30. The amplitude of spontaneous EPSCs in the cKO at P30 was significantly larger than that of WT. In response to synaptic stimulation with increasing intensity, evoked EPSCs in the cKO varied widely in latency, whereas in the WT the synchronized eEPSCs had a constant latency. The varying latencies of the eEPSCs could be caused by increased number of inputs. The frequency of asynchronized (delayed) glutamate release following a train synaptic stimulation (100 Hz, 20 pulses) increased significantly in the cKO at P30, indicative for compromised temporal coding.
Conclusions: Glutamatergic pathway-specific cKO of mGluR5 disrupted the cellular properties required for temporal processing at the AVCN-MNTB synapse. The results are consistent with the idea of increased excitatory inputs in the cKO, suggesting a role of mGluR5 in synapse formation and maturation during development.

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S19. Age-Dependent Characterization of Fusiform Cells

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Category: Brainstem: Structure and Function
Background: The cochlear nucleus (CN) receives the entire output from the cochlea via the auditory nerve. The CN is comprised of 3 main compartments, the posterior ventral cochlear nucleus, anterior ventral cochlear nucleus, and dorsal cochlear nucleus (DCN). The DCN integrates auditory information with somatosensory,

vestibular, and proprioceptive signals for external sound localization and inhibition of self-generated sounds (Ryugo et al., 2003; Singla et al., 2017). This function is orchestrated by a wide variety of inhibitory and excitatory neuronal circuits within the DCN. The primary excitatory output neurons of the DCN are fusiform cells. Fusiform cells change their electrophysiological properties during the onset of hearing at least up to p22 in mice (Benites et al., 2023). However, bushy cells (an excitatory projection neuron found in the ventral CN) demonstrate continued physiological changes at 6-7 months (Xie and Manis, 2013), and likewise morphological changes (Ryugo et al. 2006) out to at least 3 months. This study seeks to extend the characterization of morphological and physiological properties of DCN fusiform cells into adulthood; thus, providing foundational knowledge for how fusiform cells maintain or modulate morphological and physiological properties post-maturation. This insight is imperative to complete our understanding of how the CN, and the auditory system, reach their mature form and function.

Methods: CBA/CAJ mice at 4 age points were used for experiments: preweaning (p12-18), pubescent (p20-40), young adult (p50-90), and mature adult (p180+). Mice were euthanized and perfused with an NMDG-based solution before DCN collection. Brain slices (250 microns thick) of DCN were prepared on a vibratome. Fusiform cells in DCN were subjected to whole-cell recording and filled with tetramethylrhodamine-biocytin before fixation. Post fixation, brain slices were imaged on the Zeiss 780 confocal laser scanning microscope. Z-stack images, 30-50 microns thick, were imported into Imaris for 3D reconstruction of filled fusiform cells. Quantitative analysis of morphology was derived from individual filled cells.

Results: Preliminary results indicate an increase in dendritic complexity with age. The number of branch points per dendrite increases with age, indicating a more complex fusiform cell dendritic tree. Interestingly, although the number of branch points increases with age, the total cell surface area remains consistent. Physiologically, intrinsic firing properties are qualitatively similar across this age range.

Conclusions: In this study, we characterize fusiform cell morphology and physiology into maturity. This work will give critical insights into understanding the continued development of these key auditory neurons, and their potential changes that can affect auditory processing with age.

S20. Three-Dimensional Imaging of Granule Cell Regions in Cochlear Nucleus of NF107:Ai32 Mice

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Category: Brainstem: Structure and Function

Background: Axons of spiral ganglion cells (SGC's) form the auditory nerve, projecting onto cells of the cochlear nucleus (CN). However, many details of how different molecular sub-types of SGC axons, and their endings, are distributed across the CN are not well understood. We previously presented evidence suggesting that in NF107:Ai32 mice, a subset of SGC's express ChR2-EYFP and co-localize with Pou4f1 – a putative marker for low spontaneous rate SGC's. Here, we combine GABA receptor immunolabeling to distinguish granule cell regions in these mice with axonal projections (relative to granule cell regions) to investigate if these patterns also reflect features characteristic of low spontaneous rate SGCs.

Methods: Adult NF-107-Cre mice cross bred with Ai32 (ChR2-EYFP) mice express ChR2-EYFP in a sub-population of SGC's, which can be immunostained for EYFP to study their projections to the CN. In addition, the CN was immunolabeled with antibodies to GABAA receptor α6 subunit to mark granule cell regions and examine how EYFP labeled axons are distributed relative to the granule cell regions. For this, CN tissue was processed and cleared for imaging using light sheet fluorescent microscopy. Images were imported into Imaris software for visualization in three-dimensional (3D) space.

Results: The granule cell region has an extensive distribution across the CN, similar to other animals. It encapsulates the margins of the CN, broken only to allow fibers to enter or exit from the nucleus. Internally, a portion of the granule cell region forms the boundary between ventral and dorsal CN (VCN and DCN), abutting a region in the VCN also termed the small cell cap. Within the DCN, there is a district granule cell layer, characteristically fractured into island-like cellular segments. The small cell cap region is of particular interest for two reasons. In 3D, the granule cells in this region do not form a continuous layer, but rather a sheet punctuated by multiple lacunae. Many small lacunae were filled with EYFP positive puncta, and larger lacunae often contained numerous EYFP labeled fibers – forming what appear to be conduits for fibers coursing between the DCN and VCN, including some auditory nerve fibers. The second reason is that EYFP

labeled auditory nerve fibers form a plexus along the ventral surface of the small cell cap throughout its extent – a pattern similar to that shown in single-fiber studies of low spontaneous rate SGC's.
Conclusions: The present results, together with our Pou4f1 co-localization observations, further point to the NF107:Ai32 mice as expressing EYFP predominantly, if not exclusively, in a specific subset of SGC's. The central projection patterns from these SGC's exhibit a particularly close relationship to granule cell regions. Together, these observations suggest these mice provide a useful model for examining structure-function properties of a specific sub-type of SGC.

S21. Influences of Sensory-Motor and Predictive Mechanisms on the Frequency Following Response (FFR): Listening While Playing the Organ

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Category: Other, Auditory Perception
Background: The frequency following response (FFR) is typically considered as a passive response to auditory stimuli that can be used to quantify the quality of auditory encoding. However, predictive coding theory suggests that perception is the outcome of top-down prediction interacting with sensory (bottom-up) processes. Therefore, self-produced sounds may generate different FFRs than passively generated sounds, and previous auditory-motor experience may interact with these mechanisms. Our study aims to explore these interactions, as well as the improvements related to music training in auditory perception, by examining the FFR specifically in the context of auditory-motor integration.
Methods: Using newly developed procedures that combine electroencephalography [EEG] and a modified digital organ, our protocol compares the FFRs of highly trained pianists and non-musicians while they play a short melody and while they hear the same sounds passively. To discriminate between motor and perceptual factors, the listening/playing tasks included conditions in which the auditory feedback was also predictable or unpredictable (2x2 design). This study encompassed two experiments. In a first experiment, we compared the specific FFR components that are modified (F0 amplitude, pitch tracking, latency) by the motor activity, predictability of the auditory pattern, or by both factors. In a second experiment, the procedures were replicated, but the motor conditions were performed with the feet on the pedal keyboard of the organ, instead of the left hand. The aim of experiment two was to make comparisons with the hand condition to identify if FFR modifications may be linked to motor processes associated with a specific effector or to generic motor responses. Comparing both sessions also allowed to observe learning effects.
Results: Among the most salient findings, experiment 1 (fingers) shows reduced F0 amplitudes in the auditory-motor versus the purely auditory condition. The unpredictable conditions also showed reduced amplitudes compared to the predictable conditions (n=14). These predictability effects are in line with predictive coding as well as sensory-motor interaction models. Experiment 2 (feet) showed amplitude gains in the auditory (predictable) condition compared to the auditory-motor (predictable) condition. Learning effects were also observed between the fingers and feet experiments, suggesting auditory-motor consolidation or transfer learning effects. Such neural response modifications support the perceptual nature of the FFR, rather than limiting its function as a neuro-acoustic representation.
Conclusions: To the best of our knowledge, our protocol is the first to compare actively produced music tones stimuli (auditory-motor conditions) to the same passive tones stimuli (auditory conditions). Taken together, these results allow to better understand how auditory-motor and predictability mechanisms may modify the FFR, which will help to develop better theoretical models of auditory cognitive processing.

S22. Compartmental-Specific CD47 and Microglial SIRP-α Expression in the Developing Lateral Cortex of the Inferior Colliculus

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Category: Midbrain: Structure and Function

Background: The lateral cortex of the inferior colliculus (LCIC), a multisensory midbrain structure, is organized into two distinct subregions: modular zones which receive somatosensory inputs, and a surrounding matrix which receives auditory inputs. This interfacing of afferent streams with its compartmental structure develops during an early postnatal critical period (postnatal day 0-12). Microglial cells (MGCs) are known to serve a variety of developmental functions, including a prominent role in synaptic remodeling. Such sculpting is thought to involve a balance between MGC recognition of "eat me" tags to remove unnecessary connections, coupled with "don't eat me tags" that serve to protect contacts being actively utilized. The classic complement signaling cascade (C3-CR3) has been implicated in unimodal systems in identifying and pruning exuberant connections, while CD47-SIRP-α interactions detect active synapses that should be avoided and maintained. MGCs expressing the SIRP-α receptor recognize CD47 "don't eat me" tags selectively expressed on connections that are highly utilized, thereby protecting them from subsequent engulfment. Whether CD47-SIRP-α signaling is involved in shaping multisensory circuits during early critical periods of development remains unaddressed. The present study investigates CD47 and SIRP-α expression in relation to the developing modular-matrix framework of the LCIC.

Methods: Fluorescence immunocytochemistry for CD47 (BD Pharmingen [BD Biosciences], #555297, 1:500, RRID:AB_395713) and SIRP-α (QED Bioscience, #2428, 1:50, RRID:AB_130075) was performed in a developmental series of GAD67-GFP mice (P0, P4, P8, P12, and P20). This mouse line enables easy visualization of emerging LCIC modular zones that are GAD-positive. Additional labeling was performed in CX3CR1-GFP mice to determine whether MGCs co-express the fractalkine receptor (CX3CR1) and SIRP-α. Widefield epifluorescence imaging was performed on a Nikon Eclipse Ti-2 microscope and an extended depth of focus (EDF) algorithm was used for two-dimensional renderings of acquired Z-stacks.

Results: CD47 expression is robust and homogenously expressed throughout the LCIC early on (P0-P4), prior to it exhibiting a matrix bias that is most heavily concentrated ringing modular confines (P8-P12). SIRP-α expressing MGCs are present in the LCIC at birth and localize specifically to the matrix as the compartmental arrangement emerges by P4. This organization is apparent through the critical period peak (P8), prior to its downregulation shortly thereafter. SIRP-α and CX3CR1-positive microglia were non-overlapping, suggesting microglial heterogeneity and subsets with distinct expression profiles during early critical periods of development.

Conclusions: These findings implicate CD47-SIRP-α signaling in the sculpting of early LCIC circuits, and that deficits in such signaling may yield multisensory network maps that are subject to over-pruning. Ongoing experiments will test the hypothesis that CD47 and/or SIRP□ deletion results in increased engulfment of labeled LCIC terminals and decreased overlap of its multimodal afferent patterns relative to controls.

S23. Cholinergic Excitation and Inhibition of NPY Neurons in the Inferior Colliculus

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Category: Midbrain: Structure and Function

Background: The inferior colliculus (IC), a major integration hub in the central auditory pathway, receives dense cholinergic projections from the pedunculopontine tegmental nucleus (PPT), a brain region involved in arousal and attention. However, how cholinergic signaling modulates inhibitory neurons in the IC remains largely unknown. We recently identified Neuropeptide Y (NPY) as a marker for a class of GABAergic IC neurons. Here, we show that the excitability of NPY neurons can be enhanced or inhibited by acetylcholine (ACh). Using electrophysiology and pharmacology, we are testing the hypothesis that this bidirectional regulation of NPY neuron excitability is due to the differential expression of muscarinic (mAChRs) and nicotinic ACh receptors (nAChRs) in subpopulations of NPY neurons.

Methods: To selectively target recordings to NPY neurons we utilized NPY-IRES2-FlpO x Ai65F mice in which NPY neurons express tdTomato. We used brain slice electrophysiology in acutely prepared IC slices to record the changes in membrane potential of NPY neurons during puff applications of 1 mM ACh. After recording control responses to ACh, we perfused various muscarinic and nicotinic receptor antagonists for 10 minutes and assessed how responses to ACh puffs changed.

Results: We found that 1mM ACh puffs onto NPY neurons induced depolarizing and hyperpolarizing responses. To test the roles of muscarinic and nicotinic receptors in mediating these responses, we are sequentially applying selective mAChR and nAChR antagonists on IC brain slices and assessing their ability to block responses to ACh puffs. Our preliminary data suggest that individual NPY neurons can express mAChRs, or nAChRs.
Conclusions: We find that ACh elicits depolarizing and hyperpolarizing responses in subpopulations of NPY neurons. Our results to date suggest that nAChR and mAChR expression regulate the depolarizing and hyperpolarizing effects, respectively, of ACh on NPY neuron excitability. We, therefore, propose that cholinergic signaling in the IC may use selective and differential modulation of inhibitory circuits to enhance certain IC computations while inhibiting others.

S24. Altered Predictive Processing in the Inferior Colliculus of a Rat Model of Autism

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Category: Midbrain: Structure and Function
Background: Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by atypical social behaviour and restrictive, repetitive interests. Latest diagnostic criteria included unusual reactivity to sensory inputs (e.g., aversion or engagement to specific sounds) among the restrictive, repetitive behaviours. Interestingly, the predictive coding theory can explain these sensory issues. This theory postulates that the brain actively predicts upcoming sensory inputs, and that prediction errors occur when these are violated. This updates the predictions to the new sensory information. The predictive coding theory of ASD proposes a decreased ability to anticipate upcoming sensory information due to overly precise predictions. Especially when situations (e.g., social) or stimuli (e.g., sounds) become highly dynamic, which explains the difficulties in interpreting and responding to context-dependent stimuli in ASD.
Methods: Here, we studied the predictive processing of auditory stimuli at the subcortical level in a rat model of ASD. To induce ASD in the offspring, pregnant rats were injected with valproic acid. The mechanism that underlies the mismatch negativity generation was investigated, as it has been found to be different in autistic individuals. Specifically, female and male rats in pubertal (PD 30-48) and adult (PD 65 and 120) stages were used, addressing sex and neurodevelopmental differences in neurotypical and neuroatypical animals. We made single-unit recordings in the inferior colliculus in response to a classical oddball paradigm, which elicited the violation (deviant) of the regularity generated by the repetitive stimuli (standard).
Results: Results so far show alterations in neuronal mismatch negativity responses at the midbrain level, supporting atypical low-level predictive processing in this model of ASD. The time course of adaptation is also different, suggesting atypical dynamic of adaptation to the regularity.
Conclusions: These results support the notion of atypical predictive processing in autistic individuals, which explains the unusual adaptability to unexpected events.

S25. Subcortical Plasticity During Auditory Perceptual Learning

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Category: Midbrain: Structure and Function
Background: Sensory perception is highly dynamic, capable of both rapid context-dependent shifts, as well as slower changes that emerge over time with extended training. Previous research has shown that these perceptual fluctuations are driven by corresponding changes in the sensitivity of auditory cortical neurons to sound. However, it is unclear whether these changes emerge in the ascending auditory pathway and are inherited by the auditory cortex, or arise in the cortex de novo.
Methods: As a first step towards answering this question, we implanted Mongolian gerbils with chronic microelectrode arrays in either the central nucleus of the inferior colliculus (CIC) or the ventral medial geniculate nucleus (vMGN). We recorded single-unit activity as animals trained and improved on an aversive

go/no-go amplitude modulation (AM) detection task, and during passive exposure to the same AM sounds. AM-evoked firing rates, power, and vector strengths were calculated and transformed into the signal detection metric d'. Neural thresholds were obtained for each training day by fitting d' values across AM depths and determining the depth at which d' = 1. Thresholds were compared between periods of task performance and passive sound exposure across several days of training to determine whether there were context-dependent and/or learning-related changes in activity.
Results: Neural thresholds obtained from CIC and vMGN were lower (better) when animals performed the behavioral task, compared to when they were passively exposed to sounds. A subset of neurons also exhibited a context-dependent change in coding strategy, with AM stimuli encoded by vector strength during passive exposure and by firing rate during task performance. In the CIC, learning-based improvements in firing rate- and power-based neural thresholds were observed when animals were actively performing the task. In the vMGN, however, learning-related changes were gated by behavioral context, such that improvements were only observed when the animals performed the task.
Conclusions: These data suggest that extended perceptual training improves neural sensitivity by acting at or below the level of the auditory midbrain, and that rapid, context-dependent sensitivity enhancements are strengthened with learning in the auditory thalamus. Our results contribute to a deeper understanding of the circuits supporting perceptual flexibility, and may ultimately inform strategies for improving sound perception in hearing-impaired individuals.

S26. Probing Correlations between Different Types of Feature Selectivity in Inferior Colliculus of Awake Rabbit

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Category: Midbrain: Structure and Function
Background: The inferior colliculus (IC), a key structure in the auditory midbrain, integrates information from several parallel ascending pathways. Although many types of feature selectivity, such as tuning to amplitude modulation and sensitivity to interaural differences, have been characterized in IC neurons, less attention has been paid to how individual neurons simultaneously encode multiple features. Few studies have explored how different types of feature selectivity are correlated across the population of IC neurons.
Methods: To address this gap in the literature, we re-analyzed an existing dataset of extracellular single-unit recordings from awake rabbit IC. Two human raters classified sensitivities of single units based on trends in average discharge rate in response to: (1) pure tones at different frequencies and levels (response maps; RMs), (2) amplitude-modulated (AM) noise at different modulation rates (modulation transfer functions; MTFs), (3) gaussian noise presented to the ipsilateral, contralateral, or both ears at different sound levels, (4) gaussian noise presented at different interaural level differences (ILDs) and interaural time differences (ITDs), and (5) fast frequency sweeps with varying sweep direction and velocity. Resulting classifications were analyzed to explore correlations between different types of feature sensitivity. Relationships between feature tuning and absolute threshold, spontaneous rate, and characteristic frequency (CF) were also characterized.
Results: Consistent with prior results (Kim et al., 2020), most neurons were sensitive to AM frequency, with roughly even proportions of neurons with enhanced, suppressed, or both enhanced and suppressed rates over different ranges of modulation frequencies (band-enhanced [BE], band-suppressed [BS], and hybrid, respectively). Hybrid MTFs were either enhanced at lower modulation frequencies and suppressed at higher modulation frequencies, or vice versa. Neurons with BS MTFs tended to have RMs with tuning bandwidth that remained fairly constant with increasing sound level (i.e., I-type RMs). Neurons that were excited exclusively by binaural sound tended to have BS MTFs. Interestingly, neurons with hybrid MTFs that were suppressed over higher modulation-frequency bands exhibited patterns similar to BS neurons, whereas hybrids were enhanced over higher modulation-frequency bands exhibited patterns more similar to BE neurons. As expected, neurons that were excited by the contralateral ear and inhibited by the ipsilateral ear (EI) tended to have ILD sensitivity; however, a substantial proportion of non-EI neurons also exhibited ILD sensitivity.
Conclusions: The varied response sensitivities of IC neurons provide a rich database for probing interconnections between physiological mechanisms. The analyses revealed intriguing correlations between sensitivities to conceptually unrelated features of sound, such as AM tuning and interaural sensitivities. Our

results are a step towards understanding how neural populations in IC simultaneously encode multiple features of complex sounds in realistic spatial environments.

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S27. Investigating Potential Sources of Modulation Enhancement in Noise Through Physiologically Recorded and Model Neural Responses

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Category: Midbrain: Structure and Function

Background: The presence of noise typically challenges hearing, especially for those with hearing loss or hearing assistive devices. There is a substantial amount of variability in the severity of noise impacts on listening ability that cannot always be linked to standard audiometry. Hence, characterizing the auditory system's ability to naturally suppress noise is paramount. Interestingly, recent human studies indicate that in some circumstances, the presence of mild noise (20+dB SNR) can lead to enhanced coding of modulated stimuli, evidenced by increased envelope-following responses (EFRs) and perception. It has been suggested that the unmasking effect of the medial olivocochlear (MOC) efferent system could play a significant role in these observations. Here, we explore this hypothesis further using a combination of computational auditory modeling and animal physiological experiments.

Methods: A subcortical auditory model that incorporates the MOC efferent dynamic gain control system with inputs from the auditory brainstem and also the midbrain (Farhadi et al.2023) was used to assess simulated responses at the level of the auditory nerve and inferior colliculus (IC). We expanded this model and leveraged physiological data from chinchillas to simulate EFRs for a more direct comparison of simulated and experimental findings. We explored responses to various modulated stimuli (e.g., sinusoidal/rectangular AM, tone complex) in a background of speech-shaped noise. To date, we have collected awake and anesthetized data in two chinchilla models – normal hearing and selective inner-hair-cell (IHC) dysfunction - based on the expectation that anesthesia and reduced IHC-driven output each limit efferent activation.

Results: Preliminary experimental data suggest modulation enhancement in noise is stimulus- and level-dependent, and is strongest in awake normal-hearing chinchillas. The low-frequency envelope coding of tone complexes appears preserved, despite noise substantially reducing the preservation of spectral cues. Modeling of these responses similarly predicts a stimulus and level-dependence of this effect, suggesting the strongest enhancement occurs with lower overall stimulus levels (~40 dB SPL). The enhancement observed in the modeling results exists only with the activation of the efferent system.

Conclusions: Computational modeling assists our ongoing data collection to effectively characterize efferent-related contributors to modulation enhancement, though it is possible there are additional cortical contributors. Preliminary physiological findings in chinchillas corroborate this, suggesting an active efferent system is necessary to observe this neural-coding enhancement.

S28. Micro-Goldwire Electrode for Neural Signal Recording

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Category: Midbrain: Structure and Function

Background: Microelectrode arrays are essential tools for auditory physiologists, unlocking the complexities of neural processes in hearing. Their introduction has prompted in-depth studies into auditory processing within the central nervous system (CNS), shedding light on top-down and bottom-up mechanisms. This knowledge is pivotal for mapping auditory pathways and refining neuroprosthetic devices such as cochlear implants (CIs), auditory brainstem implants (ABIs), and auditory midbrain implants (AMIs).

Several studies showed that anaesthesia can significantly alter neural responses. It has been demonstrated distinct neuronal activity in the inferior colliculus of anesthetized rabbits compared to awake ones. Additionally, another research showed changes in the frequency tuning of neurons in the rat primary auditory

cortex under anaesthesia. With these findings, it is evident that accurate neural recordings require awake subjects. Long-term recording becomes crucial, allowing for continuous observation of neural activities and a deeper understanding of the dynamic nature of neural networks. Electrodes designed for this purpose need to be biocompatible, minimize tissue response, and withstand the mechanical stress from subjects' movements to ensure consistent and precise data collection.

Various electrode materials, such as stainless steel and tungsten, have been examined in the past. Among them, micro-gold wire has emerged as a particularly promising candidate due to its flexibility and compliance, which minimizes potential tissue damage. In the present study, we focus on assessing the mechanical and biological attributes of micro-gold wires when used to capture neural activity in the ICC. Our objective is to design electrodes optimized for chronic neural recordings in the auditory field and beyond; and evaluate their performance.

Methods: We used insulated micro-gold wires of 25 micrometres in diameter (GF32175569, Sigma Aldrich, USA). Prior to implantation, these wires were soldered to a board. To facilitate the insertion process, we coated the wires with a biocompatible gel, designed to dissolve after implantation. The tips of these wires varied intentionally in length, a design choice to capture neurons with a range of characteristic frequencies. For our subjects, we chose normal hearing guinea pigs. Neural recordings from ICC were made using our micro-gold wires and the NeuroNexus electrode comprising 16 recording channels (A1x16-5mm-100-177), allowing for a direct comparison within individual animals.
Results: The study is ongoing. We successfully fabricated an implantable multi-channel micro-gold wire electrode tailored for neural recordings within the brain.
Conclusions: Preliminary results show that this micro-gold wire electrode is proficient in capturing neural activity within the ICC.

S29. Effects of Auditory Learning on the Activity of Parvalbumin-Positive (PV+) Interneurons and Perineuronal Nets in the Auditory Cortex

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Category: Other, Auditory Learning
Background: Positive parvalbumin (PV+) interneurons modulate auditory cortical responses and contribute to learning. Perineuronal nets (PNNs) are an extracellular matrix structure surrounding PV+ cells. Reduced expression of PV and PNN has been associated with decreased GABAergic signaling and high cortical plasticity. We hypothesize that both PV and PNN expression are regulated during auditory learning, indicative of shifts in the activity of PV cells of the primary auditory cortex (A1) during auditory training.
Methods: Mongolian gerbils were trained on an aversive go/no go amplitude modulation (AM) detection task. Training was divided into two stages. During procedural training, animals learned to drink water during non-AM noise and to stop drinking during a highly salient (0 dB re: 100% depth) AM noise to avoid a shock. After animals achieved a d' greater than 2 for two consecutive sessions, the animals progressed to the perceptual training stage, during which weaker AM depths were presented over several days. PV and PNN expression was assessed via immunohistochemistry in untrained animals, animals that completed procedural training, and animals that completed two or seven days of perceptual training. In a different group of animals, we used a Miniscope to monitor the calcium responses of individual PV+ A1 cells throughout both procedural and perceptual training.
Results: Auditory training significantly affected PV expression in layer (L) 2/3, L4, and L5 of A1 but had no significant effect in L6. PV expression was lower after procedural training than in untrained controls (L2/3: p=0.0002, L4: P=0.002, L5: p=0.0001). In contrast, PV expression was higher after two days of perceptual training than after procedural training (L2/3: p=0.0001, L4=0.001, L5: p=0.0001) or after seven days of perceptual training (L2/3: p=0.0002, L4: p=0.0001, L5: p=0.0002). Auditory training also significantly affected the proportion of PV+ cells surrounded by PNNs in A1. After procedural training, the proportion of PV+/PNN+ cells was reduced compared to untrained controls (L2/3: p=0.03, L5: p=0.01). In contrast, the proportion of PV+/PNN+ cells was higher after seven days of perceptual training than after procedural training (L2/3: p=0.0077, L4: p= 0.0477; L5: p=0.0022), or two days of perceptual training in L2/3 (p=0.0274).

Conclusions: Our results suggest that PV and PNN expression decreases initially, opening a window for neural plasticity. After extended training, overexpression and renormalization of PV and PNN levels may stabilize network modifications and newly acquired auditory expertise. PV+ calcium responses will be analyzed within the context of these findings.

S30. A Multimodal Convolutional Neural Network Reveals Position-Dependent Changes in Single-Neuron Representations of Natural Sounds during Free-Movement.

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Category: Primary Auditory Cortex

Background: Hearing, and sensation more generally, are active processes. Humans and animals often change their position or tilt their head in order to hear and localize sound sources more accurately. These behaviors may play an integral role in real-world selective attention, and understanding how motor activity and listener's position affect auditory neural coding will be beneficial to improved auditory prostheses. Despite these observations, most previous research has focused on how the auditory system extracts information about the environment from a head-fixed posture. The current study explores the relationship between top-down input from self-motion and spatial representations of sound, thereby elucidating dynamics of the auditory system during active exploration.

Methods: Single unit neural activity was recorded from auditory cortex (AC) of two ferrets while they performed a simple tone detection task that required movement back and forth across a small arena (80 x 100 cm). Semi-chronic Neuropixels implants permitted multiple recording sites in each animal (10-15 insertions/animal, 20-50 units/insertion). During recordings, a sequence of natural sound samples was presented continuously from two spatially separated speakers outside the arena, and animal position was recorded using an overhead video camera. Markerless pose estimation was used to recover head position and angle, which was then aligned temporally to stimulus and spike events. An acoustic model based on sound level throughout the arena and a head-related transfer function was used to infer the sound spectrogram reaching each ear as the animal moved through the arena (approximately 15 dB level variability). A multimodal convolutional neural network (CNN) was trained to predict the time-varying spike rate of each neuron as a function of sound and head position/angle. Permutation analysis was used to evaluate the contribution of each modality to model performance. Dynamic spectro-temporal receptive field (dSTRF) analysis was applied to the CNN to compare spectro-temporal tuning at different arena positions.

Results: The multimodal CNN was able to predict single-trial neural activity above chance for about 60% of AC neurons. Both the addition of the acoustic model and addition of information about head position improved model accuracy over a model based only on the sound spectrogram. dSTRF analysis revealed that the spectro-temporal tuning of individual neurons was modulated by arena position. Incorporating the HRTF/acoustic model reduced tuning variability across space only for some neurons.

Conclusions: This study demonstrates the feasibility of measuring the encoding properties of auditory neurons in free-moving animals, opening up the possibility of assessing dynamic control of sound processing in this naturalistic context. Current results indicate that an animal's position modulates spectro-temporal tuning, consistent with the idea that individual neurons are modulated by distinct hippocampus-like place signals. Further analysis will assess the relationship between position-related and task event-related changes in spectro-temporal tuning.

S31. Murine GRXCR1 Has a Different Function Than GRXCR2 in the Morphogenesis of Stereocilia

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Category: Hair Cells: Anatomy and Physiology

Background: Mutations in human Glutaredoxin domain-containing cysteine-rich protein 1 (GRXCR1) have been linked to hearing loss in humans. Although both GRXCR1 and its paralog GRXCR2 are required for the morphogenesis of stereocilia in cochlear hair cells, multiple evidence have suggested that they have distinct

functions in hair cells. First, in contrast to Grxcr2-deficient hair cells, Grxcr1-deficient hair cells have stereocilia that are extremely thin and contain less F-actin (Liu et al., 2021). Second, GRXCR1 is diffusely distributed throughout the stereocilia, while GRXCR2 is concentrated at the base of the stereocilia (Liu et al., 2021). Third, while reducing the expression level of taperin rescues the morphological defects of stereocilia and hearing loss in Grxcr2-deficient mice (Liu et al., 2018), it does not rescue the defects of hair cells in Grxcr1-deficient mice (Liu et al., 2021). To investigate the functions of GRXCR1, an unbiased yeast-two-hybrid screening was performed using full length GRXCR1 as bait. We identified ~100 positive clones from ~7 million transformants. Remarkably, many of these GRXCR1-interacting proteins are associated with the actin cytoskeleton, indicating that GRXCR1 is involved in stereocilia morphogenesis by regulating actin filaments.
Methods: In this study, we analyzed the localization of GRXCR1 and GRXCR2 in hair cells. We reduced taperin expression level in Grxcr1 mutant and characterized different mouse lines bearing Grxcr1 and/or taperin mutations. In addition, we purified murine GRXCR1 protein and measured the glutaredoxin activity. Our results suggest that GRXCR1 has different functions than GRXCR2 during the morphogenesis of stereocilia.
Results: First, in contrast to Grxcr2-deficient hair cells, Grxcr1-deficient hair cells have stereocilia that are extremely thin and contain less F-actin. Second, GRXCR1 is diffusely distributed throughout the stereocilia, while GRXCR2 is concentrated at the base of the stereocilia. Third, while reducing the expression level of taperin rescues the morphological defects of stereocilia and hearing loss in Grxcr2-deficient mice (Liu et al., 2018), it does not rescue the defects of hair cells in Grxcr1-deficient mice.
Conclusions: Although GRXCR1 and GRXCR2 have similar amino acid sequences, only GRXCR2 binds to taperin. In addition, different localization patterns of these two proteins in stereocilia also suggest that they probably have different binding partners in hair cells. To extensively illustrate the functions of GRXCR1 and mechanisms of Grxcr1 deficiency-induced hearing loss, it will be of interest to screen interacting proteins of GRXCR1 and investigate the extent to which those binding partners are required for GRXCR1 functions and stereocilia morphogenesis in hair cells.

S32. Ultrasonic Characteristics of Prestin Complex Nonlinear Capacitance (cNLC) in the Mouse: Mutation and Molecular Dynamics (MD)

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Category: Hair Cells: Anatomy and Physiology
Background: We measured ultrasonic frequency responses of prestin's cNLC under voltage clamp in guinea pig (GP) OHC membrane patches where cellular loads are absent (Santos-Sacchi et al., J Neuro, 2023). Real and imaginary components of cNLC report on prestin's influence on cochlear amplification, since prestin's charge movements drive electromotility. The frequency where the imaginary component intersects the real component (Fis), reveals this cut-off, near 20 kHz for GP. Fis depends on prestin's kinetics, which is influenced by intracellular chloride levels (Santos-Sacchi and Song, BJ, 2016) and membrane fluidity (Santos-Sacchi et al., BJ, 2022). In the mouse, we test a knock-in prestin mutation that may alter chloride binding influence on ultrasonic conformational switching. We also use MD to probe the potential influence of phospholipids on cNLC.
Methods: cNLC was measured (4-120 kHz) as described for GP membrane macro-patches, using admittance-based techniques (Santos-Sacchi et al., J Neuro, 2023). Control OHCs and knock-in mouse OHCs with the prestin chloride binding site mutation (S396E) were compared. For MD we used the structure we determined by cryo-EM (Butan et al., BioRxiv, 2021; Butan et al., Nature Comm., 2022). Simulations were run on Anton2 at 296K and 333K, ranging up to 12 μs.
Results: On average, Fis for both control and S396E OHCs (n=4-5 patches) have similar cut-offs, namely 27.8 and 25.8 kHz. These values are greater than for GP (20 kHz), possibly resulting from differing membrane characteristics between species. To evaluate phospholipid influence on prestin charge movement we employed MD, where we monitored the separation (center of mass of 3 end terminal residues) of TM12 and TM6 over time. After about 2 μs following imposition of a negative potential across the membrane, these TM helices separated near the inner leaflet, permitting the intercalation of two phospholipids from the bilayer into prestin.

At higher temperatures, the intercalation occurred sooner. Those helices possess charged residues that we suggested to contribute to prestin's voltage sensor (Bai et al., BJ, 2009). Thus, unusually intimate interactions between protein and lipid may impact on prestin's complex charge movements.
Conclusions: Voltage-driven charged residue movements trigger electromotility, and the phase of those movements relative to driving voltage varies across frequency, as revealed by measures of cNLC. We show that chloride binding, absent in the S396E mouse, has little effect on cNLC high frequency response. Lower frequency activity could be impacted to a greater extent since we found that salicylate blocks low frequency components of NLC (Santos-Sacchi and Tan, iScience, 2019). We suggest that the stretched-exponential nature of prestin's NLC results from viscoelastic interaction with membrane components, whereby the evolution of the imaginary component may correspond to phospholipid intercalation within the protein itself, which changes the restrictive environment through which prestin charge moves.

S33. Natural History of Vestibular Dysfunction in Mice With Mutations in Tmc1, Tmc2 and Tmie

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Category: Hair Cells: Anatomy and Physiology
Background: The transmembrane channel-like 1, 2 (Tmc1 and Tmc2) and transmembrane inner ear (Tmie) genes encode proteins are components of the mechanotransduction channel complex in auditory and vestibular hair cells. Mutations in Tmc1, Tmc2 and Tmie have been shown to be associated with hearing loss and balance disorders. While the effects of the mutations on auditory function have been extensively studied, their effects on vestibular function remain to be elucidated. The goal of this study is to investigate how mutations in Tmc1, Tmc2 and Tmie affect vestibular function throughout the lifespan of a mouse.
Methods: WT mice (C57BL/6) and mice with mutations in Tmc1 (Tmc1-/-), Tmc2 (Tmc2-/-) and Tmie (Tmie-/-) were used in this study. Vestibular function was assessed by measuring the rotational and translational vestibulo-ocular reflexes (rVOR: 0.2-4Hz; tVOR: 0.2-2 Hz) using an ISCAN eye tracking system across a range of ages, from 1 month to 12 months.
Results: Preliminary analysis showed that Tmc1-/- mice exhibited WT-like rVOR responses. However, they displayed reduced tVOR responses. Tmc2-/- mice demonstrated comparable tVOR responses to WT mice, but showed reduced rVOR responses, particular at 12 months of age. Tmie-/- mice exhibited minimal rVOR and tVOR responses across all ages tested.
Conclusions: While preliminary, these results suggest that mutations in Tmc1, Tmc2 and Tmie result in distinct patterns of dysfunction within the canals and otoliths. Future studies will incorporate single unit recordings of vestibular afferents in the mutants, along with a more comprehensive characterization of their rVORs and tVORs.

S34. G-a Interacting Protein, C-terminus 3 (GIPC3) Regulates Vesicular Trafficking in Mammalian Auditory Hair Cells

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Category: Hair Cells: Anatomy and Physiology
Background: GIPC3 is a small adaptor protein essential for hearing in both mice and humans (Rehman et al., 2011; Charizopoulou et al., 2011). GIPC proteins contain a PDZ binding domain, potentially facilitating many molecular interactions within auditory hair cells. The roles of GIPC proteins in vesicular trafficking and cell signaling are well-established in other systems, particularly GIPC interaction with myosin VI (MYO6) (Naccache et. al., 2006; Reed et. al., 2005). Data suggests that GIPC has the unique ability to facilitate both clathrin-mediated (coated) and uncoated endocytosis though coupling of MYO6 and its cargo (Naccache et. al., 2006). Interestingly, both GIPC3 and MYO6 are enriched along the cuticular plate and pericuticular necklace (Hasson, 1997, Chatterjee et. al., 2023), where trafficking and endocytosis are highly active in

cochlear hair cells (Kachar et. al., 1997, Griesinger et. al., 2004). Previous data also suggests that vesicular apicobasal transport in hair cells is MYO6-dependent (Harasztosi et. al., 2020). Consistently, we have strong biochemical and structural data for interaction between GIPC3 and MYO6 (see companion poster by Vander Kooi et al.). While the role of GIPC3 and MYO6 in shaping the cuticular plate of hair cells was recently shown (Chatterjee et. al., 2023), here we test the hypothesis that GIPC3-MYO6 complex may additionally play an important role in vesicular trafficking and endocytosis in the apical region of mammalian auditory hair cells.
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-/- hair cells in fast-frozen freeze-substituted preparations. SEM was used to visualize the apical surface of hair cells. To determine the potential mis-localization of MYO6 in Gipc3-/- mutants, we used MYO6 immunolabeling and fluorescent confocal microscopy.
Results: We have observed abnormal accumulation of vesicles and multiple disrupted Golgi apparatuses in the apical compartment of outer hair cells in Gipc3-/- mice. Similar but less profound abnormalities were observed in Gipc3-/- inner hair cells. SEM revealed increased membrane blebbing around the pericuticular necklace in both young and adult Gipc3 mutant mice, a well-known site of endocytosis in the hair cells. Immunolabeling revealed abnormal localization of MYO6 within the pericuticular necklace and kinocilium regions of Gipc3-/- hair cells.
Conclusions: We conclude that GIPC3 is required for proper apex-to-base vesicular transport within hair cells, most likely through its interaction with MYO6.

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S35. Does Chronic TRPA1 Deficiency Affect Auditory Hair Cell Ribbons?

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Category: Hair Cells: Anatomy and Physiology
Background: TRPA1 channels are involved in detecting pain-like signals in nociceptive neurons. They are activated directly and indirectly via several ways, primarily related to tissue damage. TRPA1 channels are also present in the cochlea in inner hair cells (IHC), outer hair cells (OHC), and many supporting cells. We recently found that mice lacking TRPA1 exhibit a shorter temporary threshold shift (TTS) and permanent changes in their auditory brainstem response waveform after noise exposure (Velez-Ortega, et al. Nat Commun, 2023). In addition, we have also observed abnormal cochlear innervation patterns in Trpa1-/- mice. Here, we tested whether TRPA1-deficient mice exhibit changes in IHC and OHC ribbon synapses with age or after noise exposure.
Methods: We used C57Bl/6 Trpa1 knockout mice and wild-type littermates. Anesthetized young (4 week old) mice were exposed to 100 dB SPL broadband noise for 30 minutes, and temporal bones collected 2 weeks after noise exposure. Cochlear epithelia were immunolabeled with antibodies against CtBP2/RIBEYE, myosin VIIA, and neurofilament heavy chain (NF-H). Confocal images were obtained with a Leica SP8 upright confocal microscope. Ribbon counts and positions were quantified using ImageJ software.
Results: Our results show a decrease in the OHC ribbon counts after noise exposure in both wild-type and in TRPA1-deficient mice. This conflicts with a previous study done on wild-type mice before and after noise exposure of awake mice where no differences in the total number of ribbons in OHC was found (Wood et al., JARO, 2021). Quantifications of OHC ribbon position in young and older mice are still ongoing.
Conclusions: Additional examinations of OHC ribbon counts and positions are required to fully understand their dynamic changes after noise exposure in anesthetized and awake conditions. Whether TRPA1-dependent signaling pathways are involved in the relocation of OHC ribbon synapses after noise exposure is still under exploration.

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S36. Integrating Experiments, AlphaFold2 Predictions, and Simulations to Investigate Inner-Ear Tip Links

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Category: Hair Cells: Anatomy and Physiology

Background: Sensory perception in the inner-ear takes place in hair cells and relies on the mechanical activation of ion channels by the pulling of tip-link filaments. Mature tip links are composed of cadherin-23 (CDH23) and protocadherin-15 (PCDH15) proteins, while the transmembrane channel-like proteins (TMCs) 1 and 2 comprise the pore-forming subunits of the transduction apparatus, which features other coupled accessory proteins. How tip link proteins respond to mechanical force to activate transduction channels is poorly understood.
Methods: Here we present structural, biochemical, and computational studies aimed at elucidating the molecular mechanisms underlying mechanical function of hair-cell tip links.
Results: Data from X-ray crystallography, small-angle X-ray scattering, analytical ultracentrifugation experiments and low-resolution cryo-EM along with AlphaFold2 predictions aided us in building atomic-resolution models of the entire tip link ectodomain. Steered molecular dynamics simulations of these models predicted the strength of structural domains relevant for tip-link mechanics.
Conclusions: We conclude that dimerization and membrane adjacent domains (MADs) account for rigid and elastic responses of the tip link responsible for modulation of force propagation in the hair-cell transduction apparatus. These data and models, obtained from a combination of experimental and computational approaches, are providing a rigorous molecular view of tip-link function in hair-cell mechanotransduction.

S37. Identification of a Novel Otoferlin Isoform and Its Role in Auditory Function

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Category: Hair Cells: Anatomy and Physiology

Background: Previously, we identified a novel otoferlin isoform, transcribed from exon 6B, with inner ear specificity using single cell RNA isoform sequencing. This work will investigate the possible roles of this novel isoform.
Methods: To investigate the functional consequences of otoferlin isoforms, we generated: 1) exon 2 knockout (KO) mice with disrupted expression of the canonical isoform (Otof-ΔC); and 2) exon 6 KO mice targeting expression of the short isoform (Otof-ΔS). Otof -/- mice (exon 14-15 deletions) served as negative control. Electrophysiological recordings and morphological studies were performed both in vitro and in vivo.
Results: 1) a novel function of otoferlin in endocytic membrane retrieval, which has not been well studied, 2) impairment of synaptic vesicle recycling as a novel mechanism of occult hearing loss, and 3) otoferlin isoform distributions exhibited a longitudinal gradient along the length of the cochlea, which also changed in response to environmental stress and ageing.
Conclusions: In summary, these results provided new insights into the mechanisms of auditory physiological and pathophysiological processes at the isoform level.

S38. The Transmembrane Channel 1 (TMC1) Interacts With the Piezo1 Mechano-Sensitive Channel and Function

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Category: Hair Cells: Anatomy and Physiology

Background: Transducer ion channels are the gateway for the sensory information-brain interface. Among the sensory systems, the role of mechanoelectrical transducer (MET) channels in engaging sensory stimuli in inner ear hair cells to control sound and balance remains incomplete.
Methods: The Piezo (Pz) and transmembrane channels (Tmc) are potential components of the MET complex. We assessed the murine model's utilization of Pz1 as a MET partner and its interactions with other components of the MET complex, including Tmc1 in Neuro-2a mouse neuroblast cells. Neuro-2a cells were transfected with mPiezo1-mClover3 and mTmc1-mRuby3.
Results: Cells were also transfected with mPiezo-mClover3 and truncated mTmc1-mRuby3 constructs, including C1-TM4 (a.a. 1-455), EC3-TM8 (a.a. 457-616), and TM10-C6 (a.a. 692-717). We used Fluorescence Resonance Energy Transfer (FRET) and Total Internal Reflection Fluorescence (TIRF) to assess the interactions and stoichiometry in the protein complex of mPiezo1 and mTmc1. FRET of single transfections of Neuro-2a cells by donor constructs was performed as controls for the bleaching of donor fluorescent signals. We found that mPiezo1 and mTmc1 interacted with a FRET efficiency of ~11%. Truncated constructs of mTmc1, C1-TM4, and TM10-C6 had no significant FRET with mPiezo1, but EC3-TM8 had significant FRET with mPiezo1, suggesting the specific interaction region in mTmc1. Moreover, a stoichiometric ratio of mTmc1 to mPiezo1 was estimated to be close to 2 1.
Conclusions: Our findings demonstrate that the MET channel comprises the Pz1 channel associating and interacting with mTmc1 and other MET complex proteins, suggesting the critical role of the Pz1 channel in inner ear mechanotransduction and hearing generation.

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S39. Intracochlear Morphology Quantified: Considerations for Cochlear Implant Surgery

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Category: Inner Ear: Anatomy and Physiology
Background: Cochlear variability has been studied up to two turns, limited to the resolution of current clinical/laboratory-based computed tomography (CT). Therefore, the anatomical variations along the cochlear length are not well quantified, and it is unclear if all cochleae can accommodate deep cochlear implant (CI) electrode insertions. Furthermore, it has not been established how deep CI electrodes can be inserted and accommodated in the ST anatomy. However, deep insertions have been correlated with improved outcomes. Synchrotron-radiation phase contrast imaging (SR-PCI) has proven to be an effective approach to imaging the entire cochlear length. It obviates the need for staining or slicing required in histological analysis, and provides improved soft tissue contrast and reduced metal artifacts compared to micro-CT. The objectives are to use SR-PCI on un-implanted and implanted human cadaveric cochleae to quantify cochlear morphology, along the entire cochlear length, and assess CI electrode trajectories.
Methods: Thirty-five un-implanted and three implanted human cadaveric cochleae were scanned using SR-PCI at a 9 μm isotropic voxel size. For each SR-PCI image, the scala tympani (ST), scala vestibuli combined with the scala media, and the CI electrode sheath (for the implanted samples) were segmented. The three-dimensional segmentations were aligned to the cochlear coordinate system, then were radially sliced at 5° increments about the modiolar axis. The radial mid-modiolar cross-sections were analyzed from the cochlear base to the apex to obtain measurements of the cross-sectional diameter, defined by the largest inscribed circle, and measurements of the cross-sectional area along the entire cochlea. Additionally, the horizontal trajectory of the largest inscribed circle, the most lateral fitting inscribed 0.4 mm circle (representative of a CI electrode tip diameter), and the CI electrode relative to the modiolus were assessed for both un-implanted and implanted cochleae.
Results: The cross-sectional diameter and area generally decreased as angular depth increased up to the cochlear apex. At 720°, the largest inscribed circle reached a diameter of 0.4 mm, representative of a CI electrode tip, indicating this is the deepest an electrode can be accommodated in the ST. The horizontal trajectory of the largest inscribed circle and the most lateral fitting inscribed circle generally moved closer to the modiolus as angular depth increased for both non-implanted and implanted samples. For all implanted samples, the horizontal trajectory of the CI electrode array closely followed the path of the most lateral

inscribed circle. This is clinically significant because it begins to identify the lateral positions an array can occupy within the ST morphology.
Conclusions: This is the first study to visualize and quantify the entire cochlear morphology using SR-PCI. This work indicates that the ST cochlear anatomy can accommodate a 0.4 mm diameter electrode insertion up to two turns for CI recipients, increasing the restored hearing range.

S40. Effects of Acoustic Crossover Between Ears on ABR Thresholds and Waveform in Mice (Mus Musculus)

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Category: Inner Ear: Anatomy and Physiology
Background: Acoustic crossover refers to a transmission of sound stimulus between the ears including both air conduction and tissue/bone conduction in closed field stimulation. Acoustic crossover stimulation can evoke responses in the unstimulated ear and is well-understood in human psychophysics and clinical audiology. However, the amount of acoustic crossover in the mice (Mus musculus) has not been thoroughly characterized and is often underestimated. Here, we characterized the amount and effects of acoustic crossover in the mouse from air and tissue/bone conduction.
Methods: C57Bl/6 mice (n=12) with normal cochlear function were unilaterally deafened by insertion of a glass pipette through the round window and sterile water injection. ABR and DPOAE were recorded with the mice on their side and an acoustic system making a semi-closed seal to their ear canal. Responses were compared with and without an earplug in the contralateral ear. ABRs were recorded both with the standard vertical electrode montage (vertex [positive] to base of ipsilateral pinna), and horizontal montage (base of contralateral pinna [positive] to base of ipsilateral pinna). Finally, the intact ear was deafened then ABR and DPOAE were recorded to confirm initial deafening.
Results: Following unilateral deafening, complete loss of DPOAE was observed in the deafened ear. In contrast, using the vertical electrode montage ABR was present with thresholds ~40 dB higher than the intact ear over 4–32 kHz when tested without an earplug. With an earplug, thresholds were 60–70 dB higher than the intact ear with higher thresholds for higher frequencies. We further investigated whether ABR waveform shape and latency can be utilized to identify the ear generating the signal using the horizontal electrode montage. Comparing waveforms in response to the deafened and intact ears at equal levels relative to threshold revealed that the ABR waveform in response to stimulation in the deafened ear is an inverted waveform of the response to the intact ear. No DPOAE or ABR signal was detected after deafening the intact ear, confirming the previously observed response in the unilaterally deafened ear was due to the acoustic crossover to the intact ear.
Conclusions: We report approximately 40 dB attenuation between ears without earplugging and an average of 60–70 dB with earplugging. ABR waveform shape confirms the response to stimulation in the deafened ear is generated by the intact ear. Acoustic crossover can lead to erroneous conclusions in studies investigating treatment effects in damage or transgenic models or studies evaluating safety of treatment in normal hearing animals. Measures to ameliorate or avoid acoustic crossover response (earplugs, contralateral deafening, etc.) should be utilized when possible, and results should be interpreted with consideration for acoustic crossover.

S41. Estimating Human Cochlear Health in Situ From the Basal High-Frequency Region With High Spatial Resolution Optical Coherence Tomography (OCT)

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Category: Inner Ear: Anatomy and Physiology
Background: Considerable efforts are being devoted to improve or restore hearing through molecular methods that regenerate cochlear structures. These potential future therapies rely on the existence of

supporting cells or normal cochlear cytoarchitecture. However, there remains no present way to assess cochlear health with clinical imaging modalities such as MRI and CT. With OCT in fresh human specimens, we imaged the basal cochlear partition in situ through the intact round window membrane (RWM) and visualized internal structures of the cochlea to evaluate cochlear health.
Methods: Fresh (10-40 h postmortem) human temporal bones (N=18) were obtained from Massachusetts General Hospital donors and the basal region was imaged through the RWM 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). Measurements of the organ of Corti (OoC) height, tectorial membrane – reticular lamina (TM-RL) gap height, TM size, and other quantifiers of cochlear health were recorded with ImageJ software. Anatomical cochlear health was scored on a semiquantitative health scale from 0-3 adapted from (Kaur et al., 2023 Hear Res 435:108815). Patient health history included age, sex, postmortem time, history of drugs, and hearing loss (when available).
Results: OCT images showed good detail to enable assessment of microanatomy related to human cochlear health. Six cochleae had a perfect health score of 3 with a robust OoC and relatively small TM-RL gap height. Eight cochleae received a score of 2, indicative of recognizable cytoarchitecture with some loss of cellular structures. Two cochleae received a score of 1 that had a small, short mound of OoC, and 3 cochleae received score of 0 where there was just the basilar membrane but no OoC. Two available audiograms of normal hearing and severe hearing loss corresponded to ears with health scores of 3 and 0 respectively. Statistically significant age effects were found for OoC height and the TM-RL gap height, where older patients had smaller OoC height and a relatively larger TM-RL gap height. Older donors also had lower and more varied cochlear health scores than younger donors.
Conclusions: We were able to determine the health of human OoC with OCT imaging in fresh cadaver specimens. We demonstrated that OCT could be used to visualize clinically relevant cochlear anatomy via the middle-ear cavity approach without opening the cochlea. With future development of high-resolution endoscopic OCT for in vivo imaging, it may be possible to identify and target candidates for regenerative drug therapies that restore hearing. Supported by grants R01 DC013303 and T32 DC000038 (Training for Speech and Hearing Sciences) from the NIDCD/NIH, and the Amelia Peabody Scholars Fund.

S42. Single-Cell Analysis and Fate Mapping Studies Reveal Strong Heterogeneity of Cochlear Macrophages

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Category: Inner Ear: Anatomy and Physiology
Background: Recent advances in fate mapping and single-cell technologies have revealed how tissue-resident macrophages are shaped by their environment to fulfill specific functions and maintain their host-tissue homeostasis. However, while it is established that macrophages inhabit the cochlea, cochlear macrophages remain understudied, and little is known about their diversity, cell dynamics, transcriptomic signatures, and functions. Previous reports have suggested that cochlea macrophages are replenished by circulating monocytes over time, however, these studies have used an irradiation and bone-marrow transplantation paradigm that is now known to disrupt barriers and lead to the artificial entry of peripheral cells into tissues.
Methods: Using more physiological approaches, such as fate-mapping and parabiosis experiments, we sought to determine the developmental origin of macrophages in the cochlea, as well as their self-renewal capacity. In addition, we performed single-cell transcriptomic analysis to uncover myeloid cell diversity in the cochlea and identify specific gene signatures and putative functions attributed to each subpopulation.
Results: Fate mapping analysis during development has shown that cochlear macrophages partially originate in the embryonic yolk sac. At the adult stage, most of the cochlear macrophages are long-lived resident macrophages, except in the spiral lamina and the spiral limbus which both displayed a slow and progressive contribution of circulating monocytes to the cochlear macrophage pool over time. Single-cell transcriptomic analysis identified five different myeloid cell populations in the cochlea (three macrophage subsets, one

monocyte subset, and one dendritic cell subset), all exhibiting distinct transcriptional signatures and functions. Immunofluorescent staining confirmed the presence of the three macrophage subsets with distinct distribution across cochlear compartments.
Conclusions: We have shown that macrophages exhibit an unappreciated diversity in terms of developmental origin, cell dynamics, and transcriptional identity in the cochlea during steady-state conditions.

S43. Cochlear Aqueduct Post-Natal Growth in Human: A Computed Tomography Study

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Category: Inner Ear: Anatomy and Physiology
Background: The cochlear aqueduct (CA) is a bony canal located at the base of the scala tympani of the cochlea. It connects the inner ear perilymph fluid to the cerebrospinal fluid of the posterior cerebral fossa. Its function is not well understood, as it seems to be present in only a fraction of adult patients. Indirect observations argue in favor of the CA to be more present in children.
Methods: To study the CA morphology in infants, and to find out a potential age-related change of CA morphology, we performed a retrospective single-center study of 72 temporal bone scans of infants with a mean age of 2.26 +/- 1.92 years (47 days of life up to 18 years). A total of 38 infants were divided into 3 different age groups and each temporal bone was considered as an individual sample. CA were classified into four different types. Group 1 included infants less than 1 year of age (21 scans), group 2 between 1 to 2 years (17 scans), and group 3 infants more than 2 years (34 scans). The CA morphology measurements included its type, its total length, and its funnel length and width (wider intracranial portion).
Results: Our results showed a statistical correlation between the CA morphology as a function of age. The CA total length increased in size rapidly in the first years of life and then follows a slow growth until adulthood. The types of CA were not different between groups. The total length of group 1 (0.73 mm +/- 0.10), group 2 (0.92 +/- 0.10) and group 3 (1.16 +/- 0.16) were statistically different (p less than 0.0001). The funnel length was different between group 1 (0.27 +/- 0.46) and 2 (0.40 +/- 0.20) (p=0.0037) but not between 2 and 3 (0.44 +/- 0.14, p=0.51). The funnel width was not different between group 1 (0.23 +/- 0.05) and 2 (0.31 +/- 0.08) (p=0.15) but was between 2 and 3 (0.41 +/- 0.15, p less than 0.0001).
Conclusions: Our study shows significant postnatal growth of the cochlear aqueduct, particularly fast before the age of 2 years. A significantly shorter and wider CA were observed at its base in young infants. These findings highlight the differences between the CA morphology in adults and children and raise the question of differences in function. Moreover, it is important to take these differences into account when designing future drugs or gene delivery into the human inner ear. In fact, administration of drugs or viral vectors at the base of the cochlea could present an increased diffusion toward the subarachnoid space in children. Reversely, diffusion from the subarachnoid space could be facilitated. Further studies are required, both on the histology of temporal bones and on the function of the CA in children.

S44. Bmi1-Regulated Genome-Wide H3K27me3 Modification Pattern Modulates Auditory Sensory Epithelial Cell Proliferation and Cell Death in the Mouse Inner Ear

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Category: Inner Ear: Cochlear Mechanics
Background: Bmi1, a core member of the Polycomb repressive complex 1 (PRC1), functions as a transcriptional repressor by regulating chromatin structure. Previous studies have highlighted the crucial role of Bmi1 in neonatal mouse cochleae and its depletion leading to hair cell loss. However, the specific mechanism through which Bmi1 influences cell proliferation remains elusive.

Methods: To address this, we performed a genome-wide analysis of the distribution of H3k27 tri-methylation (H3k27me3) marks and performed RNA-sequence analysis of auditory sensory epithelium from neonatal Bmi1-deficient and control mice.
Results: The RNA-seq analysis revealed significant transcriptional changes in auditory cells due to Bmi1 deficiency, affecting genes associated with cell proliferation, senescence, and death. Whole-genome analysis of H3K27me3 marks exhibited enrichment in genes relevant to gene expression profiles in mouse cochleae. Combining the H3K27me3 ChIP-seq and RNA-seq data unveiled a notable decrease in the number of H3K27me3 peak enrichments in Bmi1-deficient mice, which correlated with elevated expression levels.
Conclusions: This study provides valuable insights into the connection between histone modification mapping and gene expression profiles in transgenic mice, shedding light on the mechanisms underlying Bmi1's impact on auditory development.

S45. Aminoglycoside Triggers the Translocation of RIPOR2 and Phosphatidylserine Externalization by Different Mechanisms

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Category: Inner Ear: Cochlear Mechanics
Background: Aminoglycosides (AGs) are widely used to treat severe infections. However, systemically administered AGs preferentially kill cochlear hair cells, resulting in irreversible hearing loss. Recently, we found that AGs induce a rapid translocation of RIPOR2 in hair cells, which activates the autophagy/mitophagy pathway, resulting in the irreversible death of hair cells and permanent hearing loss. Notably, AGs also trigger a rapid phosphatidylserine (PS) externalization in hair cells, which is due to inhibition of the mechanotransduction channels.
Methods:
Cochlear explants were dissected and cultured in DMEM/F12 media at 37 ◦C. Then, explants were treated with 1mM AGs. Samples were fixed with 4%PFA, blocked with HBSS containing 5% BSA and 0.5% TritonX-100, and then incubated overnight at4 ◦C with primary antibodies in HBSS containing 1%BSA. Tissues were washed in HBSS and incubated with secondary antibodies for 2 hours at room temperature. Then, images were captured by a DM6 FS automated deconvolution microscope (Leica).

Results: To determine whether AG-triggered rapid RIPOR2 translocation and PS externalization are independent, RIPOR2 cycling and PS externalization were systematically investigated in wild-type hair cells treated with AG for different amounts of time. Additionally, to investigate the extent to which PS externalization requires RIPOR2, PS externalization was studied in wild-type, Ripor2+/- and Ripor2-/- hair cells.
Conclusions: Our results suggest that AG triggers the translocation of RIPOR2 and PS externalization by different mechanisms.

S46. A Hybrid Fem and Experimental Study on the Relation Between Temporal Bone 3D Motion and Intracochlear Pressure Under Bone Conduction

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Category: Inner Ear: Cochlear Mechanics
Background: Numerical and experimental investigation of the 3D motion across the temporal bone, particularly the otic capsule, during bone conduction (BC) stimulation at different frequencies, and its correlation with the intracochlear pressure difference across the cochlear partition are being investigated experimentally and numerically.
Methods: Experimental data has been collected from six samples from three fresh frozen cadaver heads, including: 3D velocity at 130-200 points across the lateral and medial surfaces of the ipsilateral temporal bone and skull base; 3D motion of a single point at the promontory and stapes; differential intracochlear pressure.

Excitation was provided sequentially to the ipsilateral mastoid and classical BAHA location via a percutaneous coupling, in the 0.1-20 kHz frequency range. The experiment was digitally recreated by a custom finite element model (FEM), based on the LiUHead, with the addition of a middle ear and cochlea. The Young modulus of the bone domain within the FEM was varied between 4, 8, and 20 GPa.
Results: Predicted differential intracochlear pressure, normalized by the promontory motion, was within the confidence intervals of the experimental data for most frequencies. The spatial variation of the amount of deformation across the skull base, and the otic capsule in particular, was dependent on the material properties of the FEM and closely matched the experimental data. The model indicated that the relation between intracochlear pressure and the rigid body motion of the cochlear was affected by the Young modulus of the skull, and it followed the experimentally observed trends.
Conclusions: Both methods indicated that the otic capsule acted as a rigid accelerometer within the temporal bone, thus exerting primarily inertial load on the cochlear fluid even above 10 kHz.

S47. Pou3f4 is Necessary for Normal Patterns of Cochlear Spontaneous Activity and IGF Signaling During Development

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Category: Inner Ear: Cochlear Mechanics
Background: POU3F4 is a transcription factor expressed by otic mesenchyme cells (OMCs) and is associated with X-linked deafness. Loss of Pou3f4 leads to reduced endocochlear potential and spiral ganglion neuron (SGN) guidance and survival defects. Until the onset of hearing, the immature inner ear in newborn rodents cannot process sound waves, but the cochlea still generates spontaneous action potentials. This spontaneous activity originates in inner support cells, and activates hair cells, SGNs, and the ascending auditory pathway. Spontaneous activity is crucial for multiple aspects of auditory development, such as the maturation of auditory circuits and SGN differentiation and survival. Our data preliminarily show that Pou3f4 knockout cochleae lack spontaneous activity on postnatal day 1 (P1). In addition, single cell RNAseq results suggest Insulin-like growth factor (IGF) signaling is altered in the Pou3f4 knockout mice. We predict that POU3F4 (in OMCs) regulates the differentiation of cochlear epithelial cells by promoting IGF signaling.
Methods: Ca2+ imaging was performed to evaluate SGN spontaneous activity at P1 and P4 in Pou3f4 mutants and controls. Pou3f4y/--Snap25-GCaMP6s mice and Pou3f4y/+; Snap25-GCaMP6s littermate controls were generated and used to measure SGN activity. In samples lacking Snap25-GCaMP6s, Fluo4-AM dye was used to evaluate Ca2+ imaging on cochlear epithelial cells. Immunohistochemistry and Western blotting were used to evaluate IGF receptor activation in Pou3f4y/- and control cochleae.
Results: Overall, Pou3f4y/--Snap25-GCaMP6s mice showed reduced SGN activity compared to controls. Similarly, our Fluo4-AM experiments showed reduced activity in inner support cells, where spontaneous activity originates. In Pou3f4 mutants, spontaneous activity was absent at P1, but returned at P4, albeit with considerable variability. Immunohistochemistry revealed that Pou3f4y/- cochleae show increased levels of phosphorylated IGF1R (pIGFR) on the surface of outer hair cells, but lower levels of pIGFR elsewhere. In Western blots, cochleae from Pou3f4y/- mice showed lower pIGF1R levels compared to controls.
Conclusions: Our findings show that Pou3f4 knockout cochleae show reduced levels of spontaneous activity and altered patterns of IGF receptor activation. These data may support a model by which POU3F4 promotes IGF signaling upstream of cochlear spontaneous activity and SGN survival.

S48. Single-AAV Packaged RNA Base Editor Cures Hearing Loss Induced by OTOF Gene Mutation

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Category: Gene Therapy
Background: Mutations in the OTOF (otoferlin) gene are the most common reasons for auditory neuropathy induced hearing impairments and deafness. Nonsense mutation c.2485C greater than T (p. Q829X) in the

OTOF gene is responsible for about 3% of all cases of recessive prelingual deafness in the Spanish population. Although previous studies have adopted a dual-AAV (adeno-associated virus) gene therapy approach using two different recombinant AAV vectors to overexpress the otoferlin, the efficacy was limited.
Methods: AAV mediated base editor was injected into neonatal or adult OTOF Q829X/Q829X mice, hearing and histology examination was performed from 1 month to 7 months.
Results: We developed an enhanced mini-dCas13X RNA base editor (emxABE) delivered by an AAV serotype 9 variant with optimal transfection efficiency on hair cells (almost 100%) for the treatment of OTOFQ829X, achieving high A-to-I (TAG greater than TGG, Q829 greater than W) conversion efficiency (~80%) in humanized Otof Q829X/Q829X mice. After a single scala media injection of emxABE targeting OTOFQ829X (emxABE-T) to the postnatal day 0-3 (P0-3) of OTOF Q829X/Q829X mice, otoferlin was restored in nearly 100% of the inner hair cells (IHCs), and auditory function was rescued to near wild-type levels for at least 7 months. We also observed the restoration of auditory function in P5-7 and P30 OTOF Q829X/Q829X mice via round window injection of emxABE-T.
Conclusions: Our findings demonstrate not only a preferential therapeutic strategy for potentially curing OTOF-Q829X induced hearing loss, but also suggest emxABE as the promising toolkits for the treatment of other monogenic diseases with premature termination codons.

S49. Upregulation of Atoh1 by Crispr Activation Promotes Vestibular Hair Cell Regeneration and Function Recovery in Mice

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Category: Gene Therapy
Background: The vestibular system is important for maintaining balance. Hair cells (HCs) are the major sensory receptor cells which convert hear movements ionto electrical signals. HCs owe limited regeneration capacity, supporting cells are able to transdifferentiate into HCs by Atoh1 activation. However, in most cases vestibular function cannot recover spontaneous or by viral mediated Atoh1 upregulation. Gene editing is widely used in treating hereditary diseases recently, and gene activation by gene editing is unveiled in inner ear. This project proves the vestibular HC regeneration and function recovery after injury by regulating Atoh1 expression using CRISPR activation system.
Methods: The vestibular system is important for maintaining balance. Hair cells (HCs) are the major sensory receptor cells which convert hear movements ionto electrical signals. HCs owe limited regeneration capacity, supporting cells are able to transdifferentiate into HCs by Atoh1 activation. However, in most cases vestibular function cannot recover spontaneous or by viral mediated Atoh1 upregulation. Gene editing is widely used in treating hereditary diseases recently, and gene activation by gene editing is unveiled in inner ear. This project proves the vestibular HC regeneration and function recovery after injury by regulating Atoh1 expression using CRISPR activation system.
Results: To evaluate the curative effect, the mice were tested 1 month and 6 months after administration. Compared to the damaged group, the number of VHCs of treatment group got a significant increase, and most of them had bundled stereocilia and neuron connection. The vestibular electrophysiological function and balance function were largely recovered. The VHCs can regenerate and largely recover the balance function after injury by regulating Atoh1 expression using CRISPRa.
Conclusions: The VHCs can regenerate and largely recover the balance function after injury by regulating Atoh1 expression using CRISPRa.

S50. Mini-PCDH15b Gene Therapy Rescues Visual Deficits in a Zebrafish Model of Usher Syndrome Type 1F

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Category: Gene Therapy

Background: Usher syndrome is a devastating hereditary deafness and blindness caused by mutation of any of nine genes. Mutations in one gene, PCDH15, cause Usher syndrome type 1F (USH1F), manifesting as profound deafness and lack of balance at birth, and blindness developing over several decades. Currently, treatment for Usher 1F is limited to cochlear implants, and there is no treatment for the blindness. Gene addition therapy could be an attractive treatment, however the PCDH15 coding sequence of ~5.8 kb is too large to fit into a single AAV capsid. We used rational, structure-based design to engineer a mini-PCDH15 gene in which 5 of the 11 extracellular cadherin repeats were deleted, but which demonstrated proper protein localization and remarkable rescue of hearing in mouse models of USH1F deafness. To test mini-PCDH15 gene therapy for blindness, we used a zebrafish USH1F model which exhibits retinopathy, including early and progressive defects in photoreceptor morphology and impairment of visual function.

Methods: We used Pcdh15b-mutant zebrafish which have a 7-bp deletion in exon 8 leading to a premature stop codon. No Pcdh15b is expressed. Using a transposon-insertion strategy at the one-cell stage, we introduced mini-Pcdh15b, expressed under a photoreceptor-specific promoter, into Pcdh15b-mutant zebrafish. Mosaic mini-Pcdh15b animals were grown to adulthood and bred with wild-type individuals. A stable transgenic line was generated. Progeny were fin-clipped and sequenced to confirm germ-line transmission.
We assayed rescue of visual function with electroretinogram (ERG) and optokinetic reflex (OKR) tests. We performed immunofluorescence and electron microscopy studies to localize the mini-Pcdh15b within photoreceptors in treated fish, and compared results with untreated mutant fish.

Results: Pcdh15b-mutant zebrafish exhibit an early and progressive defect in photoreceptor morphology and visual function. Immunohistochemistry and electron microscopy of mutant photoreceptors revealed abnormal calyceal processes and distorted outer segments. In 7-dpf mutant larvae, ERGs showed attenuated a- and b-wave amplitudes, and OKR responses were less robust.
Pcdh15b mutant zebrafish expressing mini-Pcdh15b demonstrated rescue of vision to wild type levels, as assessed with ERG and OKR recording. With immunofluorescence and immunogold SEM, strong mini-Pcdh15 signal was detected along calyceal processes of photoreceptors. Immunohistochemical and SEM analysis showed robust rescue of photoreceptor morphology, comparable to that in normal larvae.

Conclusions: Mini-Pcdh15b expression restores vision in a zebrafish model of Usher 1F, and mediates expression and normal localization of Pcdh15b in photoreceptors, suggesting that a mini-PCDH15 gene therapy is a promising approach for the treatment of the progressive blindness in human Usher 1F. Furthermore, this work demonstrates that shortened versions of genes based on detailed knowledge of atomic structure may be used to treat certain forms of vision loss for which the gene product is too large for AAV's packaging limit.

S51. Preclinical Evaluation of the Efficacy and Safety of AAV1-HOTOF in Mice and Non-Human Primates

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Category: Gene Therapy

Background: Pathogenic mutations in the OTOF gene cause autosomal recessive hearing loss 9 (DFNB9), one of the most common forms of auditory neuropathy. Previous studies have reported the rescue of hearing in DFNB9 mice using a dual AAV-mediated OTOF gene therapy strategy. However, the safety was unknown. Due to the imperative of assessing the safety of the strategy before its clinical application, we evaluated the efficacy and safety of AAV1-hOTOF gene therapy in animal models.

Methods: We first designed an OTOF gene therapy agent, a dual-AAV1-Hyb (AK) approach in which the human OTOF CDS was driven by the hair cell (HC)-specific promoter Myo15, named AAV1-hOTOF. To evaluate the efficacy of AAV1-hOTOF in treating OTOF –/– mice, we injected newborn and adult mice via the round window membrane (RWM), immunohistochemistry was used to identified otoferlin expression and Auditory Brainstem Response (ABR) was performed to test the hearing recovery of the mice. We further report the assessment of the safety of the AAV1-hOTOF in wild-type (WT) mice and non-human primates (NHPs) through pharmacodynamics measurements, behavioral tests, and histopathology analysis.
Results: AAV1-hOTOF significantly recovered hearing in OTOF –/– mice without affecting normal hearing in WT mice. AAV1 was predominately distributed to the cochlea although it was detected in other organs such as the central nervous system and the liver. The normal behavior and the parameters of routine blood test and serum chemistry were not affected, and no obvious pathological changes or signs of inflammation or fibrosis were observed in WT mice. Delivery of AAV1-GFP via RWM in the NHP inner ear resulted in efficient expression in HCs, and systemic toxicity was not detected by serum chemistry or routine blood test, with the values mostly within the normal reference range after injection.
Conclusions: Our study have established a therapeutic agent – AAV1-hOTOF – for DFNB9 and demonstrated its efficacy and safety in mouse models. Additionally, we explored the transduction and tolerance of AAV1-GFP in NHPs. These findings strongly support the clinical development of AAV1-hOTOF.

S52. CHORD: A Phase 1/2 Open-Label, Multi-Center Trial to Evaluate Intracochlear Administration of DB-OTO Gene Therapy in Pediatric Patients With Profound Sensorineural Hearing Loss Due to Biallelic Otoferlin Mutations

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Category: Gene Therapy
Background: Otoferlin is a calcium-sensing protein involved in inner hair cell vesicular transport and exocytosis, a process critical for inner hair cell signal transmission to afferent auditory nerve fibers. Biallelic mutations in the otoferlin gene (OTOF) typically produce auditory neuropathy characterized by prelingual severe-to-profound sensorineural hearing loss. In these patients, the auditory brainstem response (ABR) is absent or severely reduced with normal outer hair cell function as indicated by the presence of otoacoustic emissions (OAE). Although cochlear implants are effective, replacement of the otoferlin gene is hypothesized to provide instatement of natural, physiologic and high-quality hearing. DB-OTO is a dual adeno-associated virus (AAV1) vector with a Myo15 promoter that drives inner hair cell-selective expression of cDNA for human OTOF. Nonclinical GLP studies with intracochlear DB-OTO have been completed in otoferlin-deficient mice and in non-human primates to inform the clinical dose and safety. In this first-in-human clinical trial with DB-OTO (CHORD), the safety and preliminary efficacy of DB-OTO administered by intracochlear injection is evaluated in pediatric patients with profound hearing loss caused by OTOF mutations.
Methods: Pediatric patients (≤2 years of age in Spain, UK; less than 18 years of age staggered by age in the US) with biallelic pathogenic OTOF mutations and profound sensorineural hearing loss (≤90 dB HL) will be enrolled. Eligible patients will have experienced minimal benefit from amplification and meet the cochlear implantation criteria in the ear to be injected with DB-OTO. Present outer hair cell function is confirmed via the presence of OAE (patients ≤2 yr of age) or cochlear microphonic (patients greater than 2 years and less than 18 yrs of age). Patients with a history of prior treatment with gene therapy, anatomy that would preclude the planned surgical approach, or the presence of cochlear implants (CI) in the ear to be injected with DB-OTO are excluded. In Part A, the initial dose escalation phase of the study, DB-OTO will be administered unilaterally by intracochlear injection through the round window using a typical facial recess approach similar to CI surgery.
In Part B, an expansion cohort will receive bilateral DB-OTO administration with the selected dose in Part A. After administration of DB-OTO, patients are assessed for safety (labs, vital signs, antibody and shedding

assays, physical exams, otoscopy, tympanometry, DPOAEs, vestibular assessments) and efficacy (ABR, behavioral audiometry, speech audiometry, hearing-related questionnaires) over a 5-year follow-up period.

Results: This trial has begun enrolling patients globally. Available safety and efficacy results in patients dosed by January 2024 will be presented.

Conclusions: We report on the trial design of a first in human, Phase 1/2 gene therapy trial with DB-OTO in pediatric patients with otoferlin related hearing loss. The trial is actively enrolling and key initial safety and efficacy results will be presented.

S53. Ultrastructure of Noise-Induced Cochlear Synaptopathy

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Category: Inner Ear: Damage and Protection

Background: Noise-induced cochlear synaptopathy, in which synapses between auditory nerve fibers (ANFs) and inner hair cells (IHCs) degenerate though hair cells do not, has been extensively studied via confocal light microscopy. However, the disappearance of immunostained pre- and/or post-synaptic puncta provides an incomplete view of the underlying pathology. Here, we present a serial-section ultrastructural study of the IHC innervation 24 hours and 1 week after exposure to a synaptopathic noise producing 50% loss of synapses despite only transient elevations of cochlear threshold.

Methods: CBA/CaJ mice at 7 weeks were exposed to an 8-16 kHz noise band for 2 hours at 98 dB SPL. After 24 hours or 1 week, cochleas were extracted after intravascular perfusion with glutaraldehyde and paraformaldehyde, decalcified, micro dissected, en bloc stained by serial incubation in osmium, lead aspartate, ferrocyanide and thiocarbohydrazide and embedded in plastic. After cochlear mapping, the 32 kHz region (where synaptic loss peaks) was removed by razor cuts and mounted for FIB-SEM and serial milling (at 20 nm per slice) in a Zeiss Crossbeam. Age-matched control mice received no acoustic overexposure. Images were acquired at 5 nm resolution in x and y, spanning the unmyelinated terminals from the habenula to the IHC synapse. ANFs, IHCs, mitochondria and synaptic ribbons were segmented in Dragonfly software, aided by the Artificial Intelligence Deep Learning Tool.

Results: In control ears, 71/75 reconstructed ANFs made synaptic contact with a single IHC; of the remaining four, two contacted an IHC without a synapse and two failed to reach a hair cell. In exposed ears, at both survival times, ANF terminals were normal in number, but synaptic counts were reduced by half. Most non-synapsing fibers remained in contact with, or in close proximity to IHCs (within 2 □m) and contained healthy-looking organelles. ANFs showed a transient increase in mitochondrial content and efferent innervation at 1 day post exposure. Fibers maintaining synaptic connections showed hypertrophy of pre-synaptic ribbons at both 1 day and 1 week. Non-synaptic fibers were lower in mitochondrial content and typically on the modiolar side of the IHC, consistent with selective damage to ANFs with high-thresholds and low spontaneous rates. Even 1-week post-exposure, most unmyelinated ANF terminals remained intact despite loss of synaptic specializations.

Conclusions: Prior in vitro work on excitotoxic challenge suggested that ANFs immediately retract away from the IHC, and in vivo confocal work on noise-induced synaptopathy was ambiguous in this regard. Here, we show that, even 1 week post-exposure, most synaptopathic ANFs remain in intimate contact with the IHC, with intact efferent innervation and intracellular organelles, including mitochondria. Thus, attempts to enhance regeneration do not require neurite extension, even when the trauma-treatment interval is as long as 1 week.

S54. Development of Piezoelectric PVDF Device for Cochlear Implants

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Category: Inner Ear: Damage and Protection

Background: Sensorineural hearing loss (SNHL) is one of the most common sensory deficits in humans, affecting millions of people worldwide, and has been primarily attributed to inner ear dysfunction. Cochlear implants (CIs) have revolutionized the care of patients with severe to profound SNHL and have catalyzed the development of multi-electrode devices to replace the loss of inner ear hair cell function by applying direct electrical stimulation to the cochlear neurons. However, existing minimally invasive technologies are greatly limited by their inability to preserve residual normal cellular structures and hearing due to their traumatic cochlear insertion. Furthermore, current devices fail to reach the apical end of the cochlea, which is responsible for transducing speech frequency sounds and where most of the residual functional neurons are located. Addressing these limitations may lead to improved hearing outcomes and quality of life for patients with severe to profound SNHL.
Methods: In this work, we developed piezoelectric microfibers made of polyvinylidene fluoride (PVDF) polymer as a potential device for neuronal stimulation in the inner ear. The piezoelectric PVDF microfibers with gold layers fabricated by excimer laser micromachining technology enable generating electrical charges on its surface upon acoustic stimulation, stimulating neuronal cells. The gold layers on both sides serve as electrically conductive as well as biocompatible surfaces.
Results: The Atomic Force Microscope (AFM) results showed that the microfibers with sizes of 30 μm × 90 μm and 15 μm × 90 μm possess the great piezoelectric properties. The in-air testing results using the scale-up PVDF fibers with the size of 0.5 cm × 3 cm demonstrated that the amplitude of the output voltage of the fiber varies with the frequencies, and it could reach out to 76 mV at around 900 Hz. Biocompatibility and toxic tests demonstrated that the microfibers are safe and have no toxic effects on cell proliferations. Furthermore, both in vitro and ex vivo experiments demonstrated that the PVDF fibers can effectively stimulate the rat brain cortical neurons under the acoustic stimulations.
Conclusions: The proposed device has an excellent merit with holding a great potential to be used in cochlear implants.

S55. Investigating the Role of Aryl Hydrocarbon Receptor Signaling in Protection and Regeneration of Cochlear Hair Cells

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Category: Inner Ear: Damage and Protection
Background: Investigating compounds that can provide protective effects and/or induce regeneration of inner ear hair cells is crucial for addressing permanent hearing loss occurring from acoustic trauma, ototoxin exposure or aging. Aryl hydrocarbon receptor (AhR) signaling is involved in various cellular processes such as development, differentiation and proliferation, in addition to its anti-inflammatory and protective functions in response to xenobiotics. Recent studies have demonstrated that AhR signaling is regulated by Wnt/Beta-catenin, a pathway known to promote the regeneration of hair cells after damage. Increased AhR expression in response to neomycin-induced hair cell damage was also observed in a RNAseq study done in chicken basilar papilla suggesting that AhR might have a role in the cellular processes that occur after hair cell injury and during regeneration. However, no studies have been done to explore the potential role of AhR in hair cell development, regeneration or protection. Here, we used different AhR ligands to investigate the effect on hair cell numbers after neomycin-induced hair cell damage using a cochlear explant model.
Methods: The organ of Corti from C57BL/6 mouse pups at postnatal day (P) 2 or P3 were microdissected to establish cochlear explant cultures and incubated overnight in media containing 10% fetal bovine serum (FBS) and N2 supplement. The following day, the cochlear explants were exposed to 600uM neomycin (in media containing 2 % FBS and N2 supplement) for 24 hours to induce a mosaic pattern of hair cell death. Next explants were washed in media 3 times and treated with Oto-39, an endogenous AhR ligand, at the concentrations of 1-100 nM. After 96 hours, the explants were fixed, immunostained with the hair cell marker, Myosin VIIa, and analyzed by confocal microcopy to quantify the number of inner and outer hair cells in the middle and basal turns of the cochlea.
Results: Toxicity screening using naïve explants showed that 1-100 nM Oto-39 did not cause hair cell damage. Treatment with 100 nM Oto-39 after neomycin exposure resulted in a significant increase in outer hair cell numbers compared to controls treated with neomycin only. Specifically, this increase was 35% in the middle turn and 41% in basal turn. Similar explant studies are ongoing with additional compounds that are AhR

ligands or that increase AhR expression. We also plan to use fate-mapping of supporting cells in our explants to track the lineage of the increased numbers of outer hair cells. This will help determine whether the AhR ligands are mediating protective or regenerative effects.
Conclusions: These preliminary results suggest that AhR ligands are involved in the cellular processes that occurs after hair cell injury and indicate a novel role for AhR signaling in the inner ear.

S56. Novel Electrophysiological Marker for Focal Spiral Ganglion Neuron Degeneration

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Category: Inner Ear: Damage and Protection
Background: Varying spiral ganglion nerve (SGN) density along individual cochleae is a candidate factor for impaired hearing outcomes in cochlear implant (CI) users. Diagnostic tools for SGN loss or degeneration are therefore of high clinical relevance. We used an animal model to derive a non-invasive electrophysiological marker to identify lesions in individual ears.
Methods: Therefore, the SGN of normal-hearing guinea pigs were allowed to degenerate for ~9 days, after a focal micro-lesion. Via µCT-imaging and histology, we individually identified the CI-contact closest to the lesion, the degeneration profile (i.e., peripheral to central) and the lesion size, in 12 ears. The diameter of the lesion was histologically verified and had an average apical-basal extent of 475 µm. Electrophysiological responses of chronically lesioned ears were compared to intact, control ears (n=8). After acute, pharmacological elimination of hair cells, we recorded electrically-evoked compound action potentials (eCAP) to biphasic pulses (50µs/phase) in monopolar mode via a 6-contact, species-adapted CI (MedEl comp.). We characterized the input/output functions of the clinically relevant early N1P1 component to averaged polarities and confirmed results via the late N2P2 eCAP component that allows separation of anodic-leading and cathodic-leading stimuli. Applying median-splitting categorization we assessed the accuracy of the eCAP measures to identify lesioned ears.
Results: These small lesions lead to significant threshold elevation and reduced peak-to-peak amplitudes compared to healthy control ears for averages over all stimulation contacts. We introduced the inefficiency-of-stimulation index [A/V], which is the ratio of the introduced current to the resultant maximum eCAP amplitude at maximal outputs. This ratio is expected to reflect the maximal excitable tissue for stimulation with a given CI contact. From all eCAP measures analysed, including their respective polarity effects (PE), this Inefficiency index was the only measure that allowed for an individualized separation of healthy and focally lesioned ears, with a classification accuracy of more than 80%. For 10 out of 12 cases, the Inefficiency index could identify the contact closest to the lesion within 1.4 mm (~0.54 octaves) from the histologically defined lesion site. Furthermore, the variation in lesion size explained more than 60% of its variance. Reanalysing previous data on acute micro-lesions, we revealed that the changes in Inefficiency-index predicted the presence of a chronic degeneration, but not an acute damage with remaining excitable structures, central to the lesion. Whereas the threshold PE was significantly elevated only in the presence of damaged somata and not in damaged primary afferents, the Inefficiency-index was applicable independently of the degeneration profile.
Conclusions: The Inefficiency index informed about the presence, site and size of the lesion, without any a priori assumptions, and showed high classification accuracy irrespective of the type of chronic SGN degeneration. Thus, we propose the Inefficiency index as valuable tool for clinical diagnostics.

S57. Role of Lipid Mediators in Eosinophilic Otitis Media (EOM)-Associated Sensorineural Hearing Loss: An in Vitro and in Vivo Analysis

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Category: Inner Ear: Damage and Protection
Background: Eosinophilic otitis media (EOM) is an intractable otitis media characterized by highly viscous middle ear effusion and is frequently associated with bronchial asthma. There is an increased risk of persistent and progressive sensorineural hearing loss (SNHL) over time in patients with EOM. However, the mechanisms driving this progression remain unclear. We hypothesized that lipid mediators present in the middle ear effusion of EOM patients might impact the inner ear through the round window and oval window. To test this hypothesis, we analyzed these lipid mediators using mass spectrometry and assessed their effects on the inner ear both in vitro and in vivo.
Methods: Middle ear effusions were collected from patients with EOM (n=10) and patients with otitis media with effusion (OME) (n=10). Lipid mediators were profiled using liquid chromatography/tandem mass spectrometry. Detected major mediators, like prostaglandin E2 (PGE2) and leukotriene B4 (LTB4), were exposed to HEI-OC1, a mouse auditory cell line. Additionally, LTB4 was introduced to the mouse middle ear, after which hearing functionality and histological alterations were observed.
Results: Expression of lipid mediators in the middle ear effusion of patients with EOM were significantly higher than those of the patients with OME. LTB4 demonstrated concentration-dependent cytotoxicity to OC1 cells. Furthermore, in mice, LTB4 administration to the middle ear affected the wave-I amplitude at 32kHz without altering the auditory brainstem response (ABR) threshold. This observation was further supported by the diminished number of synapses in the cochlea.
Conclusions: Lipid mediators, especially LTB4, are upregulated in the middle ear effusion of patients with EOM and exhibit cytotoxicity to the inner ear both in vitro and in vivo. These results imply that lipid mediators in EOM's middle ear fluid may play a role in causing sensorineural hearing loss.

S58. Spatial Distribution and Relationship of PSD95, GluA2 and CtBP2 Puncta and Their Reorganization After Noise-Induced Cochlear Synaptopathy in Mice

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Category: Inner Ear: Damage and Protection
Background: AMPA-type glutamate receptors (AMPARs) of spiral ganglion neuron (SGN) postsynaptic densities (PSDs) consist of GluA2, GluA3 and GluA4 subunits. AMPARs containing GluA2 have low Ca2+-permeability. GluA2-lacking Ca2+-permeable AMPA receptors (CP-AMPARs) play a major role in synaptic excitotoxicity and noise-induced cochlear synaptopathy (NICS). Although every synapse has GluA2, it is distributed unevenly within the PSD and there are GluA2-deficient domains that can allow Ca2+ entry. This heterogeneity is presumably due to GluA2 trafficking in and out of the PSD, which might be affected by noise exposure. To assess this possibility and determine whether the distribution recovers after noise exposure, we measured the volumes and spatial relationships of PDS95, GluA2 and CtBP2 puncta, based on 3D-analysis of confocal images of cochlear wholemount preparations.
Methods: 12-14 week-old CBA/CaJ male mice were divided into the following groups: non-noise-exposed control, immediately post-noise (post-noise day zero, PND0), PND1, and PND14. NICS was caused by a 2-hr exposure to 100 dBSPL 8-16 kHz octave-band noise and confirmed by ABR measures and synapse counts. The organs of Corti were removed intact and the wholemounts visualized by confocal microscopy. Presynaptic ribbons, PSDs and AMPAR-GluA2 subunits were labeled with, respectively, anti-CtBP2, anti-PSD95 and anti-GluA2 antibodies. 3D images were quantitatively analyzed with Bitplane Imaris software. The volume of each punctum was measured. The spatial location of each punctum associated with an inner hair cell (IHC) was determined in reference to a 3D coordinate centered at the nucleus of that IHC.
Results: We confirm that ribbons exhibit size gradients within the IHC, with decreasing size along the modiolar-pillar and the basilar-reticular axes; in contrast, GluA2 puncta have increasing size along these axes. We further show that PSD95 puncta follow the size gradients as GluA2. These gradients and spatial distributions were significantly disrupted by moderate noise exposure that causes ~30% synapse loss. Among the surviving synapses, on PND1, the size gradients for ribbons, PSDs, and GluA2 puncta along the modiolar-pillar axis are abolished. By PND14, the size gradients for the structural elements of the synapse, ribbons and PSDs, have been reestablished. Notably, the size gradient for GluA2 puncta was not reestablished which suggesting long-term disruption in trafficking of GluA2 subunits postnoise. We observed a bimodal distribution of synapses across the modiolar-pillar axis, with greater numbers on the modiolar and pillar sides

relative to the middle. This distribution pattern is also abolished at PND1 but largely restored by PND14. However, we did not observe a preferential synapse loss on either the pillar or modiolar side of IHCs.
Conclusions: In general, noise exposure disrupts spatial distribution patterns and relationships for structural elements and GluA2 subunits of synapses. Some features can be restored postnoise, others may stay in long-term disruption. (Supported by NIH R01 DC015790, DoD CDMRP grant RH210047).

S59. Concussion-Induced Vestibular Peripheral Functional Deficits in Mice

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Category: Inner Ear: Damage and Protection
Background: Vestibular dysfunction is prevalent following concussion, with about 30-80% of individuals affected reporting dizziness, imbalance, and vertigo. Concussions involve not only the impact on the central nervous system caused by the rapid accelerations and decelerations of the head but also potentially affect the peripheral vestibular system within the bony labyrinth of the inner ear. In previous studies, we have developed a mouse model of concussion-induced vestibular deficits, in which a closed head impact model of engineered rotational acceleration (CHIMERA) was employed to generate consistent, repeatable concussions in mice. We found that multiple impacts caused substantial reductions in the sensitivity of the vestibulo-ocular reflexes. In the present study, to elucidate the mechanisms underlying the concussion-induced vestibular dysfunction, we further investigated the impact of concussion on the peripheral vestibular function by analyzing single unit vestibular afferents activities.
Methods: C57BL/6J mice were anesthetized and subjected to three repetitive head impacts over three consecutive days, each at an intensity of 0.52J. A control group received sham injuries. Single-unit recordings of vestibular afferents were conducted at two time points: one day and twenty-eight days following the last impact. Under ketamine anesthesia, a craniotomy was performed to provide access to the 8th nerve using a microelectrode. We analyzed the spontaneous firing rates, regularity, and sensitivity of vestibular afferents to both head rotation and translation. Additionally, to assess signal to noise ratios of afferent responses to head movement, we computed a distortion index based on FFT (Fast Fourier Transform) analysis. Afferents with distortions larger than 30% in responses to both rotation and translation were categorized as 'no-response' units.
Results: A total of 904 units were recorded. The head impacts resulted in a significant reduction in the spontaneous firing rates of both canal and otolith afferents. The sensitivities of regular otolith afferents to 1Hz sinusoidal head translation significantly declined 28 days after the injuries. There was an increased proportion of 'no-response' afferents observed at both 1 day and 28 days following the injuries. Moreover, the percentage of vestibular afferents with a low distortion index (less than 5%) also decreased following the head impacts, indicating that the compromised vestibular nerves exhibited a lower signal-to-noise ratio in their responses to head movement.
Conclusions: In summary, our results demonstrate that repeated concussions, as modeled by the CHIMERA system, led to impairments in both the canal and the otolith function. These results indicate that peripheral vestibular injuries play a significant role in the development of acute and sub-chronic vestibular symptoms following a concussion.

S60. Immune Response in the Spiral Ganglion Following Aminoglycoside-Induced Hair Cell Loss

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Category: Inner Ear: Damage and Protection
Background: Spiral ganglion neurons (SGNs) slowly die after neonatal aminoglycoside deafening of rats, resulting in the death of greater than 80% of all SGNs over the course of ~14 weeks. Our lab has previously shown that an inflammatory response involving components of both the innate and adaptive immune systems occurs in the rat cochlea after hair cell loss. Additionally, using a mouse Pou4f+/huDTR strain, we have shown

that knockout of MHCII prevents SGN death after hair cell ablation, implicating the adaptive immune system. To further assess the role of the adaptive immune response in SGN death, we compared SGN survival between T cell deficient RNU nude rats and control littermates.
Methods: Sprague-Dawley (SD), Crl:NIH-Foxn1rnu/rnu (RNU nude, T cell deficient) and Crl:NIH-Foxn1rnu/+ (RNU+, normal T cells) 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 detect hair cells, neurons, macrophages, and T and B lymphocytes.
Results: We have previously shown using SD rats that macrophage abundance and activation increase in the spiral ganglion weeks prior to the start of significant SGN death. Here, we report an increase in the population of spherical CD45+/IBA1- cells (presumably lymphocytes) in SD rats within the spiral ganglion concurrent with the start of post-deafening SGN death. This population includes CD4+ helper T cells and CD8+ cytotoxic T cells. The RNU nude rats had a small number of spherical CD45+/IBA1-/CD19- cells in the spiral ganglion that are presumably NK cells (a possibility we are further investigating). To determine if T cells are necessary for SGN death after deafening, we assessed neuronal survival in RNU rats and found that neuronal survival in RNU nude rats is not significantly different from that in RNU+ rats.
Conclusions: In SD rats, a macrophage-dominated immune response is elicited in the spiral ganglion weeks prior to significant SGN death, indicating that the immune response is not responding to dead/dying neurons but rather may be causal to SGN death after deafening. This is accompanied by a delayed response from CD4+ and CD8+ T cells that appear in the ganglion concurrent with significant SGN death. 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. Future studies are planned to identify the population of CD45+/IBA1-/CD19- cells seen in the RNU nudes. In addition, we will assess neuronal survival in SRG rats (T, B, and NK cell deficient) to assess whether other components of the adaptive immune system are required for SGN death after hair cell loss.

S61. Early Players Involved in Both Cisplatin-Induced Ototoxicity and SENS-401 Protection in Intact Organ Cultures

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Category: Inner Ear: Damage and Protection
Background: Cisplatin is a widely used effective chemotherapeutic agent, which at the same time causes irreversible hearing loss by damaging fundamental inner ear structures. Most of the deleterious effects described are cellular loss of essential auditory hair cells and spiral ganglion neurons, representing mainly later stages of cisplatin impact. With SENS-401, R-azasetron besylate, we are aiming at preventing and protecting from cisplatin-induced ototoxicity (CIO) by acting on early-stage pathways. The objective of this study is to characterize early pathways involved in both CIO and protective effects of SENS-401.
Methods: Organotypic explant cultures, including both spiral ganglion and organ of Corti intact tissue, were prepared from P3 to P5 Wistar rat pups. Kinetics of early signalling involved in CIO were studied by immunofluorescence staining using markers, such as cleaved caspase 3, an early apoptosis marker. Targets of SENS-401, 5-HT3 receptors and calcineurin A (CaN A), were also analyzed by Western blot and immunofluorescence staining. To characterize the full mechanisms of action of both CIO and SENS-401, we also performed unbiased proteomics approaches.
Results: Here, we provide the timeline of cisplatin action. One of the early- to mid-apoptotic pathways initiated in response to cisplatin is via cleaved caspase 3 activation. Immunofluorescence staining shows early cisplatin-induced apoptosis occurring around 16 to 24h after lesion onset at a concentration as low as 20 µM. While at this concentration a small number of hair cells and spiral ganglion neurons are affected, the initial impact of cisplatin is predominantly seen on supporting cells of the surrounding sulcus of the organ of Corti. In addition, clinically relevant doses of SENS-401, applied prior to cisplatin, protected from CIO. We also describe the presence of 5-HT3 receptors and CaN A, both known as targets of azasetron. Here, we provide proteomics, Western blot, and immunofluorescence outcomes that characterize 1) the broad signature of early impact of cisplatin and 2) investigate target engagement and the protective mechanisms elicited by SENS-401 in the context of CIO.

Conclusions: The present study provides both a broad overview of early CIO events and pathways involved in protective and preventive therapeutic effects of SENS-401.

S62. Ubiquitin Protein Ligase NEDD4L is Essential for Hair Cell Maintenance and Hearing Function

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Category: Inner Ear: Damage and Protection

Background: NEDD4L (Neural precursor cell expressed developmentally downregulated gene 4-like), a member of the NEDD4 family of HECT domain E3 ubiquitin ligase, is known to play a crucial role in regulating protein degradation through the ubiquitin-proteasome pathway. Recently, several studies have suggested that NEDD4L directly regulates the ubiquitination of ULK1, thereby modulating cellular autophagy. Mutations in the NEDD4L gene have been associated with neural defects and hearing loss in humans. However, the molecular mechanism by which NEDD4L deficiency causes hearing loss remains unclear.

Methods: To elucidate the mechanism of hearing loss associated with NEDD4L deficiency, we characterized conditional knockout (cKO) mouse models of Nedd4l. Nedd4l-floxed mice were crossed with three different Cre recombinase lines; Pax2-Cre for both inner ear epithelium and spiral ganglion, Emx2-Cre for inner ear epithelium, and Bhlhe22-Cre for spiral ganglion. Hearing function was assessed by measuring auditory brainstem responses (ABRs) and distortion product oto-acoustic emissions (DPOAEs). Hair cell bundles morphology and the presence of the autophagy markers were observed by scanning electron microscopy and immunofluorescence staining, respectively.

Results: Fusion and degeneration of stereocilia were observed at 3 weeks of age in both Pax2-Cre; Nedd4l f/f and Emx2-Cre; Nedd4l f/f mice, but not in Bhlhe22-Cre; Nedd4l f/f mice. The morphological abnormalities of the stereocilia progressively worsened with age. The degree of hearing loss in Nedd4l cKO mice was correlated with the fusion and degeneration of the stereociliary bundles. Specifically, Pax2-Cre; Nedd4l f/f and Emx2-Cre; Nedd4l f/f mice showed elevated thresholds at 3 weeks of age, progressing to profound deafness by 8 weeks. In contrast, Bhlhe22-Cre; Nedd4l f/f mice showed normal hearing at 3 weeks and elevated thresholds at 8 weeks. In addition, the autophagy marker LC3 was increased in the cochlea of Pax2-Cre; Nedd4l f/fand Emx2-Cre; Nedd4l f/f mice compared to control mice at early postnatal stages, suggesting an overactivation of autophagy in the absence of NEDD4L function. We are currently investigating differential roles of NEDD4L in the inner ear epithelium and spiral ganglion neurons.

Conclusions: This study shows that NEDD4L plays a more prominent role in the inner ear epithelium than in spiral ganglion neurons. Our results suggest that NEDD4L E3 ligase mediated regulation of autophagy may be critical for maintaining the integrity of hair cell stereociliary bundles and preserving auditory function.

S63. Open Board

S64. Gonadal Hormones Depletion Reduces Auditory Impacts of Lgals3 Knockout in Female Mice

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Category: Inner Ear: Damage and Protection

Background: Galectin-3, a β-galactoside-binding lectin, plays a pivotal role in various biological processes, including inflammation, apoptosis regulation, fibrosis, and host defense. Our prior research demonstrated that the absence of galectin-3 due to Lgals3 knockout Preferentially impacts the auditory function of female mice. This study investigates the influence of gonadal hormones on these knockout effects.

Methods: We used 24 B6.Cg-Lgals3tm1Poi/J mice (16 females, 8 males) aged 1-6 months, categorized into three groups: ovariectomized females (OVX), sham surgery females, and males. Female mice underwent ovariectomy or sham surgery at 5 weeks of age. Hearing assessments for auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE) were conducted before surgery (at 5 weeks) and at

three subsequent time points (4, 5, and 6 months of age). We measured ABR thresholds, DPOAE thresholds, and the area under the curve (AUC) of DPOAE responses, which reflects the performance and dynamic range of outer hair cell function. After the final hearing assessment, cochleae were collected to quantify the number of missing hair cells.
Results: At 5 weeks of age, the ABR thresholds were similar across the three groups, but DPOAE thresholds were lower, and the AUC of DPOAE responses was higher in males compared to the two female groups (sham and OVX). This aligns with our previous findings indicating a preference for Lgals3 knockout effects in female mice. As mice aged, the OVX group exhibited the least ABR threshold shifts, while the sham group displayed the greatest shift, with the male group falling in between. The most notable difference was observed at 24 kHz. By the age of 6 months, ABR threshold shifts were 41.6 ± 14.6, 25.0 ± 14.8, and 34.7 ± 17.0 dB for the Sham, OVX, and male groups, respectively. Similarly, the assessment of DPOAE thresholds revealed the most significant changes in the sham group, followed by the male group, and then the OVX group at the 24 kHz testing frequency. By the age of 5 months, the AUC had decreased from the pre-surgery level by 72.6 ± 32.3%, 31.8 ± 28.6%, and 56.1 ± 35.1% in the sham, OVX, and male groups, respectively. In line with the functional results, hair cell quantification showed a significant difference in hair cell pathogenesis among the groups, with the numbers of missing outer hair cells being 216 ± 77, 131 ± 70, 171 ± 90 for the sham, male, and OVX groups, respectively, in the cochlear range between 70% and 90% from the apex, where active sensory cell pathogenesis occurred.
Conclusions: Gonadal hormones significantly influence Lgals3 knockout effects in female mice. This finding underscores the potential role of female hormones in modulating the effects of galactin-3 deficiency on auditory function in female mice.

S65. Synthesis and Characterization of Hyaluronan-Antioxidant Conjugates as Potential Therapeutics for Noise-Induced Hearing Loss

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Category: Inner Ear: Damage and Protection
Background: Mechanistically, the production and recruitment of reactive oxygen species (ROS) is a major contributor to noise-induced hearing loss (NIHL). Therefore, targeting the reactive oxygen species from NIHL seems a viable prophylactic and therapeutic approach. However, due to the complexity of the ear anatomy, treatment and prophylaxis of hearing loss are difficult and extremely limited. Experimental approaches are often focused on invasive topical drug delivery methods or systemic drug delivery leading to damage of ear structures, off target effects, and low concentration reaching the cochlea. Our group has previously investigated several hyaluronan (HA) conjugates with antioxidants that show promise in accessing the inner ear structures and providing protection to inner ear cells against oxidative damage. In this study we explore a new series of HA-antioxidant conjugates (HAXs), for increased protective effects and potential development as a minimally or non-invasive prophylactic for sensorineural hearing loss (SNHL).
Methods: An HA intermediate enriched in carboxyl functionalities was used as starting material for all HAX synthesis. The synthesized HAXs were characterized via 1H-NMR and HPLC. HAXs were screened for oxidative protection in multiple acellular assays to determine our lead conjugates. Cytocompatibility of lead HAXs were assessed with MTS colorimetric assay as well as LDH cytotoxicity assay in cochlear (HEI-OC1) cells. The oxidative protection properties of lead HAXs were evaluated using an ROS detection assay (CM-H2DCFDA) after H2O2 stress in HEI-OC1 cells. Protection was assessed by both pre-treating cells with HAXs and simultaneous treating with HAXs while stressing with H2O2. Permeation and cytocompatibility were also assessed in previously developed RWM and TM models.
Results: A series of HAXs were successfully synthesized and characterized. HAXs were evaluated for oxidative stress mitigation in various acellular assays. Two lead candidates were chosen based on these data and evaluated in further cellular studies. The lead HAXs were cytocompatible, provided oxidative protection to stressed HEI-OC1 cells, and was able to permeate the RWM model. Our additional data indicate that conjugation of the antioxidant to HA enhances these protective effects when compared to the anti-oxidant agents alone.

Conclusions: Our data so far highlights the practicality of chemically conjugating HA with antioxidants for oto-therapeutic purposes. Such therapeutics have the potential to be further developed into a new generation of biocompatible and biointegrating topically deliverable agents against hearing loss.

S66. Modulation of Hair Cell Synaptic Elements by Glutamate and Gaba Receptor Ligands

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Category: Inner Ear: Damage and Protection

Background: Hidden Hearing Loss (HHL) is a subtype of sensorineural hearing loss that impairs speech comprehension in noisy environments despite normal hearing using standard audiometric tests. Despite its prevalence in the aging population, the mechanisms of HHL are not fully understood. One likely mechanism of HHL is synaptopathy, the disconnection of hair cells from afferent spiral ganglion neurons (SGN). Synaptopathy maybe caused by overactivation of ionotropic glutamate receptors due to increased glutamate signaling, leading to deterioration of both pre- and post-synaptic elements. However, the precise relationship between glutamate receptor activation and synaptic damage is unknown. Further, there is little known about how GABAergic inhibitory neurotransmission may modulate synaptopathy in the auditory periphery. Here, we use the larval zebrafish lateral line as a model to quantify the dose by time relationship between glutamate and GABA receptor activation and synaptic damage.

Methods: We incubated larval zebrafish in AMPA, NMDA, or GABA agonists at varying concentrations, treatment durations, and post-exposure recovery times. We utilized a transgenic zebrafish line expressing green fluorescent protein (GFP) tagged Ribeye protein to visualize presynaptic hair cell ribbons in the lateral line. Nuclei of hair cells were labeled with DAPI, and the postsynaptic elements on afferent neurons were identified using MAGUK antibody labeling. Colocalization, decoupling, and area of the synaptic elements were quantified using sequential confocal microscopy and a custom macro run using FIJI software.

Results: Preliminary findings indicate receptor agonism influenced receptor area and number. Our research demonstrates that short-term exposure to high concentrations of AMPA caused decoupling of synaptic elements that were only seen after an extended recovery time (48-72 hrs), suggesting that intracellular signaling events mediate this synaptic damage. Additionally, glutamate and GABA agonists did not alter the number of ribbons per hair cell, but a 15 minute GABA exposure significantly increased ribbon area within 90 minutes of recovery and the effect persisted through the 72 hour recovery period.

Conclusions: Future research will determine the degree to which afferent neurons recede after synaptic decoupling and explore downstream mediators of synaptopathy to identify potential therapeutic targets. We will also investigate if can mitigate glutamatergic receptor decoupling. These results could provide valuable insights in the pathology of HHL.

S67. Partial-Reprogramming Contributes to an Anti-Inflammatory Associated Hearing Recovery in Acoustic Trauma

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Category: Inner Ear: Damage and Protection

Background: Hearing functions through the mechanical transduction of inner ear sensory cells, hair cells, and their connections to the auditory neurons, which convert the stimuli into electrical signals to be detected by the brain. Noise-induced hearing loss (NIHL) is usually caused by exposure to excessively loud sounds and cannot be medically or surgically corrected. Strategies to overcome the apparently irreversible noise-induced hearing loss (NIHL) in mammals become paramount for hearing treatment. So far, there is no FDA-approved drug to treat noise-induced hearing loss.

Methods: With the transgenic rtTA/tet-Myc/tet-NICD and rtTA/tet-NICD mouse models, we studied hearing by reprogramming of adult inner ear with the activation of Myc/Notch1 and Notch1 before and after noise exposure, to determine protection and/or repair for NIHL. Valproic acid (VPA), an FDA-approved small

chemical compound with a role in Notch activation, was further evaluated against NIHL by middle ear delivery.
Results: In the rtTA/tet-Myc/tet-NICD mice, Dox-induced Myc/Notch activation reprogrammed the inner ear and significantly protected hearing against NIHL. With the noise that caused the permanent threshold shifts (PTS), an average reduction of in ABR thresholds of 40 dB was detected at 11.32 kHz and 30 dB at 8 kHz and 16 kHz, with the effect that is sustained. To discern the specific role of Myc and Notch1, we studied transgenic mice in which MYC or NICD was activated individually. We found that the activation of Notch1 but not Myc was responsible for the preservation of hearing after noise exposure. We showed that a small chemical compound Valproic acid (VPA), an FDA-approved drug, is sufficient to replace the Notch1 gene and protects the inner ear from noise-induced PTS in wild-type mice. A maximum of 40 dB reduction in ABR thresholds at the middle frequency was obtained by middle ear delivery of VPA. Mechanistically, we determined that VPA "reprogrammed" the adult mice cochlea by regulating anti-inflammatory pathways. The blockade of the pathways greatly attenuates the benefits of VPA treatment. By single-cell RNAseq study, we detected the down-regulation of the ATF4 stress-response signals via VPA reprogramming. Finally, the application of VPA before noise exposure protected mice from noise-induced accelerated age-related hearing loss, with hearing protection extended to 5 months after the treatment. Application of VPA after noise exposure does not improve hearing.
Conclusions: Our work demonstrates that the activation of Notch in the mature inner ear is sufficient to offer robust hearing protection against NIHL. An FDA-approved small molecule drug VPA can effectively replace Notch to achieve hearing protection. Notch-based hearing protection is partially mediated by anti-inflammatory pathways. Our findings have the potential to protect hearing against NIHL in humans by repurposing an FDA-approved drug, VPA.

S68. Cochlear Damage in the Mouse Model of Lassa Virus Infection

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Category: Inner Ear: Damage and Protection
Background: Lassa fever is endemic to West Africa and is caused by infection from Lassa virus (LASV). It is associated with sensorineural hearing loss in up to one-third of patients following infection. Although prevalent, the exact mechanism of hearing loss following infection remains unclear.
Methods: Stat1 knockout mice or wild type (WT) mice were infected with LASV or phosphate-buffer saline (PBS) as a mock control. Auditory brainstem response (ABR) and Distortion Product Otoacoustic Emissions (DPOAE) hearing tests were performed on each mouse weekly from 3 to 8 weeks post infection. The mice were euthanized at 60 days post infection (dpi). An immunohistochemical analysis of the inner ear of these mice was done. The temporal bones were processed into paraffin blocks, thin sectioned, and processed for H and E staining, or labeling with anti-CD3 antibody, or anti-LASV nucleoprotein antibody.
Results: In the mice infected with LASV, structural damage, primarily in the spiral ganglion, was observed. Lymphocytic infiltrate as well as fibrosis was observed in the scala tympani. CD3 IHC labeling was positive mainly within the spiral ganglion, as well as throughout the scala tympani. LASV antigen was observed around the neurons and spiral ganglion cells. The Stat1 knockout mice in the negative control group injected with PBS showed no damage in any of the inner ear structures.
Conclusions: The Stat1 knockout mice infected with LASV all had severe hearing loss. Stat1 knockout mice infected with LASV had moderate to severe fibrosis and lymphocytic infiltrate in the scala tympani, while WT mice infected with LASV had less. All mice infected with LASV had bulging Reissner's membranes of varying degrees, suggestive of endolymphatic hydrops. All mice with hearing loss had a similarly damaged spiral ganglion, all of which had CD3 positive lymphocytes infiltrated adjacent to the spiral ganglion cells. This suggests that hearing loss following LASV infection is likely due to damage within the spiral ganglion as a result of the immune response.

S69. Difference of Nestin Expression by Age After Noise Exposure in Mouse Cochlea

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Category: Inner Ear: Damage and Protection

Background: For the treatment of sensorineural hearing loss, approaches targeting the regeneration of inner ear hair cells are considered to be one of the feasible strategies. Since spontaneous hair cell regeneration is not observed in the mammalian cochlea, hair cell regeneration by artificially inducing differentiation of inner ear stem cells in the cochlea has attracted attention. It has been reported that stem cell potency of supporting cells altered when hair cells are damaged. However, the long-term changes in stem cell potency due to aging and the changes in stem cell potency after hair cell damage are not fully understood. In this study, we targeted Nestin, a neural stem cell marker in the central nervous system, and examined the change after noise exposure and the effect of aging.

Methods: We used Nestin-EGFP (enhanced green fluorescent protein) transgenic mice to detect the presence of stem cell potency in the mouse cochlea. We examined the cochlea of mice on the following postnatal days (P): P30, P100, P200 and P300. Experimental groups were exposed 121dB of white noise for two hours; the control groups were not exposed. All mice were evaluated for hearing at 8kHz, 16kHz, and 24kHz by ABR (auditory brainstem response) before and after noise exposure. We compared of differences in the localization of Nestin expression in cochlea by age using immune-staining. Furthermore, we examined the change of hearing thresholds and Nestin expression after noise exposure, and compared the difference of these by age.

Results: Hearing thresholds by ABR tended to increase at higher frequencies and with increasing age. Comparing hearing thresholds by ABR before and after noise exposure, hearing thresholds were increased after noise exposure at all frequencies in each mouse.

In the inner ear specimen, Nestin was expressed at Rosenthal's canal and base of supporting cells. Comparing Nestin expression before and after noise exposure, Nestin at the spiral ganglion and base of supporting cells was enhanced after noise exposure in P100 mice. However, Nestin expression was decreased after noise exposure in P200 and P300 mice.

Conclusions: Noise exposure caused hearing damage regardless of age. After noise exposure, Nestin expression increased at P100 mice but decreased at P200 and P300 mice. These data indicate that the regenerative potential of the supporting cells varies with age. It has been reported the location of Nestin expression also changes during development. Therefore, it was considered that Nestin expression and reaction after noise exposure also varies by age in the adult mice inner ear. We plan to examine the differences of Nestin expression by frequencies.

S70. Hair Cells Are Preserved in Mice With Severe Hearing Loss Caused by Lassa Virus Infection

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Category: Hair Cells: Anatomy and Physiology

Background: Lassa Fever (LF) is caused by Lassa Virus (LASV) infection, an endemic hemorrhagic fever in West African countries. Up to one third of subjects infected with LASV develop sudden severe hearing loss after clearing the acute disease phase. In our LF mouse model we used a combination of tissue clearing and 3D reconstruction of the temporal bone to investigate the spatial-temporal changes in inner ear cochlear cells.

Methods: Stat1 knockout mice were infected with LASV or PBS, underwent serial weekly ABR and DPOAE testing, and temporal bones were harvested at different time points up to 90 days post infection. The temporal bones were fixed in 10% formalin for at least 7 days before being processed for tissue clearing. Whole temporal bones underwent tissue clearing using a modified Sca/e S protocol (Hama et al. Nat Neuosci, 2015. 18(10):1518-29). The temporal bones were labeled with myosin VI antibody and phalloidin and fluorescent secondary antibodies, visualized and serial images captured with lightsheet microscopy. The images were reconstructed into 3D for further analysis of inner ear cells. The contralateral temporal bones were embedded in paraffin, thin sectioned, and processed for immunohistochemistry analysis for comparison.

Results: Mice infected LASV showed severe hearing loss, while mice administered with PBS had no change in hearing performance. Both the mice infected with LASV and PBS showed minimal changes to all rows of the inner ear hair cells from the apical turn to the basal turn in the tissue cleared 3D temporal bones. Immunohistochemistry analysis in the thin sections showed damage to the spiral ganglion cells and infiltration of T-lymphocytes in the cochlea of the mice with LASV infection.
Conclusions: The combination of Sca/e S tissue clearing protocol with the use of myosin VI antibody and phalloidin labeling was suitable for 3D imaging with single-cell resolution of the LF model mice temporal bones. The mechanism of LF induced hearing loss does not involve direct damage to the inner ear hair cells.

S71. The Potential of Mitochondrially-Targeted Tetrapeptide in Protecting Against Noise-Induced Hearing Impairment

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Category: Inner Ear: Damage and Protection
Background: Noise-induced hearing loss (NIHL) constitutes a significant health concern for which there is currently no effective treatment. The loss of auditory receptor cells, specifically cochlear hair cells, and associated synaptopathy are common causes of NIHL. One of the primary mechanisms proposed to be involved in NIHL is the accumulation of reactive oxygen species (ROS) that ultimately overwhelms cochlear cells. ROS have been detected in the cochlea immediately after noise exposure, persisting for at least a week following the exposure. Within cells, ROS are primarily generated in mitochondria as a product of cellular metabolism. Elamipretide, also known as SS-31, MTP-31, and Bendavia, is a small tetrapeptide capable of reaching and concentrating in mitochondria, improving mitochondrial function and reducing ROS production. We hypothesized that elamipretide treatment could mitigate noise-induced hearing loss.
Methods: Male and female 16-week-old CBA/J mice were obtained from Jackson Laboratories, ME. Groups of mice were exposed to 8-16 kHz octave-band noise at 98 dB SPL for 2 hours. Intraperitoneal elamipretide treatment was initiated immediately following noise exposure and continued for two weeks. Auditory brainstem response (ABR) thresholds, peak amplitudes, and latencies were analyzed using data from ABR recordings of the treated and control groups.
Results: Noise-exposed mice exhibited a mild elevation in ABR thresholds at 32 kHz and a reduction in ABR wave peaks I and II amplitudes measured at 60-70 dB SPL. Peak latencies were not affected. Elamipretide treatment prevented the elevation of ABR thresholds and the attenuation of peak amplitudes induced by noise exposure.
Conclusions: Our results provide proof of concept that mitochondrial-targeted elamipretide, which enhance mitochondrial function and reduce ROS production, can prevent noise-induced hearing impairment in a mammalian model.

S72. Machine Learning-Driven in Silico Screening Reveals Novel Drug Hits Against Cisplatin-Induced Hearing Loss

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Category: Inner Ear: Damage and Protection
Background: Cisplatin is a first-line chemotherapy prescribed to 20% of all cancer patients.
However, more than 80% of patients receiving cisplatin treatment developed permanent hearing loss. Despite this debilitating side effect, currently, there is only one FDA-approved drug (sodium thiosulfate or STS) to prevent cisplatin ototoxicity among pediatric cancer patients.

Methods: Machine learning (ML) based classifiers and quantitative structure activity relationship (QSAR) models were used to identify drug hits that prevent cisplatin-induced hair cell loss. A diverse compound library (diversity set) containing over 1500 drug-like compounds was screened using the zebrafish lateral line neuromast assay to generate our in-house training dataset for building the ML models. A high-throughput

computational screen of a clinical compound library was performed using high-performing ML models, which identified a number of interesting FDA-approved drugs. The top-ranked drug hits were validated using the in vivo zebrafish assay.
Results: Among the top 25 FDA-approved drugs that our in silico screens discovered, DXU3056 demonstrated complete protection against cisplatin-induced hair cell damage at 50uM. Our ML models also identified a key chemical core structure responsible for the protective effect. Based on the chemical core structure, additional compounds containing the core structure were shown to partially or completely block cisplatin uptake in zebrafish hair cells.
Conclusions: We have demonstrated the potential of synergizing in vivo zebrafish neuromast hair cell assay and machine learning to accelerate high-throughput drug discovery for otoprotection against cisplatin ototoxicity. Out top hit DXU3056 is an antineoplastic agent. When combined with cisplatin, the drug combination may offer the benefit of enhanced anti-tumor efficacy and reduced cisplatin ototoxicity. A rodent study is underway to further validate the otoprotective effect of the combination therapy in the mammalian system.

S73. Investigating the Protracted Inner-Ear Proteomic Signatures in Response to Systemic Lipopolysaccharide and Kanamycin

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Category: Inner Ear: Damage and Protection
Background: Aminoglycosides are cheap and reliable antibiotics that are used globally to fight severe gram-negative bacterial infections. They have been used for decades and are reliable. Unfortunately, aminoglycosides have a narrow therapeutic window and carry a significant risk for causing debilitating hearing and vestibular dysfunction. In addition, lipopolysaccharide (LPS), a component of gram-negative bacterial cell walls, has been shown to potentiate aminoglycoside-related ototoxicity. We have developed a preclinical model utilizing systemically administered LPS and kanamycin that demonstrates reproducible hearing loss in mice. Here we utilized the ultra-sensitive label-free UHPLC-MS/MS technique to explore the proteomic changes that occurs in the cochlea of mice exposed to LPS and kanamycin alone and in combination with one another.
Methods: We utilized a clinically relevant aminoglycoside model in B6N(Cg)-Cdh23tm2.1Kjn mice. In this model, animals were treated with Lipopolysaccharide (LPS) at 1 mg/kg I.P. 3 times over a 14-day period in combination with subcutaneous injection of Kanamycin (KM) at 500 mg/kg two times a day, 6 hours apart for 14 days. Experimental animals were co-treated with KM and Fedratinib - 50 mg/kg via oral gavage (OG). (JAK2 specific inhibitor). Functional hearing assessments were collected via auditory brainstem response (ABR) and distortion product otoacoustic emissions (DPOAE). ABRs were tested at 4,8,16,22,32,45, and 64 kHz from 100 dB SPL to 20 dB SPL in 5 dB increments. DPOAEs were tested at 5.6, 8, 11.3, 16, 22, and 32 kHz from 75 dB SPL to 10 db SPL in 5 dB increments. For label free mass-spectrophotometer analysis, 1 ug peptides were injected from each sample. The LC Thermo Scientific UltiMate 3000 RSLCnano system was connected with Thermo Scientific Orbitrap Exploris 480 MS instrument. Precursor ions were detected in Orbitrap with resolution of 60,000 at m/z 200. The precursor ions were fragmented further with normalized HCD collision energy (%) 30, and the fragment ions were detected in orbitrap with resolution of 15000 at m/z 200. The raw files were subjected to label-free analysis with Progenesis QI software.
Results: This method uncovered several genes and pathways that were significantly altered utilizing this method. This method may be valuable in identifying relevant high-yield pathways that are altered with other ototoxic conditions. In addition, this method has uncovered a small group of targets that we plan to further interrogate with the goal of identifying and interfering with pathways that lead to permanent hearing loss caused by aminoglycosides.
Conclusions: UHPLC-MS/MS is a viable method for interrogating the proteomic signatures of ototoxicity in the cochlea. It provides reliable genome wide data that increases the understanding of the pathways that are implicated in ototoxicity. These high yield pathways should be further interrogated for potential therapeutic targeting with the goal of protecting patients hearing from ototoxic drugs such as aminoglycosides.

S74. Exploring the Role of Supporting Cells in Inner Hair Cell Survival During Cyclodextrin-Induced Ototoxicity

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Category: Inner Ear: Damage and Protection

Background: Cyclodextrins belong to a group of cyclic oligosaccharides known for their utility as complexing agents, effectively enhancing the aqueous solubility of poorly soluble drugs while also improving their bioavailability and stability. Our prior research has revealed that this compound preferentially targets outer hair cells, leading to immediate cell death after a single dosage of the drug treatment. Conversely, inner hair cells display a delayed onset of cell death. In contrast, inner hair cells undergo delayed cell death. This pattern of cochlear pathogenesis offers an excellent opportunity to explore the factors influencing the survival of inner hair cells in the presence of outer hair cells. The current study aims to investigate the role of supporting cells in preserving the viability of inner hair cells using a rat model of cyclodextrin-induced ototoxicity.

Methods: In this study, adult rats of both sexes were utilized as experimental subjects. These rats were administered a single dose of cyclodextrin at varying concentrations: 1, 2, 3, and 4 mg per gram of body weight. Cochleae were subsequently harvested at different time points following the administration of the drug. The collected cochleae were prepared for surface preparations or cochlear sectioning. To visualize tissue structures, a range of staining techniques was employed, including hematoxylin staining, silver nitrate staining, succinate dehydrogenase staining, and toluidine blue staining.

Results: The administration of cyclodextrin resulted in a dose-dependent outer hair cell pathogenesis. In the high-dose group, significant outer hair cell loss was observed within three days after treatment. However, even in cochleae displaying extensive outer hair cell loss in the high-dose group, supporting cells and inner hair cells remained present at the two-week mark following treatment. By the fourth week, supporting cell death became evident. In the low-dose group, supporting cell damage was primarily localized to the basal region of the cochlea, coinciding with the area where outer hair cell loss had occurred. In contrast, the high-dose group exhibited more extensive supporting cell lesions, affecting both the middle and apical portions of the cochlea. Notably, Deiters cell lesions were frequently larger than pillar cell lesions, and pillar cell lesions were observed exclusively in regions with existing Deiters cell lesions, suggesting a sequential occurrence of pillar cell death following Deiters cell death. Furthermore, the loss of inner hair cells was observed only in areas where pillar cell death had occurred, indicating a strong spatial correlation between these two types of lesions.

Conclusions: The results from this study suggest that the preservation of inner hair cells relies on the well-being of pillar cells in the acute outer hair cell death model. Understanding the precise mechanisms governing the role of pillar cells in safeguarding inner hair cell survival remains a topic for future investigation.

S75. Mucoadhesive Nanoparticle-Mediated Inner Ear Drug Delivery

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Category: Inner Ear: Drug Delivery

Background: To facilitate the entry of drugs injected into the middle ear into the inner ear, they must penetrate through the round window or oval window. However, there exists no inherent driving force to propel these drugs from the middle ear through these windows. Drugs that float freely within the middle ear without coming into contact with the middle ear mucosa cannot contribute to inner ear drug delivery. Therefore, if drugs inserted into the middle ear can adhere well to the middle ear mucosa, it is expected that the efficiency of drug delivery can be significantly improved. In this experiment, we assessed the suitability of mucoadhesive nanoparticles as inner ear drug delivery vehicle in comparison to non-adhesive nanoparticles.

Methods: Dopa (3,4-dihydroxyphenylalanine) is recognized as a key chemical signature of mussel adhesion and has been adopted into diverse synthetic polymer systems. We fabricated nanoparticles using Polyvinyl alcohol (PVA) and poly lactide and poly (D,L-lactide-co-glycolide) (PLGA), and coated them with DOPA to create mucoadhesive nanoparticles. We evaluated the in vitro and in vivo toxicity of the nanoparticles and subsequently loaded them with a fluorescent dye to compare drug delivery efficiency based on the presence

of DOPA coating. Next, we loaded dexamethasone into the nanoparticles and compared the amount of dexamethasone delivered to the cochlea in practice.
Results: The in vitro toxicity analysis was conducted using HEI-OC1 cells, and PVA/PLGA nanoparticles showed no cytotoxicity up to 10mg/ml, while DOPA/PVA/PLGA nanoparticles did not exhibit toxicity up to 5mg/ml. For in vivo toxicity evaluation, both types of nanoparticles were injected into the cochlea at a concentration of 5mg/ml, and hearing was assessed at the end of the second week. In both groups, there was no apparent hearing damage compared to the saline control group. Subsequently, lipophilic coumarin was encapsulated within the nanoparticles and administered to the cochlea. Cochleae were collected 1 hour, 3 hours, and 6 hours after administration, crushed in 100% methanol to create lysates, and fluorescence intensity was analyzed. At all three time points, DOPA/PVA/PLGA nanoparticles exhibited superior fluorescence intensity. Subsequently, dexamethasone was encapsulated within the nanoparticles in the same manner and delivered. DOPA/PVA/PLGA nanoparticles demonstrated superior dexamethasone delivery compared to PVA/PLGA nanoparticles or dexamethasone sodium phosphate.
Conclusions: This study demonstrated that mucoadhesive nanoparticles coated with DOPA, specifically DOPA/PVA/PLGA nanoparticles, exhibited excellent biocompatibility and enhanced inner ear drug delivery compared to non-adhesive nanoparticles. These findings suggest that mucoadhesive nanoparticles hold promise as a potential strategy to improve drug delivery to the inner ear by promoting adhesion to the middle ear mucosa.

S76. Kv7.4 Activator INT002-140 Protects Against Noise-Induced Hearing Loss

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Category: Inner Ear: Drug Delivery
Background: Noise-induced hearing loss (NIHL) is one of the most common type of sensorineural hearing loss and estimated to affect 12% or more of the global population. Despite its prevalence, there is still limitation of the treatment options for NIHL. The exposure to excessive noise has been suggested to play a role in NIHL by reducing KCNQ4 function on the surface membrane. Therefore, the discovery of small compounds that activate or enhance the KCNQ4 is an important strategy for the therapeutic treatment of NIHL. Here, we demonstrated the efficacy of a novel and potent small-molecule Kv7.4 activator, INT002-140, on NIHL.
Methods: Eight-week-old rats were exposed to 2 hours of 120 dB SPL, 8-16 kHz. Group 1 received CF1 formulation only. Group 2 was given 10 mg/mL INT002-140 in CF1 formulation. The INT002-140 was formulated as suspension in thermoreversible gel and trasntympanically injected immediately after the noise exposure. The auditory function was assessed by ABR at day 1, 7, 14, 21 after noise exposure. The cochlear exposure of the INT002-140 from a formulation was determined in a pharmacokinetic study by analyzing perilymph at 1, 4, 24 hours and at day 7 after trans-tympanic injection in guinea pig.
Results: The INT002-140 as a novel and potent Kv7.4 activator has EC50 of 0.06 μM. For CF1 formulation, INT002-140, delivered in the middle ear and rapidly reached the inner ear fluids (IEF) at an hour post-administration. The CF1 formulation reached a greater IEF level of INT002-140 at 4 hours (13090.4 μg/mL). Then, the concentration slowly decreased within 24 hours to finally reach 32.0 μg/mL at 7 days after a injection. Hearing thresholds shift in rats exposed to noise showed permanent hearing loss with more than 60 dB thresholds shift. ABR threshold shifts by a mean of 70 to 80 dB relative to baseline were determined after 1 days of vehicle over the range of frequencies evaluated. Treatment with INT002-140 reduced ABR threshold shifts after 1 days, particularly at click and 4 kHz by 14 to 22 dB compared to the vehicle group. The efficacy became more pronounced after day 7 over the range of frequencies evaluated and lasted at 3 weeks after the treatment.
Conclusions: This study is notable in that we in vivo experimentally clarified the therapeutic effects of Kv7.4 activator used to treat NIHL. The CF1 formulation developed by iN Therapeutics lead to higher concentration of INT002-140 in the IEF after administration and allowed a sustained and long-lasting delivery of INT002-140 in the IEF. In summary, A single local treatment with the novel small-molecule Kv7.4 activator INT002-

140 using the CF1 formulation could allow to have sufficient level of the INT002-140 in the cochlea, which was therapeutic effective in NIHL.

S77. Congenital Cytomegalovirus Infection: Assessing the Feasibility of Inner Ear Therapy for Hearing Loss Using Mice

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Category: Inner Ear: Drug Delivery

Background: Congenital cytomegalovirus (cCMV) is the major cause of congenital non-hereditary sensorineural hearing loss (SNHL). Approximately 15% of the newborns will have hearing loss at birth. Moreover, the hearing function of 10% of the children will progress during childhood. They may develop hearing loss at later age ('late-onset hearing loss') and once present, the hearing loss might also improve or deteriorate. A subgroup of children is eligible for systemic therapy consisting of valganciclovir. However, the effect on hearing outcome remains uncertain and therapy carries the risk of side-effects. The aim of this study is to assess the efficacy of inner ear therapy in mice.

The pathophysiology of cCMV-related hearing loss has not yet been fully elucidated. Today, there is evidence for a direct cytopathic effect and/or inflammatory-based damage of the stria vascularis which disrupts endocochlear potential and leads to secondary damage of sensory and non-sensory inner ear structures.

To investigate cCMV-related hearing loss, mice are frequently used. The murine inner ear has a similar anatomic structure and physiological function as the human inner ear. The auditory system of a newborn mouse equals that of a human fetus. Inoculation of a newborn mouse with murine cytomegalovirus (MCMV) results in similar outcomes as a fetal cCMV infection. Audiological testing can be performed using Auditory Brainstem Responses (ABR) and Distortion-Product Otoacoustic Emissions (DPOAE).

Methods: BALB/c mice will be injected intraperitoneally with phosphate buffered saline (PBS) or MCMV Smith virus on postnatal day 0. On postnatal day 7, they will be treated by intratympanic injection of PBS, ganciclovir (antiviral effect), or dexamethasone (anti-inflammatory effect). Two outcome measures will be compared between the treated groups and the placebo group: hearing outcome and preservation of hair cells and stria vascularis. Audiological testing using ABR and DPOAE will be performed on postnatal day 20 and 60. Mice will be sacrificed on postnatal day 60 to extract the cochlea. Cochleas are fixed in 4% paraformaldehyde at 4°C overnight, followed by decalcification in 120mM EDTA for 3 days. Subsequently, the cochlea will be microdissected to separate different cochlear turns. These tissues will be blocked followed by applying the primary antibodies (anti-MYO7A for hair cells and anti-KCNJ10 for stria vascularis) and secondary antibodies overnight. The next day, the tissues will be rinsed with PBS, treated with DAPI and mounted on a medium for confocal microscopy of the different structural parts. The different cells will be manually counted using ImageJ software.

S78. Gelatin Encapsulation Improves the Therapeutic Efficacy of Tetrahedral DNA in Inner Ear Drug Delivery

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Category: Inner Ear: Drug Delivery

Background: DNA nanostructures have garnered significant attention as potential drug delivery systems due to their biocompatibility, biodegradability and versatility. Our previous research has demonstrated tetrahedral DNA nanostructures (TDNs) as nanocarriers for delivering drugs to the inner ear, due to their remarkable ability to penetrate through biological barriers (round window membrane). However, their therapeutic effect in inner ear therapies was curtailed by some limitations of TDNs including the undesirable uptake of TDNs by HEI-OC1 cells. In response, we introduced a novel drug delivery complex, TDN@GNP, wherein gelatin

nanoparticles (GNPs) were ingeniously integrated to create a compact structure housing functional TDNs, aiming to improve their inner ear drug delivery efficacy.
Methods: TDNs were encapsulated within gelatin through nanoprecipitaion. The discrepancy in uptake efficiency and mechanisms between TDN@GNP and TDNs were investigated in HEI-OC1 cells. The nuclease resilience of TDNs in TDN@GNP was assessed by electrophoresis. Additionally, the ability of TDN@GNP to evade lysosomes and delivery their payload into the cytoplasm was investigated by confocal laser microscope and transmission electron microscopy. We evaluated the overall drug delivery efficiency of TDN@GNP and TDNs by adopting a model drug, epigallocatechin gallate (EGCG). We compared the anti-lipid peroxidation efficacy of the drug-loaded nanocarriers in vitro, and established a noise-induced hearing loss rodent model to assess their hearing protective effect upon in vivo application.
Results: The incorporation of GNPs endowed TDN@GNP with enhanced structural stability compared to standalone TDNs. This innovation led to a significant restoration of TDN internalization in HEI-OC1 cells, a crucial advancement. Notably, multiple endocytic pathways participated in facilitating TDN@GNP internalization, and the lysosomal escape capability of TDN@GNP allows TDNs to evade the lysosomal pathway and maintain their integrity. Finally, we demonstrated that TDN@GNP loaded with epigallocatechin gallate (EGCG) exhibited a more potent anti-lipid peroxidation effect than bare TDNs in vitro, and demonstrated stronger efficacy in preventing noise-induced hearing loss through local administration in a rodent model.
Conclusions: In summary, our investigation revealed the limitations of TDNs as an inner ear drug delivery vehicle. Through TDN encapsulation within GNP, we successfully identified TDN@GNP as a novel drug delivery complex, effectively enhancing drug bioavailability in cochleae. Our results suggested that the TDN@GNP complex significantly enhanced the therapeutic potential of TDNs in inner ear drug delivery, which implied a great prospect of the complex combining gelatin with DNA nanostructures in clinical and preclinical applications.

S79. Bio-Responsive Nanoformulation Fabricated to Maintain Inner Ear Homeostasis via Redox and Calcium Ion Channel

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Category: Inner Ear: Drug Delivery
Background: Hearing loss resulting from chemotherapeutic induced ototoxicity (CIO) especially platinum drugs is irreversible and there are at present no treatments available to prevent or reverse CIO. Therefore, this investigation potentially provides a prophylactic cure for CIO and associated hearing loss by developing an effective bio-responsive drug delivery system to protect hair cells. The choice of drug and drug delivery system was based on oxidative stress in the cochlea and calcium ion channel dysregulation and examined the molecular mechanism of CIO on HEI-OC1.
Methods: The novel dual-drug bio-responsive nanoformulations were developed using ROS-responsive polymer synthesized in-house. The nanoformulations were optimized using Design of Experiment (DoE) – Central Composite Design (CCD) and prepared by nanoprecipitation method. The quantitative method was developed for Bucillamine (BUC)-PPS-mPEG2000-NCs and Diltiazem (DLT)-PPS-mPEG2000-NCs to determine entrapment efficiency (EE) and drug loading capacity (DL) using Ellman's assays and HPLC respectively. TEM image analysis was performed at different resolutions and scales to study NCs morphology. The cytoprotective effect of BUC-NCs, DLT-NCs and BUC/DLT-NCs, were studied at various regimens with and without cisplatin using MTT, and Live-dead cell assays on HEI-OC1 cells. The redox homeostasis and apoptosis effect of nanoformulation was confirmed by ROS-Scavenging and Caspase assay respectively. The PI3 AKT/STAT/Caspase molecular pathways associated with oxidative stress and apoptosis were studied using WB analysis on HEI-OC1 cells.
Results: The ten nanoformulations were successfully prepared for each BUC and DLT considering optimized EE, DL, size and PDI. The %EE and %DL was 55.87% and 8.38% resp. for BUC. The %EE and %DL was 81.98% and 12.30% resp. for DLT. The average sizes of BUC-NCs, and DLT-NCs, were found ~107.1 and 98.8 nm, resp. and PDI less than 0.3 confirmed that most of the NCs have the same size and appeared spherical as imaged by TEM. BUC-NCs alone or in combination with DLT-NCs have demonstrated as highly efficient as ROS scavengers in HEI-OC1 cells and could be beneficial to protect CIO. BUC-NCs showed efficient

antioxidant activity confirmed by ABTS+ radical scavenging assay. Both BUC- and DLT-NCs were found to be highly biocompatible and their cytoprotective effect after CisPt exposure on HEI-OC-1 by cell and nucleic acid stained live-dead cell image analysis. The fluorescence microscopy confirmed the inhibition of caspase 3/7 activation in the CisPt-exposed HEI-OC1 cells after NC treatment. The NC treatment regimen shields the HEI-OC1 cells from cisplatin-induced toxicity via PI3 AKT/Stat/caspase molecular pathways.
Conclusions: The rationally designed and pharmacologically targeted novel NC was successfully optimized, evaluated, and developed for the treatment of the CIO and associated hearing loss. The explicit knowledge gained from engineered dual-drug combination bio-responsive technology protecting hair cells against cisplatin will offer new avenues for other inner ear diseases such as Age-related and Noise-induced hearing loss etc.

S80. Perilymph and Plasma Pharmacokinetics of Ebselen (SPI-1005) After Oral Dosing in Guinea Pig

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Category: Inner Ear: Drug Delivery
Background: Ebselen (SPI-1005) is a novel anti-inflammatory that mimics and induces glutathione peroxidase-1 activity, which is a critical cytoprotective enzyme expressed in multiple cochlear cell types including the organ of Corti, stria vascularis, and spiral ganglia neurons. In multiple preclinical and clinical studies, ebselen treatment has been shown to protect or improve hearing and tinnitus following noise, ototoxin and/or Meniere's disease. While ebselen's pharmacokinetic (PK) profile in plasma has been studied in mice, rats, minipigs, monkey and man, the PK profile in the inner ear has not been determined. This study reports the initial PK profiling of orally delivered ebselen in guinea pig plasma and cochlear perilymph after multiple ascending doses that are known to be otoprotective and/or therapeutic in multiple models of hearing loss and tinnitus.
Methods: Adult guinea pigs (46 males and females) were purchased and housed at SPI. Animals were gavaged with either vehicle or ebselen (10, 30, or 100 mg/kg) using a stock solution of 120 mg/ml of ebselen/0.5% carboxymethyl cellulose dosed on a twice daily schedule (AM/PM). Plasma and perilymph samples were collected after the last AM dose (assuming steady-state within 5-6 half-lifes of ebselen). Blood sampling (50-100 µL) occurred at 1 and 6 hrs after the 3rd oral dose via a percutaneous venous puncture of the hindlimb. Cochlear sampling (approximately 10 µL of clear perilymph) occurred at 5 and 6 hrs after the 3rd oral dose via round window membrane. All plasma and perilymph samples were analyzed using a validated HPLC-MS method for the determination of ebselen (limit of quantitation, 2 ng/ml).
Results: At 1-hour post-dose, the plasma dose-response of ebselen was 5.58, 9.31, and 26.09 ng/mL following 10, 30, and 100 mg/kg oral dose, respectively. At 6 hours, the plasma concentrations decreased to 1.96, 2.61, and 13.91 ng/ml, respectively. At 5 hours, the perilymph dose-response of ebselen was 7.28, 26.02, and 41.46 ng/mL, respectively. At 6 hours, the perilymph concentrations decreased to 3.29, 21.16, and 27.18 ng/ml, respectively.
Conclusions: The Cmax of ebselen in plasma occurs ≤ 1 hr post-oral dose. Plasma levels of ebselen exhibited a dose response following a 3rd oral dose. The Cmax of ebselen in perilymph occurs ≤ 5 hours after a 3rd oral dose. The ebselen concentration achieved in the cochlear perilymph at 5 hrs exceeded the ebselen concentrations achieved at 1 hr in plasma following all test doses (10-100 mg/kg). These data further support the idea that ebselen is rapidly absorbed and distributed throughout the plasma and cochlea in significant therapeutic concentrations.

S81. Assessing Factors Influencing the Efficiency of Drug Delivery via the Semicircular Canal

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Category: Inner Ear: Drug Delivery
Background: Drug delivery to the inner ear is a growing area of interest for researchers as it provides access to new experimental approaches for evaluating function, and lays a foundation for future therapeutic

interventions. The inner ear's closed system, characterized by a fluid-filled bone-encased structure, poses a significant barrier to the efficient delivery of drugs to its intended target. The semicircular canal has been proven to be a successful delivery route to the cochlea, but there is still some debate regarding the optimal factors for this approach. Here we review our success to date in developing a parameter set that optimizes success, limits variability, and maximizes ease of use.
Methods: Either pharmacological agents or trypan blue was injected via posterior semicircular canal in 4–6-week-old C57BL/6 mice. We characterized the consistency of delivery to perilymph or endolymph compartments using a pharmacological approach with drugs targeting the perilymph (CNQX to block glutamate receptors, TTX to block sodium channels) or endolymph (curare to block mechanotransduction channels). We calibrated the syringe pump apparatus at various rates and volumes. We also assessed the efficacy of delivery using trypan blue. The hearing function was measured using auditory brainstem responses (ABRs) following insertion and removal of the injecting tube. We also monitored the time to recovery and tested whether the extent of ABR shift was due to the time between making the hole and inserting the tubing for injection.
Results: Drugs were selectively delivered to the perilymph space with curare having negligible effects on ABR responses while CNQX and TTX both reduced ABRs in a tonotopic manner. The pump used for delivery was consistent across rates and volumes but sensitive to fluid viscosity and the presence of any air bubbles in the tubing. We compare data obtained using manual insertion of the tube to that of a robotic insertion and compare recovery of the ABR over time with and without injection. Preliminary data suggests that removal of the tube results in more significant and permanent ABR shifts. Preliminary data also suggests that robotic insertion of the tubing reduced response variability. Importantly successful injections were much more consistent as damage to the semicircular canal was virtually eliminated.
Conclusions: Our data suggest that semicircular canal injection is a safe and reproducible method to deliver drugs to the perilymphatic space of the cochlea. Removal of the injection tube induced a significant hearing loss that did not recover. The robotic insertion yielded greater consistency, not only in the time required for tubing insertion but also in the time needed for recovery, indicating an improved method for inner ear delivery. Also the overall success rate of injections was increased. And finally, as a tool, robotics makes the method more easily accessible to new users.

S82. Transcriptional Analysis of Early Cochlear Development and Proliferation at the Single Cell Level

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[1]*NIDCD*

Category: Development: Cellular/Systems
Background: Cell proliferation is a crucial process during developmental tissue formation. For instance, the emergence and elongation of the cochlear duct, which initiates around embryonic day (E)11 in mice, is driven, at least in part, by cellular proliferation. However, there is a limited understanding of the spatial patterning of cell proliferation during early cochlear development and of how those patterns might contribute to subsequent cochlear morphogenesis and cellular differentiation.
Methods: The cochlear duct floor was dissected from E12, E13, and E14 embryos, yielding isolated cells. These single cells were captured with the 10X Genomics Chromium Controller, lysed, and converted into cDNA with barcoding for individual mRNA molecules. Data processing was conducted through Seurat, and results were visualized using UMAP and other dimensional reduction techniques, alongside unsupervised clustering analysis. Tricycle (R package v3.17) was employed to determine cell cycle positions in cells from each embryonic stage. For single-cell analysis validation, proliferating cells were in vivo identified during each embryonic stage using 5-ethynyl-2-deoxyuridine (EdU). Samples, collected one hour post-EdU administration, were examined for EdU-positive cell localization using a combination of the Click-iT kit and immunostaining.
Results: At E12, Tricycle analysis indicated that approximately one-third of the cells within the floor of the duct were actively in the cell cycle. These proliferating cells were localized to two populations, discerned by the expression of the markers Ebf1 or Sall3. EdU analysis in vivo revealed pronounced areas of cell proliferation. Specifically, heightened proliferative activity was observed in the inner curve of the cochlear spiral in the medial non-sensory region and in the sensory and lateral regions of the base of the cochlea, corresponding to Ebf1- and Sall3-positive populations, respectively.

At E13, Tricycle analysis showed that a noticeable shift in the distribution of proliferating cells within the cochlear floor compared to E12. There was a marked increase in medial proliferating cells and a corresponding decrease in basal proliferating cells. EdU analysis at E13 demonstrated an enlarged cochlear medial non-sensory region compared to E12, with EdU-positive proliferating cells spanning from the basal to apical turns. In contrast, EdU-positive cells in the base of cochlea were significantly reduced.

Analysis at E14, using Tricycle, revealed that proliferating cells were predominantly localized within the medial non-sensory region. EdU analysis at E14 indicated a substantial decrease in EdU-positive cells within the medial non-sensory region of the basal turn, while numerous EdU-positive cells were still evident in the apical turn.

Conclusions: In the early stages of cochlear development, a substantial number of cells in the cochlear floor exhibit mitotic activity. The localization of these proliferating cells is dynamic and evolves over time. These findings suggest that proliferating cells during the early stages of cochlear development may exert a crucial influence on cochlear morphogenesis.

S83. Cochlear Deletion of Prox1 Results in Outer Hair Cell Loss and Profound Deafness in Adult Mice

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Category: Development: Cellular/Systems

Background: The Prospero homeobox 1 (Prox1) transcription factor plays a fundamental role in murine development showing broad expression and effects in many tissues. In the inner ear, Prox1 is transiently expressed in the sensory epithelia of the otolithic and ampullary organs of the vestibular system as well as in the organ of Corti (oC) of the cochlea. Prox1 expression is restricted to prosensory cells located in the abneural (lateral) compartment of the oC, with subsequent down regulation in developing outer hair cells (OHC) by Embryonic day (E) 16. In lateral support cells, Prox1 expression declines after birth and is largely undetectable by postnatal day (P) 10. The temporal and topographically restricted expression of Prox1 suggests that it regulates different aspects of development in OHCs or support cells. Previous studies on the role of Prox1 were conducted using a model that resulted in early postnatal lethality which prevented a complete and thorough assessment of the role of Prox1 in the cochlea. In this study we addressed two questions. One, how the does the deletion of Prox1 during development affect auditory physiology and anatomy? Two, how is the transcriptional profile of oC cells changed in the absence of Prox1?

Methods: To selectively delete Prox1 in the inner ear, we used Cre-recombinase driven by Fibroblast growth factor 20 (Fgf 20-Cre) which is expressed in the oC sensory epithelium as early as E11.5. In addition, the Prox1 Lox-P allele contained an enhanced green fluorescent protein (eGFP) leading to induced expression of eGFP in Prox1 deleted cells. We used standard immunohistochemical protocols at different ages to evaluate cochlear morphology and auditory brainstem response (ABR) and distortion product otoacoustic emissions (DPOAE) to assess hearing function. To examine changes in transcription, we performed single-cell RNA sequencing (SCRNA-Seq) on control and Prox1-deleted cochleae.

Results: We confirmed the targeted deletion of Prox1 based on an absence of PROX1 immunopositive nuclei and the expression of eGFP in the same cell types. As previously reported, deletion of Prox1 results in misaligned OHCs at birth. However, after the onset of hearing OHCs start to die and are completely absent by P35. Sox2 immunopositive support cells remain present, however the tunnel of Corti and reticular lamina seem malformed. Prox1 mutants lack DPOAE recordings and have ABR thresholds consistent with profound deafness and loss of OHCs.

Conclusions: These results provide evidence of a critical role of Prox1 in normal cochlear development. Our results suggest that Prox1 may act as a survival factor for OHCs while also regulating support cell development. Our SCRNA-Seq analyses will reveal the transcriptional targets of Prox1 in both hair cells and support cells and should provide insights regarding the pathways that mediate the effects of Prox1 in distinct cochlear cell types.

S84. Using Cochlear Explants as a Tool to Examine the Role of Tgfβr1 in the Developing Cochlea

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Category: Development: Cellular/Systems

Background: The organ of Corti within the mammalian cochlea contains two types of sensory cells, inner hair cells (IHCs) and outer hair cells (OHCs), which are aligned in ordered rows that run the length of the cochlear spiral. As the sensory cells for detection of mechanical stimuli, hair cells are essential for auditory perception. The factors that regulate hair cell development are still poorly understood however, previous work from our laboratory and others have suggested a role for the Transforming Growth Factor b (Tgfb) signaling during cochlear development and, more specifically, in hair cell specification. In particular, our laboratory has identified Tgfβr1 as being expressed in the cochlea as early as embryonic (E) day 12 in mice. In other systems, binding by Tgfβ2 facilitates the formation and activation of Tgfbr1/Tgfbr2 heterodimers, which lead to intracellular signaling.

Methods: Cochleae were dissected from mouse (CD1) embryos at E13.5 or E14.5 and established as explants. Explants were grown in either 10% fetal bovine serum (FBS) or 1% FBS for 6 (E13.5) or 5 (E14.5) days in vitro (DIV). All explants were fixed at the equivalent of E19.5 by immersion in 4% paraformaldehyde. To inhibit activation of the TGFb pathway, explants were treated with a neutralizing anti-TGFb-1,2,3 Monoclonal Antibody (1D11) (ThermoFisher, MA5-23795) or a Tgfbr1 antagonist, Galunisertib (Selleckchem, S2230). To activate the pathway, recombinant mouse TGF-b2 protein (R and D Systems, 7346-B2-005), which activates Tgfβr1, was added to explants. The antibody, antagonist, or recombinant proteins were added to explant culture media for 6 or 5 DIV, depending on the age of dissection. To analyze the effects of each treatment, immunohistochemistry was used to double-label the cytoplasm and nucleus of HCs with Myo7a and Pouf4f3, respectively. Lateral SCs were also labeled with anti-Prox1, and filamentous actin was visualized with fluorescent Phalloidin. After image analysis, data analysis was conducted using ImageJ and Prism.

To determine the overall level of pathway inhibition or activation, western blotting was carried using an anti-Phosphorylated SMAD2/SMAD3 antibody (Cell Signaling Technology, D27F4), an intracellular effector of Tgfβr1, and anti-Phosphorylated Tgfβr1 Antibody (ThermoFisher Scientific, PA5-40298) to detect activation of Tgfβr1.

Results: Hair cell development was comparable in explants maintained in 1% or 10% FBS. Therefore, all inhibition experiments were carried out in 1% FBS to decrease potential non-specific interactions between FBS components and the described agonists and antagonists. Preliminary analysis of explants treated with Galunisertib suggest a decrease in OHCs and IHCs in response to inhibition of Tgfβr1.

Conclusions: Activation of Tgfβr1 in vitro is required for development of the normal complement of hair cells, a key step in the development of cochlear morphology. Using cochlear explants, it will be possible to determine whether a temporal window exists for the role of Tgfβ in hair cell development.

S85. Proper Time of Atoh1 Expression for Hair Cell Subtypes and Function

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Category: Development: Cellular/Systems

Background: Atoh1 overexpression is essential for hair cell (HC) regeneration in the sensory epithelium of mammalian auditory and vestibular organs. However, Atoh1 overexpression alone cannot induce fully mature and functional HCs in the mammalian inner ear. In the current study, we investigated the effect of Atoh1 constitutive overexpression in native HCs by manipulating Atoh1 expression at different developmental stages.

Methods: To activate Atoh1 in vivo in HCs at different developmental stages, we bred CAG-loxP-stop-loxP-Atoh1-HA mice with mice expressing Cre under the control of different promoters. Tamoxifen diluted in corn oil was injected intraperitoneally once at P2 for Atoh1CreER mice and at P7 for PrestinCreER mice at 0.2 mg/g body weight. The tail-hanging reflex and swimming tests were evaluated to observe the balance function; The HAVOR and OVAR test were performed to evaluate the function of the semicircular canals and macular organs.

Results: Constitutive overexpression of Atoh1 in native vestibular HCs did not affect cell survival but did impair vestibular function by interfering with the subtype differentiation of HCs and hair bundle development. Meanwhile, Atoh1 overexpression disrupted the development of calyx nerve terminals in type I HCs. In contrast, Atoh1 overexpression in cochlear HCs impeded their maturation, eventually leading to gradual HC loss in the cochlea and hearing dysfunction.
Conclusions: Time-restricted Atoh1 expression is essential for the differentiation and survival of HCs in the inner ear, and this is pivotal for both hearing and vestibular function re-establishment through Atoh1 overexpression-induced HC regeneration strategies.

S86. The Role of Flippase ATP8A2 in Developing Type II Spiral Ganglion Neurons

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Category: Development: Cellular/Systems
Background: Flippases are proteins that maintain asymmetry of the cell membrane by "flipping" phospholipids from the extracellular leaflet of the membrane to the inner leaflet. This translocation of phospholipids drives cell membrane asymmetry and is especially critical for relegating phosphatidylserine (PS), which serves as an "eat me" signal in the context of phagocytosis and cell death, to the inner leaflet of the membrane. The flippase ATP8A2 flips PS and is known to be important for the survival of spiral ganglion neurons (SGNs) in adult mice, but its role in cochlear development has not previously been described. The development of a functional circuit between SGNs and sensory hair cells (HCs) is essential for hearing. SGNs undergo a stereotyped development during which an excessive number of neurons and synapses are initially produced that must be removed prior to maturation of the circuit. We hypothesize that this reduction in SGNs and synapses occurs via a presently unknown phagocytic mechanism. Our lab has found that the postnatal expression of ATP8A2 in HCs and SGNs undergoes significant changes that are consistent with hair cell synapse refinement. We hypothesize that expression of ATP8A2 is driven by SGN activity and that ATP8A2 functions during SGN maturation to mark the neurons and synapses that will survive into the mature circuit by preventing externalization of phosphatidylserine.
Methods: We created an ATP8A2-HA knock-in mouse to visualize localization of ATP8A2 throughout postnatal cochlear development. We also generated a hypomorphic mouse in which 33 amino acids have been deleted from the C-terminal of ATP8A2 (A2Δ33), and a complete ATP8A2 knock out (KO) mouse. Confocal microscopy was used to assess changes in the amount of pre-synaptic marker CtBP2 as a measure of synaptic pruning. We generated a mechanotransduction (MET) negative mouse (Cib2-/-) and crossed this mouse to our ATP8A2-HA line to investigate whether ATP8A2 expression is dependent on MET activity. Finally, we performed auditory brainstorm response threshold and distortion product otoacoustic emissions tests.
Results: At P6, there is broad expression of ATP8A2 throughout IHCs, OHCs, and SGN soma and processes, but by P12, ATP8A2 is largely restricted to type II SGNs and the en passant synapses of OHCs. There are fewer ribbon synapses in OHCs of A2Δ33 mice. ATP8A2 localization is altered in MET negative mice. We also find elevated ABR thresholds in A2Δ33 and KO mice.
Conclusions: The postnatal maturation of ATP8A2 in the cochlea coincides with the period of synaptic and neuronal refinement, which suggests that ATP8A2 is actively involved in this process. Our working model is that ATP8A2 expression is activity dependent and ATP8A2 expression is protective to neurons by preventing phosphatidylserine presentation to phagocytic machinery. Future work will focus on elucidating the mechanisms of phagocytosis during cochlear circuitry refinement.

S87. In Vitro Organotypic Explants and Single Cell RNA Sequencing Provide a Novel Comparison Between the Neonatal and Adult Stria Vascularis

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Category: Development: Cellular/Systems

Background: Developing biological solutions for inner ear degeneration is crucial to ensure 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 is responsible for generating the endocochlear potential, which is required for hearing, and for maintaining the blood-labyrinth barrier to prevent pathogenic infiltration into the cochlea. SV degeneration can occur due to aging, ototoxic drugs, and genetic diseases, which disrupt cochlear homeostasis and result in progressive and irreversible hearing loss. Yet, there are no treatments for SV-associated hearing loss and there is little research investigating SV regeneration. To advance the development of regenerative therapies for SV-related hearing loss, we generated a new in vitro platform to study the SV in neonatal and adult mice. In addition, we performed single cell RNA sequencing to elucidate the molecular underpinnings that differentiate the neonatal and adult SV.

Methods: We developed an organotypic explant technique to isolate and culture whole SV and associated vasculature from neonatal and adult mice. We used n = 10 male and female CD1 mice from at least three independent litters at postnatal day (P) 0-1 for our neonatal stage, and P30-35 for our adult stage. We carefully dissected the SV from the lateral wall of the cochlea and cultured the explants on Matrigel coated plates for 72 hours in the presence of BrdU, a proliferation marker, at 3.5 μg/mL. We then investigated the role of the Wnt/β-catenin signaling pathway in SV proliferation by pharmacologically inhibiting Wnt/β-catenin signalling using the TCF/LEF inhibitor, FH535. We administered FH535 at 1, 2.5, 5, and 10μM concentrations. Furthermore, we performed single cell RNA sequencing using the 10x genomics protocol. SV from four P1 and four P30 CBA/J mice were used. We conducted downstream analysis using Seurat, a single cell analysis package in R.

Results: Our in vitro results demonstrate that the neonatal SV is highly proliferative while the adult SV is not, consistent with results reported in vivo, providing a representative in vitro model comparing the proliferative vs. non-proliferative SV. We also observed that inhibiting Wnt/β-catenin signaling in neonatal explants results in a significant decrease in proliferation indicating that Wnt/β-catenin signaling plays a role in SV proliferation. Single cell RNA sequencing further revealed key genes, transcription factors, and pathways unique to P1 and P30 SV that may play a role in SV proliferation, development, and maintenance.

Conclusions: Together, our novel experimental platform to culture the whole SV and our single cell RNA sequencing data produce a robust comparison of the neonatal and adult SV. This provides new insights which we can further investigate to produce biological solutions for SV-associated hearing loss.

S88. Molecular Diversification of Zebrafish Inner Ear Hair Cells and Afferent Neurons

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Category: Development: Cellular/Systems

Background: Hair cells of the inner ear sense head acceleration through space and tilt with respect to gravity as well as sound. Zebrafish have several sensory epithelia innervated by afferent neurons in the statoacoustic ganglion (SAG) that convey vestibular and auditory information to the brain stem. In the zebrafish model, little is known about genetic differences underlying hair cell innervation, branching morphology, firing properties, neuronal excitability, and central targeting. We seek to identify and profile subpopulations of afferents that innervate different sensory epithelia, distinct epithelial zones, or disparate patterns of hair cells. We hypothesize that physiologically relevant subpopulations of zebrafish hair cells and primary afferents will have distinct genetic profiles that inform physiology and function.

Methods: We analyzed single-cell RNA sequencing data of hair cells and cranial nerves from zebrafish embryos collected at various time points between 18 hours post-fertilization and 5 days post-fertilization. Statoacoustic ganglion neurons were identified using known otic markers and then further subclustered. We then used fluorescent in situ hybridization chain reaction labeling to validate candidate genes that target specific hair cell and neuron subpopulations.

Results: Analyses using unsupervised clustering have indicated at least four different clusters of neurons in the SAG with differential expression of voltage-gated ion channels, neurotransmitters, adhesion, cytoskeletal molecules, and transcription factors, with cellular representation from all stages. Using genes identified from cluster analysis, we labeled SAG neurons in transgenic zebrafish larvae to identify the spatial organization of neuronal subpopulations and their axonal projections to hair cells. We also labeled subpopulations of hair cells in the macular epithelia to determine the development of hair cell spatial organization in tandem with innervation.
Conclusions: Using single-cell RNA sequencing, we identified perinatal diversification of SAG neurons, before the onset of vestibular and auditory behaviors, as well as distinctive expression patterns in hair cells and afferents that might predict functional diversification. We aim to identify markers for genetic models of vestibular and auditory afferent physiology and function.

S89. Casz1 is Necessary for Cochlear Inner Hair Cell Development by Regulating Gata3 Expression

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Category: Development: Cellular/Systems
Background: The perception of sound relies on two specialized types of mechanosensory hair cells (HCs) within the cochlea, known as inner hair cells (IHCs) and outer hair cells (OHCs). IHCs and OHCs originate from common progenitor cells. While the epistatic transcriptional cascade involving Insm1 and Ikzf2 is known to mediate OHC development, the molecular and genetic mechanisms underlying IHC development remain poorly understood.
Methods: Because germline Casz1-/- mice exhibit embryonic lethality attributed to heart defects, we employed HC-specific conditional Casz1 knockout model, Atoh1Cre/+; Casz1flox/flox that are subjected to immunostaining, auditory brainstem responses (ABR) and whole cell patch-clamp recording, and single cell transcriptomic analysis.
Results: Upon deletion of Casz1 in embryonic HC progenitors, the Casz1-/- IHCs undergo transdifferentiation into OHC-like cells, characterized by the downregulation of the IHC marker vGlut3 and the upregulation of the OHC marker Prestin. Atoh1Cre/+; Casz1flox/flox mice exhibit severe hearing impairment compared to control mice. Similar hearing defects are observed when Casz1 is deleted in cochleae using other Cre drivers (based on personal communication with Dr. Botond Banfi at the University of Iowa). Furthermore, single-cell transcriptomic analysis reveals that Gata3 expression is reduced in Casz1-/- IHCs compared to Casz1+/+ IHCs. Importantly, the forced expression of Gata3 alleviates the dysfunction of Casz1-/- IHCs, leading to a significant improvement in hearing ability.
Conclusions: In conclusion, our analyses uncover essential roles of Casz1 in the determination of IHCs fate through direct regulate its downstream targets Gata3. We have uncovered the importance of the transcriptional cascade from Casz1 to Gata3 in IHC development, opening new avenues for future investigations into IHC regeneration.

S90. Chd7-Sox2 Gene Regulatory Network in Development of the Inner Ear

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Category: Other, inner ear development
Background: Hereditary hearing loss and balance disorders remain 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 to understand the underlying mechanisms. CHD7, an ATP-dependent chromodomain helicase DNA-binding protein, and SOX2, an SRY-related HMG box pioneer transcription factor, cooperate to regulate semicircular canal and cochlear

development. Combined haploinsufficiency of Chd7 and Sox2 results in reduced otic cell proliferation, severe malformations of semicircular canals, and shortened cochlea with ectopic hair cells. Although the morphological changes are evident, the gene regulatory network of Chd7 and Sox2 remains elusive.
Methods: Chd7 germline mutant mice (Chd7Gt/+) and Sox2 inducible Cre knockin mice (Sox2CreER/+) were used to explore genetic interactions of Chd7 and Sox2 and a potential gene regulatory network in the developing inner ear. Microdissected E10.5 otocysts from mice of four different genotypes (Wild type, Chd7Gt/+, Sox2CreER/+, and Chd7Gt/+;Sox2CreER/+) were processed for bulk RNA sequencing. Wild type otocysts dissected from E10.5 embryos were processed for Cleavage Under Targets and Tagmentation (CUT and Tag). Regions that displayed statistically significant enrichment of CHD7 and SOX2 were identified using Sparse Enrichment Analysis for CUT and RUN (SEACR).
Results: Differential gene expression analysis of bulk RNA sequencing revealed 75 down-regulated genes and 55 up-regulated genes in E10.5 Chd7Gt/+;Sox2CreER/+ developing otocysts compared to wild type. Among these differentially expressed genes (DEGs), we found genes involved in either semicircular canal development (Aldha3 and Ffg10), cochlear development (Gjb2, Sall1, Etv5), or both (Pax2, Fgfr2, Fgf3). CUT and Tag showed occupancy by CHD7 and SOX2 (CHD7+SOX2+), CHD7 only (CHD7+) or SOX2 only (SOX2+). We identified CHD7 and SOX2 enrichment near enhancers and promotors of DEGs, some of which (Otx2, Sox2, Dusp6, Pax2, Fgfr2 and Sall1) are expressed in E10.5 otocysts and are implicated in human hearing loss. Immunofluorescence labeling of PAX2 and OTX2 confirmed the dysregulated expression in Chd7Gt/+;Sox2CreER/+ otocysts compared to wild type.
Conclusions: Data from genome-wide RNA-sequencing and CUT and Tag studies in the otocyst show that Chd7 regulates Sox2 expression and acts early in a gene regulatory network that controls expression of critical otic patterning genes, including Pax2 and Otx2. CHD7 and SOX2 directly bind independently and cooperatively at transcription start sites and enhancers to regulate otic cell gene expression. These results inform the design of novel targets for molecular therapies to treat hearing and balance disorders.

S91. SOX11 and CHD7 Act in the Same Gene Regulatory Network to Promote Inner Ear Development

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Category: Vestibular: Basic Research and Clinical
Background: Inner ear development relies on precise spatiotemporal control of gene expression. Studies have shown that two SRY-related high-mobility group box (SOX) transcription factors, Sox4 and Sox11, are involved in inner ear morphogenesis. In the brain, SOX11 and SOX4 are essential neurogenic fate determinants and direct targets of CHD7, which encodes the ATP-dependent chromodomain-helicase-DNA-binding protein 7 chromatin remodeler. Haploinsufficiency of CHD7 in humans has been causally linked to CHARGE syndrome, in which 90% of individuals present with hearing loss and balance impairment due to inner ear dysplasia. Pathogenic variants in SOX11 are associated with Coffin-Siris syndrome and inner ear malformations. The specific mechanisms by which loss of CHD7 or SOX11 influence ear development are unknown, but likely involve changes in downstream gene expression due to transcriptional regulation and nucleosome remodeling. In this study, we used mouse models to determine whether Chd7, Sox4, and Sox11 function together in a common genetic regulatory network during inner ear development.
Methods: Embryonic inner ears with germline or conditional (Pax2Cre) deletion of Sox4, Sox11, and/or Chd7 were examined for morphological abnormalities using paint-filling and histological analysis. Molecular markers normally expressed during early inner ear development (E10.5-12.5) were assessed via qRT-PCR, immunofluorescence and in situ hybridization. Proliferation and apoptosis were evaluated using BrdU incorporation and anti-Caspase3 assay, respectively.
Results: Severe abnormalities in the semicircular canals and endolymphatic duct were observed with loss of Sox11 or Chd7. In contrast, Pax2Cre;Sox4flox/flox inner ears exhibited mild anomalies with enlarged common crus and endolymphatic duct. Sox11-/- mutants exhibited delayed formation of the canal fusion plate, normal basement membrane laminin, decreased cell proliferation in the surrounding mesenchyme, and no

aberrant cell death. Chd7 expression was unchanged in Sox11-/- mutant otocysts at E10.5 while expression of Sox11 was reduced in the Chd7Gt/Gt otocyst. Bmp4 expression was ectopically expressed along the E11.5 anterior to lateral Sox11-/- mutant otocyst compared to wild type.
Conclusions: These conditional and germline mutant analyses show that development of vestibular structures in the inner ear (semicircular canals and endolymphatic duct) depends on proper gene dosage of Sox4, Sox11, and Chd7. These results also suggest a common gene regulatory pathway including Chd7, Sox11, and Bmp4 that regulates semicircular canal formation, providing a basis for designing therapeutic strategies to treat vestibular dysfunction.

S92. The Function of CaMKIV in Spiral Ganglion Neuron Development

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Category: Development: Cellular/Systems
Background: Spiral ganglion neurons (SGNs) are the primary auditory neurons that form synaptic connections with hair cells in the cochlea and transmit auditory information to the brainstem. SGN-hair cell contacts are critical for hearing and are lost in various forms of hearing loss. Ca2+/calmodulin-dependent protein kinase IV (CaMKIV) has been shown to be crucial for aspects of neuron function such as gene expression and neurotransmitter release, and is a target gene for autism spectrum disorders in humans. CaMKIV has also been shown to be important for SGN survival and neuron branch dynamics in the cochlea. Our previous studies (Wang et al., 2020 eNeuro) have shown that P2RX3, an ionotropic purinergic receptor, is expressed by SGNs around the period of the onset of spontaneous firing events. We also found that SGNs lacking P2rx3 showed elevated SGN activity at E16.5. In this present study, we hypothesized that CaMKIV activity may act downstream of P2RX3 in response to SGN activity, and that CaMKIV is an important regulator of SGN differentiation.
Methods: We performed immunohistochemistry on P2rx3 knockout mice to study the levels of CaMKIV protein expression across different developmental time points. We also used sparse labeling techniques to visualize neuronal fibers to identify any developmental defects in nerve branching that could result from a loss of P2RX3 receptors in the neurons. Calcium imaging studies using Snap25-GCaMP6s were also done in these samples to study changes in neuronal activity during development.
Results: Our preliminary data showed there was an increase in CaMKIV protein levels in the nuclei of the SGNs from mice lacking P2RX3 (P2rx3 knockout mice). The increased CaMKIV levels coincided with increased pCREB (phosphorylated CREB) activation, SGN branch arborization, and a brief increase in spontaneous activity.
Conclusions: Loss of P2RX3 in mice leads to increased activity in spiral ganglion neurons during development along with developmental defects like improper branch refinement. This has provided us with a reliable model to look at the factors affecting spontaneous activity and SGN differentiation during cochlear development. Furthermore, we will study the function(s) of CaMKIV in SGNs during development.

S93. Inhibition of Retinoic Acid Signaling Increases Striolar Fate Specification in Inner Ear Organoids

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Category: Development: Cellular/Systems
Background: Retinoic acid (RA) regulates inner ear development at several key stages. Early in development, RA governs anterior-posterior patterning and possibly sensory-nonsensory domain formation in otocysts. Later, RA is involved in regional patterning within inner ear organs, where for example high RA is thought to influence extrastriolar fate in otolith organs and low RA a striolar fate. Little is known about the impact of RA signaling in cultures of inner ear organoids. In this study, we sought to examine the effects of late-stage RA manipulation on striolar-extrastriolar fate specification in organoids.
Methods: Mouse embryonic stem cells (R1E) were used to generate inner ear organoids using standard protocols that produce otocysts around in vitro day 10 (D10) and organoids by D18. In addition, a reporter

line carrying a lacZ transgene under the control of RA response elements (RARE-lacZ) and another line carrying an Atoh1-nEGFP transgene were used. To produce RA deficiency and excess in the cultures, we added either the pan-RAR-receptor antagonist AGN193109 (100 nM) or excess all-trans RA (atRA; 500 nM) on D16 onward. RA signaling was monitored with X-gal staining of organoids from RARE-lacZ cells. Gene expression for striolar-extrastriolar regional markers was assessed using qPCR and immunofluorescence. The efficiency of organoid production was assessed as the percentage of aggregates in each independent culture with one or more protruding fluid-filled cysts.
Results: LacZ expression was observed in otic and non-otic structures within the cultured aggregates throughout D16 to D20, suggesting some endogenous RA activity under control conditions. The addition of AGN significantly decreased while atRA increased organoid production, respectively. More sensory hair cells were produced by inhibiting RA signaling yet the size of the organoid cyst was greater with the addition of atRA. The addition of AGN also significantly increased the expression of striolar markers Ocm and Tectb in whole aggregates at D20, whereas excess RA decreased the expression of these markers. Notably, Modulation of RA signaling under these conditions had no significant impact on the expression of the extrastriolar marker Spp1 or the Type II hair cell markers Anxa4 and Nhlh1.
Conclusions: In a prior study, severe deficiency or excess RA during otocyst formation led to a near complete loss of organoid formation, but here, late-stage manipulations had less dramatic effect. Inhibition of RA signaling with AGN increased the expression of striolar markers, similar to in vivo data associating low-RA conditions with the striola. The observation of lacZ expression under control conditions suggests endogenous RA activity, which may explain a default extrastriolar fate. Our data suggests that application of AGN during late-stage organoid formation may tip the balance toward generation of striolar hair cell types, providing an in vitro platform for examining the RA-responsive processes driving cell fate specification.

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S95. Thyroid Hormone Accelerates Hair Cell Maturation in Human Cochlear Organoids

Tsubasa Saeki[1], Stephen Moore[1], Isabel Aristizabal-Ramirez[2], Gregory Frolenkov[2], Eri Hashino[1], Tsubasa Saeki*[1]

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Category: Development: Cellular/Systems
Background: We recently established a novel protocol to generate cochlear organoids from aggregates of human pluripotent stem cells (Moore et al., Cell Stem Cell 2023). However, the majority of hair cells in these organoids appeared to be immature at d102 with only 0.3% of hair cells expressing Prestin. Previous studies showed that thyroid hormone directly regulates Prestin expression in mouse outer hair cells, pointing to its important role in hair cell maturation. Here, we explored whether thyroid hormone thyroxine promotes Prestin expression or hair cell maturation in human cochlear organoids.
Methods: PAX2-nGFP/POU4F3-ntdTomato hESCs were differentiated into cochlear organoids based on our established protocol, and the cultures were grown in the presence or absence of thyroxine after detection of the POU4F3 reporter expression. To determine the minimum effective dose regimen, organoid cultures were treated with thyroxine starting at d70, 90, 100 or 105 for a treatment duration of 40, 20, 10 or 5 days, respectively. In another series of experiments, cultures were treated with thyroxine at the same starting time with varying durations. All cultures were harvested at d120 for immunofluorescence, and the percentages of marker-expressing cells per all hair cells were counted. To access the motor function of Prestin, the voltage-dependent (non-linear) capacitance of POU4F3+ cells was measured in whole-cell patch-clamp recordings.
Results: In cochlear organoids lacking thyroxine supplementation, most POU4F3+ hair cells co-expressed SOX2 or GATA3 and less than 1% of POU4F3+ cells were Prestin+. In striking contrast, more than 10% of POU4F3+ cells expressed Prestin in cochlear organoids treated with thyroxine for 10 days, while the percentage of hair cells expressing SOX2 and GATA3, immature cochlear hair cell markers, was significantly lower than that in control organoids. Interestingly, some thyroxine-treated hair cells exhibited a non-linear capacitance, similar to the one observed in developing mouse outer hair cells. Moreover, expression of SOX2, but not GATA3, was maintained in supporting cells of treated organoids, recapitulating cochlear maturation

in vivo and indicating that thyroxine does not directly regulate SOX2, but rather a hair cell specific program that facilitates the maturation process.
Conclusions: Simultaneous upregulation of Prestin and downregulation of immature cochlear hair cell markers in our experiments suggest that thyroxine is likely to accelerate overall maturation of cochlear hair cells, rather than just promotes Prestin expression. Investigation is currently underway to evaluate expression of other marker genes in thyroxine-treated and untreated hair cells using single-cell RNA-seq analysis.

S96. Exploring Tonotopic Gene Isoform Diversity in the Cochlea by Single-Cell Long-Read Sequencing

Sihan Li*[1], Natchanon Sittipongpittaya[1], Gloria Sheynkman[1], Jung-Bum Shin[1]

[1]*University of Virginia*

Category: Genetics A: Genomics and Gene Regulation
Background: Hair cells distributed along the tonotopic axis of the organ of Corti are responsible for discerning various frequencies. It is well established that frequency discrimination is largely determined by the basilar membrane (BM). However, additional cellular frequency tuning mechanisms remain to be explored. Notably, properties of hair cells vary along the tonotopic axis, including gradients in tip-link tension, stereocilia length, etc. We recently discovered that different isoforms of MYO7A are expressed in a tonotopic gradient and suggested their roles in tuning tip-link tension tonotopically. Generalizing our finding on MYO7A, we propose that differential expression of isoforms of many other genes is critical for frequency tuning in the cochlea. Therefore, our goal is to comprehensively characterize mRNA isoforms that exhibit tonotopic gradients, in both hair cells and structurally important supporting cells, and to elucidate their functional relevance.
Methods: We applied a single-cell long-read sequencing method known as MAS-ISO-seq (Al'Khafaji et al., 2021), which increases transcript yield by ~15 times compared to previous PacBio sequencing methods. Sequencing analysis was performed by using published packages: IsoSeq and IsoQuant for isoform classification/refinement; Seurat and Acorde for single-cell clustering and functional analysis. Unibind and hair-cell ATAC-seq data was applied for SIX2 binding site identification. The expression pattern of the SIX2 was characterized by Six2-cre tdTomato reporter mice.
Results: To explore the isoform diversity in the cochlea, we conducted MAS-ISO-seq of the organ of Corti at P7. Our sequencing results detected 3,835,237 reads, a ~16 fold increase compared to previously published cochlea long-read sequencing. Among these transcripts, we identified 38,697 novel isoforms. Notably, novel isoforms were identified for multiple hair-cell specific genes, such as Myo7a, Myo15a, whirlin, and harmonin. Our downstream analysis classified 16 cell clusters with distinct isoform expression patterns, allowing us to characterize isoforms that are differentially expressed. Furthermore, we are also actively identifying the upstream mechanism of tonotopic isoform regulation. Beyond alternative splicing, alternative transcriptional start sites are essential in isoform regulation. Through analysis of publicly available ChIP-seq and cochlear ATAC-seq datasets, transcription factor SIX2 is shown to interact with enhancers of multiple genes that have alternative transcriptional start sites, including Myo7a, Myo15a, and Xirp2. Additionally, SIX2 is expressed in a tonotopic pattern, increasing its expression towards the base of the cochlea. Overall, we will test the hypothesis that SIX2 is involved in regulating isoform expression along the tonotopic axis.
Conclusions: Our long-read sequencing approach was effective in sequencing RNA isoform transcripts at the single-cell level. In the future, we intend to conduct MAS-ISO-seq on basal, middle, and apical regions of cochlea for a detailed analysis of isoforms diversity along the tonotopic axis. We will also conduct SIX2 Cut and Run and long-read sequencing with SIX2 conditional KO mice to further investigate its role in isoform expression regulation.

S97. Validation of Intercellular Interactions Between Epithelial and Mesenchymal Cells in the Postnatal Mouse Utricle by Single Cell RNAseq and in Situ Hybridization

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Category: Development: Cellular/Systems

Background: The utricle, an inner ear vestibular sensory organ, relies on mechanosensory hair cells for detecting motion. In newly born mice, hair cells are made from non-sensory supporting cells and continue to be added for the first ~7 days as part of the normal postnatal developmental process. We hypothesize that there is communication between the mesenchymal and epithelial layers which could be the driving factor behind postnatal proliferation and homeostasis. Identification and validation of these interactive cell populations will provide more comprehensive detail of utricular developmental pathways.
Methods: We used published single cell RNAseq (scRNAseq) data of the utricular sensory epithelium (Jan et al., 2021) from P4 and P6 utricles. Non-sensory cells were collected at these same time points and single cell suspension was created. Following flow cytometry for cell isolation, the Smartseq2 protocol was used to generate scRNAseq data. We previously performed computational analyses using CellChat to determine what compartments of the inner ear communicate with each other. Here we validated our findings by in situ hybridization and immunohistochemistry.
Results: We were able to annotate 12 cell populations including: mesenchymal cells, type I and II hair cells, transitional epithelial cells, supporting cells, glia, roof cells, pericytes, Schwann cells, endothelial cells, macrophages, and melanocytes. CellChat analysis identified mesenchymal cells as the dominant signal sender with statistically significant pathways including WNT, pleiotrophin (PTN), and midkine (MK). To validate classified genes, we examined whole mount and fixed frozen wild type utricles. Our analysis revealed five highly interactive ligand-receptor pairs between mesenchymal and epithelial compartments. We designed probes against Lgal9/Ighm, Bdnf/Ntrk2, Itga8/Spp1, Tgf-beta2/ Tgf-betar, and Ptprz1/Ptn. Our on-going validation experiments demonstrate strong cross-talk between the epithelial and mesenchymal compartments.
Conclusions: CellChat analysis revealed communication pathways among 9 transcriptionally unique cell populations at single cell resolution. The dominant signal senders in the postnatal developing utricle are the mesenchymal cells. Our validation experiments support the computational analyses and open the door for further in vitro and in vivo cell-cell interaction studies in previously unexplored pathways.

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S99. Single-Cell RNA-Sequencing of Stria Vascularis Cell Types in the Slc26a4-/- Mouse

Jin-Young Koh[1], Corentin Affortit*[1], Ranum T. Paul[2], Cody West[1], William D. Walls[1], Hidekane Yoshimura[3], Jian Shao[4], Brian Mostaert[5], Hela Azaiez[1], Richard J.H. Smith[1]

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Category: Genetics A: Genomics and Gene Regulation
Background: The SLC26A4 gene encodes the anion exchanger pendrin. In the inner ear, pendrin is expressed in the epithelial cells and transports bicarbonate (HCO3−) into the endolymph to maintain endolymphatic pH homeostasis. Lack of pendrin in Slc26a4−/− mice results in hearing loss, vestibular dysfunction, and an enlarged vestibular aqueduct. One of the primary pathological alterations is acidification of the endolymphatic pH. However, the contribution of the stria vascularis cell types in maintaining endolymphatic pH remains poorly characterized. We aimed to identify pH regulators in pendrin-expressing cells that contribute to homeostasis of endolymph pH and to define the pathogenic mechanisms that contribute to the dysregulation of endolymph pH in Slc26a4−/− mice.
Methods: We conducted single-cell RNA sequencing of stria vascularis cells isolated from wild-type (WT) and Slc26a4−/− mice. Bioinformatic clustering analysis based on single-cell gene expression defined the different cell types within the stria vascularis. Gene Ontology (GO) enrichment analysis was performed on pendrin-expressing cells. Additionally, we investigated gene expression changes in Slc26a4−/− mice. Specific findings were confirmed at the protein level by immunofluorescence in WT and Slc26a4−/− adult mice.
Results: We found that spindle cells, which are pendrin-expressing cells, highly express extrinsic cellular components, enabling cell-to-cell communication. Additionally, we characterized the gene expression profile of the pH regulator in spindle cells. Compared to WT, the transcriptional profiles in Slc26a4−/− mice showed alteration in extracellular exosome-related genes in spindle cells. Immunofluorescence studies in spindle cells

of Slc26a4−/− mice validated the increased expression of the exosome-related protein, annexin A1 (ANXA1), and the Clathrin-mediated endocytosis-related protein, adaptor protein 2 (AP-2).
Conclusions: In this study, we identified regulators of endolymphatic pH homeostasis in spindle cells, and we show altered expression of ANXA1 and AP-2 in Slc26a4−/-.
This project was supported in part by NIH-NIDCD grants DC002842, DC012049, and DC017955 (RJHS).

S100. Hyperacusis-Behavioral Changes by Cx26 (GJB2) Heterozygous Mutation

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Category: Genetics B: General
Background: Connexin 26 (Cx26) recessive heterozygous mutation carriers occur up to ~10-20% of the general population. Our previous study found that Cx26 heterozygous deletion or mutations can induce hyperacusis (hearing oversensitivity) rather than hearing loss (Liu et al., Sci Adv. 9, eadf4144, 2023, PMID: 36753545). However, behavior evidence is still missing. In this study, the behavioral changes by Cx26 heterozygous mutation were examined.
Methods: Cx26 heterozygous deletion mice were used. Acoustic startle response (ASR) was recorded to assess animal behavioral changes. Hearing function tests were also examined by ABR and DPOAE recordings.
Results: Consistent with our previous electrophysiological recording of hearing function, we found that ASR in Cx26 heterozygous mice demonstrated an enhanced response. The ASR and enhancement were increased as the acoustic stimulation increased. At the high stimulus intensities, the ASR in Cx26 heterozygous mice was triple-time larger than that in wildtype (WT) mice. The ASR in Cx26 heterozygous mice also showed an increase as age increased. The ASRs had no significant difference between Cx26 heterozygous mice and WT mice at 2 months old. However, the ASR in Cx26 heterozygous deletion mice was significantly increased in comparison with that in WT mice at 3 months old and afterward. Moreover, oppositely, the ASR in WT mice demonstrated a slightly decreasing as age increased. Finally, there was no significant difference in the peak time of ASR between Cx26 heterozygous mice and WT mice.
Conclusions: These data demonstrated that Cx26 heterozygous mutations can produce hyperacusis changes in behavior, suggesting that persons carried with Cx26 heterozygous mutations could have clinical manifestations, leading to tinnitus, noise sensitivity, learning disabilities, and other psychological abnormalities.
Supported by NIH R01 DC019687 to HBZ

S101. Investigation of the Potential to Reverse Hearing Loss Caused by Whrn Mutations

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Category: Genetics B: General
Background: Whrn encodes the PDZ-containing protein whirlin which has previously been demonstrated to function in stereocilia length regulation as well as bundle organisation during development. In humans, mutations in the WHRN gene underlie a range of hearing disorders including non-syndromic profound deafness DFNB31 and Usher syndrome type 2D. Previously studied Whrn mouse mutants have revealed a variety of phenotypes. These range from profound deafness with vestibular defects (e.g. the whirler allele; Whrn less than wi greater than) to non-progressive profound hearing loss at high frequencies with moderate hearing loss at low frequencies and no balance defects (e.g. the tm1b allele; Whrn less than tm1b greater than). Here, we characterise a new tm1a allele of Whrn. The tm1a allele is a ‘knockout-first’ design with a LacZ-containing transcription disruption cassette between exons 3 and 4. Additionally, through a genetic approach enabling the activation of Whrn gene expression upon administration of tamoxifen, we have investigated whether the hearing impairment caused by the Whrn less than tm1a greater than allele is reversable. This will shed light on the potential for successful gene therapy for hearing loss caused by WHRN mutations.

Methods: Auditory-evoked brainstem response (ABR) recordings have been carried out at frequencies ranging from 3-42kHz on Whrn less than +/+ greater than , Whrn less than +/tm1a greater than and Whrn less than tm1a/tm1a greater than mice at P14, P28, P56, and P98. Whole-mounts of the organ of Corti from Whrn less than +/+ greater than , Whrn less than +/tm1a greater than and Whrn less than tm1a/tm1a greater than mice at P14 were immunostained for myosin-VIIa for hair cell visualisation and quantification. Finally, ABR recordings were also performed on Whrn less than +/tm1a greater than and Whrn less than tm1a/tm1a greater than mice at P28, P42, and P56, following activation o f Whrn gene expression (via the removal of the transcription disruption cassette by a tamoxifen-inducible Flpo recombinase enzyme) at P4 or P14 respectively.
Results: The Whrn less than tm1a/tm1a greater than mice exhibited a moderate to severe early-onset non-progressive hearing loss, indicated by significantly increased ABR thresholds across all frequencies tested compared to littermate controls from P14. These raised ABR thresholds were stable to at least P98. Both Whrn less than +/+ greater than and Whrn less than +/tm1a greater than mice had comparable ABRs, indicating Whrn less than tm1a greater than is a recessive allele. No overt balance defects have been observed in Whrn less than tm1a/tm1a greater than mice up to P98, suggesting no vestibular dysfunction. Despite the raised thresholds of Whrn less than tm1a/tm1a greater than mice, there was no significant hair cell degeneration at P14. No discernible improvement in ABR thresholds of Whrn less than tm1a/tm1a greater than mice have been observed up to at least P56 following Whrn activation at either P4 or P14.
Conclusions: Whrn less than tm1a greater than is a recessive allele which causes moderate to severe early-onset non-progressive hearing loss without balance defects. Raised ABR thresholds were detected from two weeks old, an age when all hair cells were present, indicating that degeneration of hair cells was not the cause of hearing impairment. Activating the Whrn gene as early as P4 did not improve ABR thresholds in homozygous mutants.

S102. The Molecular Principles Underlying Diverse Functions of SLC26 Proteins

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Category: Genetics B: General
Background: The mammalian solute carrier 26 (SLC26)/sulfate permease (SulP) family consists of homodimeric membrane proteins with diverse physiological roles. Dysfunctions of SLC26 proteins are associated with various human diseases, including syndromic and non-syndromic deafness for SLC26A4 (pendrin) and SLC26A5 (prestin), and bronchiectasis for SLC26A9. Recent studies revealed striking similarities in overall molecular architectures among the family members, even though they mediate distinct functions as an anion exchanger (pendrin), a voltage-dependent motor (prestin), and a transporter with channel-like properties (SLC26A9). To understand the molecular mechanisms that underlie their diversity, we examined several key structural features of the SLC26 proteins using both naturally occurring and artificially made missense changes introduced to pendrin, prestin, and SLC26A9.
Methods: Standard site-directed mutagenesis was used to generate amino acid changes of i) basic residues in anion binding site, ii) conserved polar residues at the interface of N- and C-terminal cytosolic domains, and iii) C-terminal residues of TM14 helices at the homodimerization interface. They are: Y20H, V509G, V510D, V510I, H723D, and H723Y in pendrin; Y16H, R399V, I500V, I500A, and H707R in prestin; Y8H, V393R, V493G, and H731R in SLC26A9. All variants were placed with a C-terminal mTurquoise2 tag in a pSBtet-Pur vector (Addgene), which allows doxycycline-dependent expression. Stable cell lines that express these variants were established using HEK293T cells as described before (Kuwabara et al., 2018). Using these stable cell lines, functional assays were performed for each variant along with corresponding wild-type (WT) controls. For pendrin anion transport function, previously established in vitro plate reader-based transporter assays were performed (Wasano et al., 2020). For prestin function, nonlinear capacitance (NLC), a proxy of electromotility, was measured. For SLC26A9 anion transport function, both electrophysiological and plate reader-based transport assays were performed. Expression and plasma membrane (PM) targeting was confirmed by fluorescence microscopy for each variant.
Results: The basic residues at the anion binding site are essential for both anion antiport in pendrin and motor function in prestin, and its conversion to a nonpolar residue is crucial but not sufficient for the fast uncoupled anion transport in SLC26A9. The conserved polar residues in the N- and C-terminal cytosolic domains are

likely involved in dynamic hydrogen-bonding networks and essential for anion antiport in pendrin but not for motor (prestin) and uncoupled anion transport (SLC26A9) functions. The hydrophobic interaction at the dimerization interface is not of functional importance for uncoupled anion transport in SLC26A9 but essential for the anion antiport and motor functions of pendrin and prestin, respectively.
Conclusions: We have uncovered the key structural features within the SLC26 family members, which greatly facilitate the understanding of molecular mechanisms that support diverse physiological roles of the SLC26 proteins.

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S104. Identification of Cytotoxic Truncating MYO6 Variants

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Category: Genetics B: General
Background: MYO6 encodes an actin-based motor protein, myosin VI, that is abundantly expressed in cochlear and vestibular hair cells. Myosin VI is essential for maintaining the ordered structural integrity of the stereocilia. To date, 129 MYO6 variants associated with either dominantly- or recessively-inherited hearing loss (DFNA22 and DFNB37, respectively) have been reported, of which 48 are truncating variants. Most of these truncating variants are presumed to result in loss-of-function, as myosin VI without the C-terminal cargo-binding domain would be of little or no physiological use. In fact, p.R1166X nonsense MYO6 variant is recessively inherited. However, there are many dominantly inherited MYO6 variants that lack the C-terminal cargo-binding domain. The pathogenic mechanisms underlying these perplexing observations remain unexplored. The objective of this study is to address this issue by experimentally testing our hypothesis that expression of truncated myosin VI proteins may impair cell viability.
Methods: Stable cell lines that express truncated myosin VI variant proteins in a doxycycline (Dox)-inducible manner were established in HEK293T cells. A self-cleaving near-infrared fluorescent protein tag (miRFP670-P2A) was included at the N-termini of the myosin VI constructs so that their Dox-dependent expressions can be fluorometrically monitored. Using these cell lines, pathogenic effects of truncated myosin VI proteins were assessed by multiplexed RealTime-Glo MT Cell Viability and CellTox Green Cytotoxicity assays. These assays were performed in a 96-well plate format using an automated plate reader with temperature (37oC) and CO2 (5%) controlling capability. A cytotoxic truncated Kv7.4 variant, p.Q71Sfs, which was identified in our recent study, was also included as a positive control.
Results: As expected, Dox-induced expression of Q71Sfs-Kv7.4 severely impaired cell viability and induced cell death. Expressions of several truncated myosin VI variants also noticeably impaired cell viability and induced cell death. We also conducted the multiplexed assays in the presence of chloroquine (an autophagy inhibitor) and MG-132 (a proteasome inhibitor). We found that DFNA22-associated R659X-myosin VI moderately impaired cell viability and induced detectable cell death, and DFNB37-assiciated W793X-myosin VI also moderately impaired cell viability but induced little cell death. Application of chloroquine significantly deteriorated the viability of both cell lines expressing R659X- and W793X-myosin VI. On the contrary, application of MG-132 had little or very small adverse effects on the viability of both cell lines expressing R659X- and W793X-myosin VI, implying a relatively small contribution of ubiquitin-proteasome system compared to autophagic pathways for clearing at least these two truncated myosin VI variants.
Conclusions: Cytotoxicity that impairs cell viability was indeed identified in multiple truncated myosin VI variant proteins, which may account for their pathogenic roles in hearing loss.

S105. Unlocking Insights Into DFNA5 Hearing Disorder Using Inner Ear Organoids

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Category: Other, Inner Ear Disease Modeling

Background: Mutations in DFNA5 gene cause dominant hearing loss in humans. While GSDME plays an important role in two types of programmed cell death, including apoptosis and pyroptosis, the role of normal or mutant GSDME in the inner ear or hearing is unknown. All DFNA5 mutations result in exon 8 skipping and DFNA5 patients manifest delayed onset progressive hearing loss. However, the mouse model of human DFNA5 does not exhibit the phenotype of hearing loss, making it particularly challenging to study DFNA5 hearing loss or develop interventions to rescue hearing. In this context, creating Inner Ear Organoids (IEOs) using human pluripotent stem cells carrying the DFNA5 mutation offers valuable tools for investigating the disease's biology and testing potential therapies.
Methods: 1) To model DFNA5 mutation, we introduced a 3-nucleotide deletion (TTC deletion) within intron 7 of wild-type 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) Expression of marker genes at different time points was assessed by IHC, RT-PCR, and bulk sequencing. The organoids were screened for apoptosis using the TUNEL assay.

4) RNA editing strategy was developed to disrupt the exon 8 skipping transcript of DFNA5 by screening of CasRx/gRNA systems. Expression vectors for hfCasRx/gRNAs with GFP were assessed for signal reduction to identify the most effective gRNA for knockdown efficacy.

5) The most effective gRNA was packaged into AAV2-hfCasRx/AAV2-gRNA-2 vectors and delivered to DFNA5-IEOs to study the treatment effect.

Results: 1) TTC deletion successfully induced exon 8 skipping in iPSCs, a hallmark observed in human DFNA5 patients.
2) DFNA5-IEOs were successfully generated by optimizing the levels of BMP-4 during the developmental phase. The DFNA5-IEOs displayed normal development of otic vesicles with the production of hair cells, supporting cells, and neurons up to date 40. Past day 40, significant loss of hair cells and supporting cells were detected, with the loss of both cell types by day 60. TUNEL assay detected significant cell death starting at day 40.

3) AAV-hfCasRx/AAV2-gRNA-2 delivered to the reporter cell line reduced DFNA5-GFP signal significantly, a demonstration of efficient RNA editing to abolish the mutant transcripts. The experiment is ongoing with the delivery of AAV-hfCasRx/AAV2-gRNA-2 into the DFNA5-IEOs for the rescue effect.

Conclusions: The human DFNA5-IEOs reveal that DFNA5 mutations primarily affect cell survival without significantly impeding the development of inner ear including hair cells, supporting cells and neurons, consistent with relatively normal hearing in young DFNA5 patients who subsequently develop progressive hearing loss. We developed an RNA editing strategy to effectively abolish the mutant DFNA5 transcripts, with the potential to develop a treatment strategy for all DFNA5 patients.

S106. Cellular and Functional Avian Auditory Regeneration

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Category: Regeneration
Background: Birds, in contrast to mammals, are capable of naturally regenerating hair cells and neuronal connections after damage, leading to hearing restoration. However, how newly regenerated hair cells become properly reinnervated remains unknown. Furthermore, conventional methods to damage the chicken auditory organ, the basilar papilla (BP), often result in partial damage, and regeneration happens asynchronized because of the cumulative effects of repetitive or prolonged insults. In this study, we aimed to characterize hair cell regeneration, reinnervation, and hearing restoration in deafened chickens following a single local infusion of an ototoxic drug.
Methods: We used post-hatch day seven chickens. To induce hearing loss, we administered sisomicin, an aminoglycoside antibiotic, through the lateral semicircular canal. At various post-sisomicin treatment (PST) time points, the BP was collected for immunohistochemistry analysis. To assess hearing function, auditory brainstem responses (ABR) were measured.

Results: We found a retraction of cochlear ganglion neurites (dendrites) following complete hair cell loss one day PST. The first regenerated hair cells emerged five days PST, while the neurites remained withdrawn near the basement membrane. Notably, the regenerated hair cells extended long basal protrusion towards the resting neurites, establishing contact. In addition, presynaptic specializations, identified with CtBP2 immunohistochemistry, appeared at the tips of the basal protrusions from day 6 PST onwards. Subsequently, the neurites regrew in coordination with the retraction of the hair cell protrusions, accompanied by relocation of the CtBP2-positive punctae. Postsynaptic-like structures also began re-establishing themselves by 9 days PST. By 14 days, when hair cells regained their normal cylindrical shape, CtBP2-positive punctae settled beneath the nuclei of hair cells. Calretinin levels, highly expressed in regenerated hair cells, tapered off toward the lateral side of the BP within 28 days PST, aligning with hair cell maturation and reinnervation. Compared to developmental innervation, the initial location of the re-emerging presynaptic specializations differed in the reinnervation process. Hearing recovery commenced at 9 days PST, corresponding to synaptic reformation initiation. Hearing thresholds consistently improved, eventually matching those of age-matched controls within 28-35 days PST.
Conclusions: We conclude that auditory restoration in completely deafened chickens is remarkably efficient, approaching pre-damage levels. The regeneration-specific synaptic reestablishment, coupled with hair cell basal protrusions, may represent a location-preserving process necessary for maintaining tonotopic fidelity.

S107. IP3 and RyR Signaling are Essential and Have Distinct Roles in Regulating Neurite Pathfinding in Response to Micropatterned Growth Cues

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[1]*University of Iowa Hospitals and Clinics*

Category: Regeneration
Background: The cochlea exploits an intricate tonotopic organization of afferent innervation to effectively process highly complex auditory stimuli. To create this precisely organized pattern, neurites from spiral ganglion neurons (SGNs) navigate a complex milieu of cells, extracellular matrix, and biochemical gradients to reach their peripheral and central targets in the organ of Corti and cochlear nuclei, respectively. Leveraging these growth cues via micro-scale patterning of surface features and biochemical cues have emerged as a potential tool to direct neurite growth into close proximity with next-generation neural prosthesis electrodes. However, the signaling events underlying the ability of growth cones to respond to these features and cues remain unclear. There is a growing body of research demonstrating the influence of Ca2+ signaling in how a growth cone senses and grows in response to various growth cues.
Methods: We study the role of IP3 and RyR signaling as sensory neurons (spiral ganglion neurons and dorsal root ganglion neurons) pathfind in response to a wide variety of engineered micropatterned substrates. To study this we leverage, the photopolymerization of biophysical substrates, patterned coatings of peptides, and real-time imaging of growth cones.
Results: IP3 and RyR signaling play crucial roles in the growth cone as neurites navigate biophysical features, thereby enabling proper guidance to these cues. Additionally, these signaling pathways are integral in SGN pathfinding in response to both chemo-permissive and chemo-repulsive patterns. In our study of the role of these signaling pathways in pathfinding in response to complex geometries, RyR signaling was found to be involved in halting growth in response to a repulsive cue. Conversely, IP3 signaling appears necessary for a growth cone to turn in response to a guidance cue.
Conclusions: Overall, this research sheds light on the fundamental biological processes governing how an SGN neurite senses and turns in response to substrate cues. These key Ca2+ signaling elements, IP3 and RyR, are essential for SGNs to pathfind in response to diverse biophysical and biochemical cues. Additionally, IP3 and RyR have distinct roles in neurite guidance. Understanding how neurites sense and respond to cues informs fundamental neural development and offers insights into translating these principles into applications such as guiding SGN neurite growth for improved neural prostheses, including cochlear implants.

S108. Comparison of Inner Ear Organoid Derived EVs at Different Developmental Stage

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Category: Regeneration

Background: The permanence of hearing loss is mainly due to the inability of the sensory hair cells to regenerate. Although cochlear hair cells are known to be not replaced in mammals, neonatal mouse cochlear supporting cells are able to proliferate and differentiate into inner organoid. During the process of developing into inner ear organoids, intercellular communication might occur through extracellular vesicles (EVs). The purpose of our study is to effectively generate inner ear organoid from mouse neonatal stem cells by isolating cells from the P2 organ of Corti, to isolate EVs from the "proliferating sphere" and "differentiated organoid" and to compare and analyze EVs derived miRNA to identify key genes and proteins related to inner ear regeneration.

Methods: We generated floating spheres from neonatal (P2) mouse supporting cells and optimized the condition to harvest the most abundant number of solid spheres. The spheres were characterized by immunohistochemistry staining, FACS and western analysis on the proliferation day 7. Then, we transferred spheres to adherent culture condition, differentiated the spheres into inner ear organoid and optimized the condition to obtain organoids efficiently. The inner ear organoids were characterized by immunohistochemistry staining, FACS and western analysis on day 21. EVs were isolated from the 5-7th day media of "proliferating spheres" and 19-21st day media "differentiated organoids". EVs were characterized with nanoparticle analysis, and western. Also, miRNA sequencing and data analysis was done to compare between two groups.

Results: Single cells were obtained from the dissected cochlea and after modifying the concentration of small molecules and seeding cell numbers, seeding 2.5 x104 cells / well were proven to be most efficient number to generate the most abundant number of solid spheres on day 7. Spheres were transferred to adherent culture system and after modifying the concentration of small molecules, the concentration of Matrigel, and incubation period, using the 20%-Matrigel with proliferation media for next 7 days, and then differentiation media for the rest 7 days were proven to be most efficient condition to generate inner ear organoid on day 21. In miRNA sequencing, 139 mature miRNAs were identified after normalization. A total of 91 miRNAs were more than 2 folds changed, and 35 miRNAs (6 miRNAs upregulated; 29 miRNAs downregulated) were statistically significant.

Conclusions: We've identified that different miRNAs were involved in the proliferation and differentiation stage in the development of inner ear organoids. Further research is going on to elucidate the role of miRNA in inner ear regeneration.

S109. The Role of Notch, Wnt, Shh and Fgf2 Signalling in the Differentiation of LGR5-Positive Progenitor Cells into Inner Ear Hair Cells

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Category: Regeneration

Background: The precise regulation of inner ear hair cell (HC) differentiation from LGR5-positive supporting cells (SC) is a pivotal process in auditory system development and maintenance. Emerging evidence underscores the indispensable roles played by intricate signaling pathways, notably the Notch, Wnt, Sonic Hedgehog (SHH) and FGF2 signaling cascades, in orchestrating this intricate cellular differentiation. Notch inhibition has been demonstrated to release the brake on cell fate determination, facilitating the transition of SC towards a HC fate, while Wnt signaling activation promotes the proliferation and positioning of nascent HC precursors while SHH signaling modulation adds an additional layer of control to this intricate process. The inhibition of FGF2, a multifaceted growth factor, is essential for specifying and sustaining the commitment of SC to the HC lineage. This study seeks to unravel the intricate interplay of these molecular mechanisms, shedding light on the potential for therapeutic interventions aimed at harnessing the regenerative potential of LGR5-positive SC in hearing restoration.

Methods: Cochleae were obtained from postnatal day 1-4 mice of either wild type or Lgr5-EGFP reporter mouse lines. The entire cochleae were enzymatically dissociated into individual cells and marked with anti-Lgr5 magnetic microbeads, allowing their isolation via MACS. Spheroids were generated from each isolated

cell and induced differentiation into 3D organoids using a combination of Wnt agonists and Notch inhibitors. These organoids were closely observed for a duration of 21 days and sampled for various analyses, including immunofluorescence (IF), RNA sequencing, and TEM. Compared to the previously published organoids isolated from manual isolation (MI), Magnetic-Activated Cell Sorting (MACS) generated organoids we further treated the organoids with FGF inhibitor and SHH agonist, aiming to gain deeper insights into the underlying molecular mechanisms.
Results: Supplementation of Notch inhibitor and Wnt agonist during differentiation process resulted in organoids from both methods to develop putative hair cells (HCs) characterized by the expression of relevant molecular markers akin to native HCs, including the presence of stereocilia bundles confirmed through IF. However, MI-derived organoids exhibited more prominent F-actin protrusions and a distinct uptake of FM1-43, indicating enhanced mechanotransduction channel functionality. These differences may be attributed to the higher FGF2 inhibition observed in MI-derived organoids as indicated by RNA-sequencing data. Interestingly, the use of an FGF2 receptor inhibitor led to smaller-sized spheroids but did not improve hair cell prevalence stereocilia characteristics through TEM imaging and electrophysiological testing in MACS-derived organoids. Conversely, early results in SHH activation in MACS-derived organoids resulted to cochlear hair cell prevalence and FM1-43 uptake.
Conclusions: In conclusion, the intricate orchestration of Notch inhibition, Wnt signaling activation, and FGF2 inhibition plays a pivotal role in driving the differentiation of LGR5-positive supporting cells into inner ear hair cells, a process of paramount significance in auditory system development and regenerative medicine.

S110. RNA-Seq Analysis of Early Transcriptional Genomic Changes of Alzheimer's Disease in the Auditory System

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Category: Aging
Background: Alzheimer's disease (AD) is a progressive neurodegenerative disorder and is also the most prevalent cause of dementia. Early detection and intervention are crucial for AD treatment and prevention. It has been estimated that delay of dementia even by one year can reduce 10% of dementia population. Recent epidemiological studies reveal that hearing loss is a major high-risk factor for AD and AD-related dementia (ADRD) development and progression. Our previous studies (Liu et al., 2020, PMID: 31870800; Mei et al., 2021, PMID: 34588972) also demonstrated that AD could produce early hearing functional changes (or biomarkers) prior to the occurrence of typical AD phenotypes, suggesting that hearing functional tests could serve as early biomarkers for AD/ADRD diagnosis and assessment. In this study, we continually examine AD-induced genomic changes, particularly, early genomic changes in the auditory system.
Methods: APP/PS1 AD mice were used. The auditory cortex (AC), inferior colliculus (IC), cochlear nucleus (CN), and cochleae at 2, 3, 6, and 9 months old were collected. Bulk Poly(A) RNA Sequencing was performed. The hearing functional tests and behavioral tests were also performed.
Results: As shown in early functional changes by hearing functional tests in our previous studies, the auditory system has early significant AD-related transcriptional and genomic changes. At 2 months old, significant changes could already be found in the cochlea. Interestingly, as age increased, transcriptional and genomic changes in the cochlea and CN were quickly reduced, whereas AD-induced changes in the IC and AC in the auditory centers were significantly increased. At 9 months, at which the typical AD phenotypes just occurred in the APP/PS1 AD mouse model, little significant changes were found in the CN and cochlea. However, significant changes were still found in the IC and AC. The significant difference was also found between female and male AD mice. Female mice showed more apparent changes in comparison with male AD mice at the early stage. RNA-Seq analysis also revealed that these changes are mainly located at pathways involving neurodegeneration, postsynaptic density, and neurotransmitters besides amyloid-beta binding. These changes were also consistent with our electrophysiological recordings and behavioral tests.
Conclusions: AD has early transcriptional and genomic changes in the auditory system. The apparent earliest changes appear in the cochlea. Female mice have more significant changes than male mice at the early stage. These data provide valuable information for AD intervention and prevention at the early stage.

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S111. Fhod3 Dysregulation Affects Actin Polymerization in the Cuticular Plate, Leading to Progressive High-Frequency Hearing Loss: Insights Into Age-Related Hearing Decline

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Category: Aging

Background: Age-related hearing loss (ARHL) ranks among the leading causes of sensorineural hearing decline in the elderly, with known genetic factors accounting for only a fraction of its heritability. To address this gap, our study employed BXD strains for a genome-wide association study (GWAS) on ARHL, successfully identifying a novel locus on chromosome 18 associated with ARHL specifically linked to a 32 kHz tone burst stimulus. We extended our investigation to assess the functional impact of Fhod3 (formin homology 2 domain containing 3), a candidate gene for ARHL discovered through our meta-analysis GWAS in mice.

Methods: Utilizing genetically modified mice, we created Fhod3-overexpressing models (Pax2-Cre+/-; Fhod3Tg/+) (TG) and HC-specific conditional knockouts (Atoh1-Cre+/-; Fhod3fl/fl) (KO). Gene expression patterns of Fhod3 in the inner ear were determined using multiplexed error-robust fluorescence in situ hybridization (MERFISH) in P5 cochleae. Immunofluorescence staining further corroborated Fhod3 localization. Auditory functions in these mutants were assessed via auditory brain stem response (ABR) tests at 6, 12 and 18 weeks of age. We also analyzed survival hair cell (HC) counts and phalloidin intensities at the cuticular plate, along with ultrastructural studies on stereocilia.

Results: Fhod3 was found to be expressed in HCs, primarily localizing to the cuticular plate (CP). TG mice exhibited progressive high-frequency hearing loss, marked by a notable loss of outer HCs and decreased phalloidin intensities at the CP. Ultrastructural evaluations revealed shortened stereocilia in basal turn cochlea. Intriguingly, these phenotypic changes were replicated in KO mice.

Conclusions: Our findings illuminate Fhod3's crucial role in modulating actin dynamics within the CP and stereocilia. This lays the groundwork for future studies to explore Fhod3-related hearing impairment mechanisms, which may pave the way for innovative therapeutic strategies targeting ARHL in humans.

S112. Neural Oscillatory Responses to Speech-In-Noise in Older Adults With Normal-Hearing, Hearing Loss, and Mild Cognitive Imparement

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Category: Aging

Background: Hearing loss is the primary risk factor for dementia, and it may affect the central auditory function and cognitive ability in the brains of older individuals. The speech-in-noise (SiN) test, involving sensory encoding and cognitive neural processing, assesses central auditory function. Electroencephalogram (EEG) has a history of quantifying neural responses to speech stimuli but has seen limited use in studying brain response changes in older adults concerning hearing and cognitive abilities. This study aims to measure brain oscillations during SiN listening and their relationship with cognitive abilities in older adults with hearing loss (HL) and/or mild cognitive impairment (MCI).

Methods: A total of 20 participants were involved in this study, including 13 individuals with mild cognitive impairment (MCI), 10 with hearing loss (HL), and 7 with both MCI and HL (MCI+HL). Additionally, 10 individuals with normal hearing (NH) served as controls. All participants underwent cognitive function assessment using the Montreal Cognitive Assessment (MOCA) test. EEG activity was recorded from 64 scalp electrodes while participants actively performed the Korean digit-in-noise test. In a passive condition,

participants listened to digit stimuli while watching silent, closed-captioned movies. We employed DICS beamforming to estimate the sources of neural activity for each frequency band.
Results: In behavioral data, both HL groups (HL only and MCI+HL) showed lower DiN thresholds compared to NH and MCI-only groups. The MCI+HL group had significantly reduced DiN accuracy during attentive listening. In active versus passive listening, the HL group exhibited alpha event-related desynchrony (ERD) enhancements in broader areas of the brain compared to other groups. Moreover, a positive correlation between alpha power in the right frontal lobe and DiN performance was found in the HL group. Conversely, the MCI+HL group displayed increased frontal lobe activity in the theta rhythm. The theta power in MCI individuals is negatively associated with DiN performance and with MOCA scores.
Conclusions: Our findings suggest that the alpha rhythm may serve as a neural correlate of auditory attention, while theta rhythm appears to be more sensitive to cognitive function. Neural activity during SiN listening is correlated not only with listening ability but also with cognitive function.

S113. Investigation of Afferent and Efferent Neuron Systems in Cochlea With Age-Related Hearing Loss Based on Three-Dimensional Imaging

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Category: Aging
Background: The degeneration of cochlear hair cells and cochlear synaptopathy are considered as peripheral mechanisms underlying age-related hearing loss (ARHL). ARHL has been associated with predisposing cognitive impairment and dementia, hence the consequence of central pathology of ARHL requires the investigations of changes in projection of peripheral and central pathways. This study aims to systematically present the age-related structural changes of afferent and efferent neuron systems in the cochlea and cochlear nucleus (CN) with 3-dimensional imaging at single cell resolution.
Methods: Transparent embedding solvent system (TESOS) technique was used to obtain the whole neural projection from cochlea to CN at single-cell resolution in 2-month and 18-month-old thy1-YFP16 transgenic mice. We employed scAAV-mcherry retrograde injections in CN to label spiral ganglion neurons (SGNs) in 2-month and 18-month-old mice, and subsequently analyzed cell size, number, morphology and 3-D spatial distribution using Imaris. Xradia Microscopy was applied to observe the bone structure of cochlea in high resolution. The number of SGNs at different frequencies in different age can be calculated based on the fibers shuttling through the habenula perforata. To further compare the synaptic connections of medial olivocochlear (MOC) efferent fibers on outer hair cells (OHCs) between 2-month and 18-month-old mice, whole-mount immunostaining was applied to stain Prestin and ChAT in whole cochlea, respectively.
Results: We first reconstructed cochlea-cochlear nucleus projection at single cell resolution in 2-month and 18-month-old Thy1-YFP16 mice. Significant changes were found in cochlea, cochlear root neurons (CRNs) and CN in ARHL model. Both the number and dispersion of CRNs notably increased in ARHL, while neuron size remained unchanged. Secondly, we discovered two distinct projection pattens of SGNs and MOC in ARHL model. Notable loss of high-frequency SGNs, fusion and swelling of neighboring spiral neuron fibers were observed in afferent systems. However, the age-related damages in the MOC were primarily at synapses, manifested as a significant reduction in synaptic contacts with OHCs across frequencies and evident disruption and shrinkage of boutons at the hook region, which happened simultaneously with the loss of OHCs. Finally, the volume of CN in ARHL notably shrink, while the number of dorsal cochlear nucleus neurons were slightly increased. The dendrites of satellite cells decreased and the terminals projected to bushy cells gradually degenerate with age.
Conclusions: The structure of cochlea-cochlear nucleus were systematically presented in TESOS technique based high-resolution 3D imaging. The age-related changes of cochlear projections to cochlear nucleus include, reduced neural fibers and synaptic contact and distinct patterns of neural projection of SGNs and MOCs. Overall age-related changes underlies the structural basis of pathology of ARHL.

S114. Preventing Age-Related Hearing Loss Using Mitochondrial Function Improving Drugs

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Category: Aging

Background: Mitochondrial dysfunction is considered to be associated with aging and age-related hearing loss. However, the detailed mechanism and pathophysiology of hearing loss remain unknown. Transfer RNAs (tRNAs) contain a wide variety of posttranscriptional modifications that are important for accurate decoding. Mammalian mitochondrial tRNAs (mt-tRNAs) are modified by nuclear-encoded tRNA-modifying enzymes (Wei, 2013).Cdk5 regulatory subunit-associated protein 1 (cdk5rap1) is responsible for 2-methylthio (ms2) modifications of mt-tRNAs. Deficiency in ms2 modification markedly impaired mitochondrial protein synthesis. This resulted in respiratory defects in cdk5rap1 knockout (KO) mice. We reported the influence of a mitochondrial dysfunction caused by the ms2 modifications of mt-tRNAs on age-related change in vivo and in vitro (Miwa, 2021). While, an endogenous indole compound, mitochonic acid 5 (MA-5), is known to enhance intracellular ATP production as a mitochondrial function-improving drug. In the present study, we conducted preliminary experiments to determine whether MA-5 can prevent the onset of age-related hearing loss in a mouse model of mt-tRNA chemical modification and deletion.

Methods: At the age of 5 months, hearing was evaluated by ABR, DPOAE, and EP, and morphological evaluation was performed by HE staining.

Results: In Cdk5rap1 knockout mice, age-related hearing loss was suppressed in the middle frequency range in ABR, and similar results were observed in DPOAE. EP also showed a marked suppression of the decrease in endocochlear potential. Not significant morphological changes were observed.

Conclusions: MA-5 showed partial suppression of age-related hearing loss in a mouse model of age-related hearing loss with abnormal mt-tRNA modifications. The suppression of endocochlear potential reduction suggests an inhibitory effect on aging in the spiral ligament or stria vascularis in cochleae.

S115. Progressive Changes in Central Gain in the Aging Human Auditory System

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Category: Aging

Background: Auditory nerve afferent activity is lower in older adults in comparison with younger adults. Despite this, older adults have equivalent or increased neuronal activity compared to young adults at the level of the brainstem and cortex, a phenomenon known as central gain. Central gain may compensate for the loss of input from the auditory nerve, but we have found that it can be maladaptive, potentially contributing to poorer speech recognition in noise. The propagation of central gain through the auditory system – from the auditory nerve to the auditory brainstem and cortex – is not well understood. The purpose of this study is to assess the magnitude of central gain at multiple points of the auditory system. We hypothesized that older adults (compared to younger adults) exhibit progressive increases in neural response amplitudes at the brainstem and again at the cortex.

Methods: The compound action potential (CAP) and auditory brainstem response (ABR) were measured in a group of younger (18-30 y/o) and older (55+) participants. Cortical auditory-evoked potentials were recorded from a subset of participants. Auditory stimuli were 100 dB pSPL clicks presented to the right ear. Central gain was assessed by comparing the effects of age on the amplitudes of the CAP N1, ABR wave V, and cortical P1 peaks.

Results: Results: CAP N1 amplitudes were significantly smaller in older adults compared to younger adults, but there were no significant differences in wave V amplitudes between younger and older adults. Cortical P1 amplitudes in older adults were significantly larger compared to younger adults.

Conclusions: By showing significant age-related deficits in neural response amplitudes at the auditory nerve but not at the brainstem or cortex, these data support our previous findings of central gain in the auditory system of older adults. Additionally, although there were no differences in the brainstem response amplitude between older and younger adults, the cortical response from older adults had a significantly larger amplitude than younger adults. The results from the current study showing progressive increases in central gain, together with previous work demonstrating that cortical central gain is driven by decreased inhibitory function at the level of the cortex, support our hypothesis that central gain mechanisms are progressive, culminating in the

largest increase in neural amplitudes at the cortex. The role of central gain in auditory processing will be discussed.

S116. Open Board

S117. Computer Simulation of Combined Stimulation of the Cochlear and Vestibular Systems Employing Realistic Human Inner Ear Anatomy and Multi-Compartment Nerve Models

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Category: Auditory Prostheses

Background: Both sensory organs in the inner ear are often affected by the same pathologies, leading to a combined impairment of both cochlear and vestibular function. Studies have proven the feasibility of a combined cochlear/vestibular implant (CVI) supporting both cochlear and vestibular input. Computational modeling of this stimulation is a valuable tool to test effects of various stimulation scenarios in-silico prior to consideration in in-vivo experiments and in patients. However, recent modeling approaches of the inner ear have mainly focused on one organ – either the vestibular system or the cochlea. The simulation of combined cochlear-vestibular stimulation offers the possibility to analyze potential cross-talk and current spread from cochlear implants to the vestibular system and vice-versa. In addition, stimulation strategies for combined stimulation can be tested and optimized.

Methods: A modeling workflow to simulate combined cochlear-vestibular stimulation based on realistic human inner ear anatomy was developed, extending computer models for the analysis of vestibular implant stimulation scenarios previously presented by our group. Naturally distributed nerve fiber trajectories were generated for both organs starting at the sensory epithelia of the ampullary nerves, the macula organs and the cochlea, respectively, as well as for the facial nerve and the inner auditory canal. Based on these fibers, a multi-compartment nerve model for vestibular fibers implemented in our previous simulation framework was extended by a multi-compartment nerve model for the myelinated cochlear nerve fibers. Extracellular electrical fields resulting from the stimulation by electrode contacts were computed using the finite element method. Stimulation scenarios with electrodes in both the vestibular system and the cochlea were tested and selective stimulation of single targeted nerve branches was evaluated. Furthermore, electrically evoked compound action potentials (eCAPs) were computed to simulate the combined neural response. Time dependent transmembrane currents originating from stimulated nerve fibers were considered as distributed current sources and resulting electrical fields are evaluated at positions of sensing electrodes.

Results: The framework was tested by considering individual and combined stimulation scenarios and comparing selective nerve stimulation of nerve branches in the inner ear. Simulation results indicate that the impact of combined stimulation of the vestibular and cochlear nerve branches is not negligible, and that potential crosstalk occurs, in particular during simultaneous stimulation of both organs. In addition, obtained ECAPs of realistic stimulation scenarios corresponded well to literature data.

Conclusions: The presented model enables the investigation of the impact of combined cochlear vestibular stimulation on both organs and their interactions. This will contribute to a better understanding of the effects of electrode locations and stimulation scenarios on focused stimulation inside the inner ear and mutual influence between the organs. Furthermore, new diagnostic options might develop from this understanding, as the described model can be used to investigate characteristic influences of pathological conditions.

S118. Charge Threshold, Array Tilt, and Longitudinal Speech Perception in Exceptional Users of the Auditory Brainstem Implant (ABI)

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Category: Auditory Prostheses

Background: A high amount of variability in sound and speech perception is observed in users of the auditory brainstem implant (ABI). Factors that influence ABI outcomes include duration of deafness, number of

auditory electrodes, charge thresholds, three-dimensional (3D) position of array, daily processor use, and active social participation. Only a small number of ABI users achieve open-set speech comprehension and reaching this level of performance takes longer compared to recipients of the cochlear implant (CI). Longitudinal outcome data for these ABI users are limited. In the following study, we correlate electrode and patient variables with speech perception in exceptional ABI users with long-term follow-up.
Methods: Retrospective review of adult ABI subjects with Neurofibromatosis type 2 (NF2) with postoperative computed tomography (CT) and perceptual data. All subjects except two were implanted unilaterally with a Nucleus ABI device (Cochlear Corp) using either a retrosigmoid or translabyrinthine approach. Exceptional patients were identified based on their post-operative constant nucleus constant (CNC) testing indicating their performance met a threshold above random chance (~4%). Using the imaging methods of Barber, S. et al. (2017, Ear and Hear. 38(6):e343-e351), 3D electrode array position was classified based on angles from the horizontal using posterior and lateral views and on distances between the proximal array tip superiorly from the basion (D1) and laterally from the midline (D2). Tilt of the ABI array was measured by grouping electrodes and calculating an angle measure based on line of best fit for their respective charge thresholds. Exceptional patients were compared to other patients on the basis of CNC performance, overall charge threshold, and overall array tilt using a Mann-Whitney U test.
Results: Seven adult subjects were identified as exceptional ABI performers, achieving above chance scores on the open-set CNC monosyllable test. These patients were compared to 17 non-exceptional users. Three of these patients underwent a retrosigmoid approach, and four underwent a translabyrinthine approach. Patient follow-up ranged from 6-13 years post-activation. Compared to other ABI users, these patients are all socially independent, full-time users, and have daily social interactions. There was a statistically significant difference in charge threshold and array tilt in exceptional patients compared to non-exceptional patients. There was no significant difference in the angle measures relative to the basion between exceptional and non-exceptional users. The mean overall charge threshold and tilt in exceptional patients measured 6.02±3.07 and 47.48.87°±22.29°, respectively, which is significantly lower compared to 11 non-exceptional ABI users (M=29.42±32.11; M=68.92°±19.29°; both with p less than 0.05). Exceptional patients had a significantly higher speech perception outcome compared to other ABI users (p less than 0.05).
Conclusions: The best performing ABI users have a significantly lower overall charge tilt, and similar social factors that influence ABI usage. Future studies should focus on longitudinal analyses of ABI patients with long term follow-up.

S119. Acute Focal Micro Lesions Reorganize Tonotopic Profiles Evoked by Cochlear Implant Stimulation

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Category: Auditory Prostheses
Background: Electrical stimulation of spiral ganglion neurons (SGNs) by a cochlear implant (CI) results in significant outcome variability, especially for measures involving a high degree of spectral processing. Typically, CIs use cathodic-first biphasic stimuli in a monopolar configuration. The two polarities of the biphasic pulse are expected to interact electrically and neurally. Furthermore they preferentially activate different parts of the SGN: The anodic phase excites SGNs at or central to the soma, while cathodic stimuli preferentially activate peripheral processes. Thus, it is hypothesised that they will distinctively be influenced by damage of the SGN, depending on the peripheral-to-central focus of the lesion.
Methods: We investigated how the inferior colliculus (IC) activation patterns reflect cochleotopic stimulation position when polarities acted separately (monophasic) or in sequence (biphasic) in 11 guinea pigs. Furthermore, we assessed how these patterns were affected by focal micro-lesions (less than 440µm) in osseous spiral lamina (OSL) or Rosenthal's canal (RC). Multi-electrode arrays were used for IC recordings, with characteristic frequencies (CF) between 6 kHz and 32kHz, corresponding to the cochleotopic position of the 6 contacts of a guinea pig adjusted CI (MedEl, apical C1: 6.41±1.03kHz; basal C6: 20.9±2.18kHz). After acoustic receptive fields and CFs had been assessed, animals were deafened with neomycin-sulfate in Ringer's solution. Deafening success was confirmed by neural measurements. IC response profiles to electric stimulation were first recorded in the intact cochlea and repeated after cochlear lesioning with a micro-needle.

Results: In intact ears, monopolar stimulation (+1dB) covered ~0.5 octaves near threshold and greater than 3 octaves at high stimulation levels (+7dB). Anodic stimuli typically had 1-2dB lower response thresholds than cathodic stimuli, with comparable response latencies. The highest IC activation occurred ~1 octave apical (lower CF) to the stimulating contact for anodic stimulation, while cathodic stimulation activated CFs ~1 octave to the base (high CF). Stimulation with biphasic pulses resulted in activation pattern corresponding to the cochleotopic position, when assessed relative to median activation levels. Acute micro-lesions in OSL or RC did not result in gaps in IC response profiles, but increased thresholds and decreased activation strength, depending on lesions size and target (i.e., OSL or RC). CI stimulation close to the lesion enhanced the observed differences between polarities by shifting the IC response profiles to lower CFs for anodic stimulation and to higher CFs for cathodic stimulation, each by ~0.3 octaves. For biphasic stimulation, lesioning reorganized the tonotopic patterns, observed in intact ears.
Conclusions: In conclusion, anodic and cathodic polarities generate distinct midbrain excitation patterns, when stimulating separately, however, place information was retained in IC response profiles for biphasic stimuli. Micro-lesions in OSL or RC disrupted place-specific information, irrespective of the stimulus, potentially explaining poor CI outcomes in the presence of patchy SGN degeneration.

S120. All You See is an Electrode? Probing CI Electrode Array Properties for Optimizing Stimulation Parameters of Cochlear Implants

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Category: Auditory Prostheses
Background: Cochlear implants (CIs) have achieved remarkable success as neural prostheses, globally employed to restore sensorineural hearing loss via direct electrical stimulation of the spiral ganglion. However, the current clinical application of CIs employs a standard stimulation protocol, which unfortunately leads to insufficient frequency resolution and low dynamic range. Notably, commercially available CI electrodes exhibit variations in contact size, wire shape, no. of contacts, total length, and configuration, all dependent on the manufacturer. Our goal is to determine and model the electrical properties of CI electrodes with subsequent extraction of tissue properties and stimulus optimization.
Methods: CI electrodes from four different manufacturers (Advance Bionics, Cochlear, MED- EL, Oticon) were investigated. Each CI electrode was inserted into a linear cochlear model filled with artificial perilymph along its length. The investigation encompassed electrochemical impedance spectroscopy (EIS), covering a frequency spectrum of 5 Hz to 13 MHz, performed among all conceivable electrode pairs. From the resultant impedance responses, an impedance matrix was obtained for all electrode pairs. The response was fitted using an equivalent electrical circuit model. The analytical model based on resistive and capacitive subcomponents was used to extract the tissue properties from CI electrode impedance spectroscopy.
Results: Impedance spectroscopic responses demonstrated broad similarities in amplitude and phase across the implants. The impedance matrices of the resistive part of the tissue showed dependence on the distance between the two measured electrodes. Based on this, our proposed equivalent circuit model, an extension of the general impedance model for biomedical electrodes showed a good fit with the recorded impedance data. From the model, the sub-components of impedance were extracted and differences across the implants were analysed.
Conclusions: Unlike conventional clinical impedance measurement, our approach is analysing the subcomponents of impedance for a better description of a CI electrode. In future, our simulation model should be able extract detailed tissue properties from impedance measurements with CI arrays. This will serve to optimize the stimulation pulse parameters to ensure that each electrode is providing appropriate levels of stimulation to the auditory nerve in the patients.

S121. Consequences of the Use of Ramped Pulses in an Animal Model of Cochlear Implants on the Discriminative Abilities of Auditory Cortex Neurons

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Category: Auditory Prostheses

Background: For several decades, cochlear implant (CI) is the most successful neuro-prosthetic device allowing thousands of patients to recover hearing sensation and speech understanding. However, the performances are usually good in silence, but CI patients have more difficulties in the presence of background noise, such as in public transportation. Potentially, these limitations stem from the large spread of currents diffusing in the cochlea's perilymph when the electrodes are activated. To reduce this large spread of current when activating the different electrodes, one potential strategy is to change the shape of the electrical pulses. Previously, studies have used asymmetric pulses rectangular pulses (Macherey et al., 2006, 2008). Recently a new pulse shape, called ramped pulse, has been proposed (Ballestero et al., 2015) but only one in vivo study has reported that anodic or cathodic ramped pulses elicited eABR responses with lower thresholds and steeper growth functions than anodic or cathodic rectangular pulses (Navntoft et al 2020). Here, we report the consequences of the use of ramped pulses on the discriminative abilities of auditory cortex (ACx) neurons.

Methods: The responses of ACx neurons were recorded in anesthetized guinea pigs. The intracochlear stimulating array was a shortened version of the EVO electrode array used by Oticon Medical (Smørum, Denmark). The array was composed of 6 ring-shaped Platinum-Iridium electrodes, each with a 0.0046 mm² surface. Center-to-center inter-electrode distance was 600 µm. Four shapes of ramped pulses were tested and 20 charges levels were tested from 3nC to 31.5nC to obtain the growth function of ACx neurons. Recordings were collected by 16-channels electrodes composed of two rows of 8 electrodes separated by 1000 µm (350 µm between electrodes of the same row). The Mutual Information (MI) was used as a metric to determine to what extent ACx neurons can discriminate between the different charge levels.

Results: The four shapes of ramped pulses elicited responses with both (i) lower thresholds and (ii) higher maximal firing rates than the rectangular pulses, both with anodic and cathodic first phases. On average, the dynamic range was unchanged as the whole growth function of most of the ACx neurons were shifted on the left. The MI values were computed for each recording (MIind) and also for the entire population of recordings (MIpop) available with a given strategy (between 50-150 recordings). Based upon individual recordings, the mean MI values were similar between ramped and rectangular pulse shapes. Based on the MIpop, the ramped pulses led to higher discrimination abilities than the rectangular pulses but only when the first phase was cathodic.

Conclusions: These results suggest that ramped pulses can be considered as a good alternative to the rectangular pulses, but the polarity of the first phase matters to benefit from these shapes.

S122. Towards Real-World Benefit With a Cochlear Implant Speech Coding Strategy That Leverages Temporal Masking Effects

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Category: Auditory Prostheses

Background: Speech perception remains challenging for cochlear-implant (CI) recipients in listening conditions containing background noise. The temporal integrator processing strategy (TIPS) can significantly improve speech perception in the presence of speech-shaped noise while reducing the power required for stimulation (Lamping et al. 2020). Here, the potential for TIPS to benefit real-world speech perception and reduce device power consumption is investigated through adaptation of the algorithm for real-time processing (a key constraint of CI devices), and computational analyses and speech perception testing in everyday listening conditions.

Lamping W, Goehring T, Marozeau J, and Carlyon, RP. Hearing research. 2020; 10.1016/j.heares.2020.107969.

Methods: TIPS leverages a model of temporal masking to identify and remove stimulation pulses that are unlikely to be perceived. First, TIPS was objectively evaluated in a range of acoustic conditions using

computational comparisons. Second, the speech-recognition performance of twelve CI listeners was measured in two double-blind within-subject experiments before and after TIPS processing. Experiments 1 and 2 investigated TIPS performance against the Continuous Interleaved Sampling (CIS) or Advanced Combination Encoder (ACE) baseline CI processing strategies, respectively. Prior to testing, participants were acclimatised for 10 minutes to the respective condition. Mean-opinion score quality ratings were obtained for speech in quiet and speech reception thresholds were measured in the presence of speech-shaped noise and multi-talker noise. The data collection and analysis are preregistered at https://doi.org/10.17605/OSF.IO/7RFTV.
Results: The computational analyses indicate that the pulse-removal behavior of TIPS is consistent across everyday listening conditions but varies with the listener's stimulation map parameters. For speech-in-noise conditions, TIPS removed speech and noise pulses in equal proportion regardless of noise type and signal-to-noise ratio, suggesting that the previously observed benefit to speech-in-noise perception did not arise from a reduction in stimulus pulses primarily due to noise. In all conditions, TIPS could considerably reduce the power required for stimulation, addressing a major limitation of current devices. A low-latency semi-causal version of TIPS was developed and validated in simulation, demonstrating the feasibility of applying TIPS in clinically-used devices. The speech perception outcomes and statistical analysis of the results from the ongoing listening experiments will be presented. Preliminary results from 11/12 participants indicate that for many of the listeners tested TIPS processing does not compromise speech-in-noise perception whilst potentially providing significant power savings.
Conclusions: The consistent pulse-removal behaviour of TIPS across a range of acoustic conditions suggests that the previously observed speech perception benefits may translate to a variety of everyday listening scenarios. The adaptation of the TIPS algorithm for real-time processing demonstrates that TIPS could feasibly be implemented in the compute-constrained CI device, benefitting CI recipients through an update of the external CI processor. The listening experiments will reveal whether additional speech perception benefits occur in everyday listening scenarios.

S123. Towards the Optimal Preserving Cochlear Implant Stimulation

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Category: Auditory Prostheses
Background: Using a wider electrical dynamic range by increasing the most-comfort level (M) may be important for the outcomes of cochlear implant recipients as this is associated with better speech perception in both quiet and noise. However, this could potentially result in current levels that exceed limits for neuronal health. Previous studies have shown mixed results in relation to this topic. The same holds true for the effect on dorsal cochlear nucleus neurons (DCN-N). A degeneration or a conservation of DCN-N has been reported. Since the various studies used different stimulation parameters, the most advantageous current range for supporting the conservation of auditory structures could not be determined until now. The present animal study therefore, aims to determine SGN and DCN-N density following chronic intracochlear stimulation with different current intensities while maintaining similar rate and strategy.
Methods: Four to five electrode contacts of a HiFocus1j electrode array (Advanced Bionics HiRes 90k®) were inserted into the first turn of left cochleae in 10 mechanically deafened adult guinea pigs. After 4 weeks, the animals were randomly divided into three stimulation groups: "LSI" (mean M level 100 CU), "MSI" (mean M level 150 CU), or "HSI" (mean M level 235 CU). T levels were adjusted to 10% of M levels. An additional group was implanted only but not electrically stimulated. All animals were acoustically exposed to a radio play at a maximum of 65 dB SPL (16 h per day) and stimulated via an Auria© sound processor. After 90 days, the animals were perfused with a fixative solution and the modioli as well as the brains were histologically processed (HE staining). The tissue slices (Rosenthal canals and DCN) were micro-photographed and the number of neurons was determined for both structures.
Results: In the electrode area, SGN density was significantly lower in the "HSI" group compared to the controls. In the electrode-free area, a significantly higher SGN density was found in the "HSI" group compared to all other experimental groups. Furthermore, there was a conservation of DCN neurons in the "HSI" group within the electrode input area.
Conclusions: These results demonstrate that chronic high-current stimulation can result in significant SGN loss close to the electrode contacts. However, the same stimulation had a protective effect for SGNs in

electrode-free regions of the cochlea, where current intensity is lower due to distance from the electrode contacts. The conservation of DCN neurons by high-current stimulation is possibly related to the increased input from related SGNs during the overexcitation. This conservation will possibly later disappear due to the degeneration of these SGNs (deafferentiation). These results suggest the existence of an optimal current range for the protection or preservation of the lower auditory pathway.

This study was supported by Advanced Bionics GmbH Hanover.

S124. The Role of Maximum Output and Necessary Dynamic Range for Active Middle Ear Implants

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Category: Auditory Prostheses

Background: The frequency specific maximum output (MO) of acoustic devices, such as conventional hearing aids belongs to the most important parameters that are crucial for speech intelligibility and patient benefit. However, in all bone conduction devices and most active middle ear implants, technical limitations prevent the use of the patients' entire residual input range due to low MO. We present a method to determine individual MO with the Vibrant Soundbridge (VSB) from clinical routine data. Further we use this method for an analysis of how much coverage of the dynamic range is required for sufficient speech intelligibility.
Methods: For our retrospective analysis, 69 patients, implanted with the VSB at the round window (RW) at the Medical School Hannover (Germany), were analyzed. Firstly, individual frequency-dependent MO were determined. Secondly, the dynamic range (DR) was calculated for each patient and frequency. Finally the word recognition score (WRS) in quiet was correlated to the absolute and weighted DR across the frequencies 0.5, 1.0, 2.0 and 4.0 kHz.
Results: The MO was similar for different coupling types with a maximum at 1.5 kHz. The average MO over speech relevant frequencies (0.5, 1.0, 2.0, 4.0 kHz) was between 71.6 ± 13.8 dB HL to 82.6 ± 7.3 dB HL for different coupling modalities to the RW. Despite minor differences in avg. MO, the individual variability of all coupling modalities was pronounced. Word recognition scores in quiet (n=66) improved with increasing dynamic range and were strongly correlated between predictor and outcome variable (R^2=0.6371-0.6403, sigmoidal function). A significant shift in performance was detected above a DR of 20 dB with a mean WRS of ≥ 78.5% (± 15.7% standard deviation) and a mean WRS of ≤ 55.0% (± 19.7 dB standard deviation) below.
Conclusions: The individual MO and DR can be successfully determined from patients' clinical data only, permitting an in-depth analysis of patient outcomes. Our approach equally applies to bone conduction devices for the prediction of necessary dynamic range and for the definition of evidence based frequency-specific indication limits.

S125. Characterizing Localization of Stationary and Moving Sound in Children Using Bilateral Bone Conduction Devices and Bilateral Cochlear Implants

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Category: Auditory Prostheses

Background: The aims of this work were to: (1) quantify spatial hearing deficits in children using bilateral cochlear implants (BCI) and children with bilateral bone conduction devices (BOCs) and (2) determine if either group effectively utilizes head movements compared to peers with normal hearing (NH). Children with hearing loss have poor access to binaural cues through bilateral hearing devices which results in impaired spatial hearing. BCI users receive electrical stimulation in each ear but have better access to interaural level than interaural timing cues. Bone conducted stimuli reach both cochleae in theory, potentially leaving BOC users with no interaural cues. Hypotheses were therefore that: 1) spatial hearing is impaired in children using BCIs and BOCs compared to NH peers but further compromised in BOC users and 2) children with BCIs move their heads more than children with BOCs to optimize interaural level differences to locate auditory sound sources.

Methods: White noise was presented from a small speaker 1-m from the listener in stationary or moving conditions (angular distances of 0°, ±20°, ±40°, where "+" indicates rightward and "–" indicates leftward) and real-time unrestrained head movements were measured using a gyroscopic sensor. Spatial hearing was measured in children with BOC in unilateral and bilateral device conditions. Speech perception and hearing asymmetry were measured using spatial release from masking (SRM) in which spondees were presented in speech-weighted-noise co-located at 0° azimuth and when noise was moved 90° left or right. Self-reported hearing was measured using the Speech, Spatial and Qualities of Hearing questionnaire (SSQ). Participants were 42 children with BCI [MAge(SD)=12.3(3.3)years], 11 children with BOC [MAge(SD)=14.7(3.5)years] and 37 children with typical hearing [MAge(SD)=12.9(2.5)years]. Mixed effects linear models, ANOVAs and t-tests were employed to assess group effects.
Results: Children with BOCs and BCIs showed higher errors when locating stationary sound than peers with NH [F(2,87)=47.7,p less than 0.001] and less accurate perception of moving sound direction [F(2,87)=118.3,p less than 0.001]. Stationary and moving sound location was not significantly different between children with BOCs or BCIs [Stationary:TukeyHSD=4.16,p=0.45; Moving:TukeyHSD=0.06,p=0.73]. Stationary sound localization improved in children with BOC when using bilateral compared with unilateral devices [F(3,57)=22.7,p less than 0.0001]. Children with BOC moved their heads more than BCI and NH peers when listening to moving sound [NH–BOC:Estimate=-78.2,SE=17.3,p less than 0.001; BCI–BOC:Estimate=-53.1,SE=16.5,p less than 0.01] but no differences were found during stationary localization [F(2,76.3)=0.10,p=0.90]. SSQ scores tended to be poorer on the spatial hearing sub-test for children with BOC than BCI peers (p=0.06). Benefits of speech detection with spatial separation of noise revealed a right ear bias [F(1,58)=38.0,p less than 0.0001] but no group differences [F(2,58)=0.46,p=0.64].
Conclusions: Results indicate children with bilateral hearing devices have impaired spatial hearing and children with BOCs may utilize head movements to compensate for poor interaural cues afforded by bone conducted hearing.

S126. Piston Diameter Influences the Output Efficiency in Oval Window Stimulation

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Category: Auditory Prostheses
Background: Placement of the piston-shaped oval window coupler attached to the middle ear implant transducer (Vibrant Sound-bridge, VSB MED-EL, Austria) after stapedotomy or stapedectomy procedure is a standard treatment of patients with conductive or mixed hearing loss, e.g., otosclerosis. In contrast to surgical reconstruction, where the piston diameter plays a minor role, the size of the piston has a major effect when stimulation is performed by an active middle ear implant (Busch et al. 2021). In this study, we experimentally investigated if an increased surface area of the piston improves efficiency.
Methods: Fresh frozen human cadaver temporal bones (N=6) were prepared to study the output of different piston sizes in oval window coupling modality using intracochlear pressure measurements. After stapedectomy, a preloaded piezo electric transducer (Physik Instrument, Karlsruhe, Deutschland) attached to the standard oval window coupler (MEDEL, Innsbruck, Austria) was used to electromechanically stimulate the cochlea. In addition to the standard coupler (⌀=0.7 mm), two different silicone adapters with larger surface areas were designed to investigate the effects of piston diameters.
Results: The output was determined by the intracochlear pressure differences across the basilar membrane (Psv-Pst). The average output in response to electromechanical stimulation with largest piston compared to the standard coupler was up to 20 dB higher at frequencies between 0.1 and 1 kHz. At frequencies above 1 kHz, the average output was approximately 10 dB higher.
Conclusions: Our analysis confirms that a larger surface of oval window coupler increases the output efficiency according to the ratio of the piston surface area to the oval window surface area.

S127. Development of a Penetrating Electrode Array for Stimulation of the Auditory Nerve in a Non-Human Primate Model for Translation to Human Patients

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Category: Auditory Prostheses

Background: The cochlear implant (CI) can provide speech understanding, at least in quiet environments, for many deaf patients. However, CI performance is severely degraded in sound environments containing multiple speakers or significant background noise. These limitations of the CI have inspired renewed interest in intraneural stimulation via a penetrating electrode array targeting the auditory nerve. Direct stimulation of nerve fibers can reduce broad current spread seen with CIs and thus allow for transmission of more complex sound information. The present work focuses on development of a novel auditory nerve implant (ANI), initially for patients who cannot benefit from a CI and later as a potential CI alternative. Critical to clinical translation is demonstration in animal models of the safety, stability, and functionality of the implant. These studies will also help inform stimulation strategies for future ANIs and develop surgical techniques that allow for long-term stability of the implant.

Methods: The NHP model (rhesus macaque) was chosen for preclinical studies, as the inner ear anatomy and upright posture of the NHP is similar to that of humans. A translabyrinthine approach was developed to gain access to the VIII nerve bundle. The device was secured by placement of an abdominal fat graft and positioning of the array cable within grooves drilled in the surrounding bony structure, which helped to stabilize array position within the nerve. Electrode impedances and auditory brainstem responses (ABRs) were recorded to confirm successful implantation and activation of the auditory nerve. Electrical stimuli consisted of charge-balanced, biphasic pulses (100 μs/phase) of alternating polarity, ranging in current level from 1 to 120 μA.

Results: Stimulation in the NHP produced robust ABRs whose waveforms varied depending upon the activated electrode site. Low thresholds were observed down to approximately 10 μA. Electrode sites placed deeper in the nerve showed lower thresholds than those closer to the surface of the nerve. The bone grooves enabled device components to be stable throughout the experiment. The array itself was positioned under a bony crest before being inserted fully into the nerve. The crest secured the device in place and minimized displacement of the array in chronically implanted animals.

Conclusions: Our NHP studies have shown that the ANI can produce strong activation at low current levels, which are substantially lower than with CIs. Activation at multiple sites across the array showed that different regions of the nerve can be stimulated. Furthermore, a safe surgical approach has been developed that enables long-term stability in implanted NHPs. To prepare for clinical translation, we are currently investigating analogous implantation and stimulation procedures in human patients. Both cadaver experiments and acute intraoperative studies are being performed to demonstrate safety and viability of the ANI for a first-in-human pilot clinical trial in deaf patients.

S128. A Novel Prototype: The Hybrid Opto-Electrical Cochlear Implant for Hearing Restoration

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Category: Auditory Prostheses

Background: Deafness and hearing loss are widespread in the world. At the moment, more than 1.5 billion people live with hearing loss. By 2050, nearly 2.5 billion people are projected to have some degree of hearing loss and at least 700 million will require hearing rehabilitation.

Implantable devices, such as cochlear implants (CIs) can help people with this disability. While CIs are widely implemented, their technology based on electrostimulation exhibits low spatial precision due to the current spreading in the cochlea. The interaction between neighbouring CI electrode contacts reduces the number of independent stimulation channels, thus affecting sound perception. Various methods have been developed to increase the number of independent channels for stimulation, including placing CI electrodes closer to the neurons and implementing multipolar stimulation to direct the electrical current towards the neurons. However, these approaches have not yielded significant advancements in CI performance.

An innovative method for neurostimulation involves using light, which has the potential to trigger responses from small groups of neurons, showing a spatial selectivity comparable with that of acoustical stimulation. Optical stimulation, specifically infra-red neurostimulation (INS), offers a promising solution to enhance CIs, without the need of light-sensitive molecules (opsins).

The integration of INS into a CI represents a promising advancement in the field of auditory neuroprosthetics. Yet, the successful implementation of this technology requires the consideration of various features, such as the number of electrical and optical stimulation channels, energy requirements, size of the CI, coding strategies, and efficient light delivery systems. The development of a hybrid opto-electrical CI (oeCI) represents a significant step towards overcoming these challenges. In this work, we describe a prototype of a hybrid oeCI.

Methods: Our implant prototype has 24 electrical channels and 16 optical channels. Aiming to have a small implant, we chose a 6-layer printed circuit board (PCB) design (dimension 3.5x3.5 cm^2). The top layer accommodates a microcontroller with a Bluetooth module. The middle layer includes two electrical drivers comprising 24 current stimulators capable of delivering pulses less than 2.5 mA.
The bottom layer contains three optical drivers that offer output currents switching from constant to pulsatile mode (1 kHz). The outputs can switch light sources between different current levels defined by input voltages. These inputs are controlled by 16 DACs.

Results: The device testing is ongoing.
Conclusions: A preclinical study will be conducted to verify the prototype of the implant.

S129. Middle Ear Ossicular Joint Changes in Type 2 Diabetes Mellitus: A Histopathological Study

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Category: Clinical Otolaryngology and Pathology
Background: Diabetes mellitus type 2 (DM) affects approximately 37 million Americans and is associated with a threefold increase in the risk of hearing loss. While previous research has indicated inner ear changes in DM patients, the underlying pathophysiological mechanisms remain unclear. Recent studies have proposed that abnormalities in the ossicular joints and ossicles may lead to sensorineural hearing loss due to altered sound transmission biomechanics. However, no prior study has explored the middle ear, particularly the ossicles and their joints, in DM patients. This study aimed to investigate whether type 2 DM is associated with middle ear changes, specifically affecting the ossicular chain and joints.
Methods: Histopathological analysis was conducted on archived human temporal bones obtained from the Otopathology laboratory at the University of Minnesota. Specimens with DM type 2 were included, while those with processing artifacts or confounding diseases were excluded. The study included 47 ears from 25 patients with DM (Male=13, Female=12, Age: 51.0±20.5) and age- and sex-matched controls (Male=10, Female=10, Age: 54.8±15.9) (p=0.991). Otopathological evaluations of the auditory ossicles and Incudomalleolar joint (IMJ) were performed using light microscopy. Various measurements, such as malleus and incus hyalinized cartilage distances, bone-line distance, total cartilage distance, calcified cartilage distance, and joint discus distance, were quantified using ImageJ software.
Results: In the IMJ of DM cases, malleus hyalinized cartilage (Malleus hC) and incus hyalinized cartilage (Incus hC) were significantly increased compared to control cases (Malleus hC; DM cases 34.17±9.71μm vs. control cases 21.96±4.16μm, p less than 0.001) (Incus hC; DM cases 35.11±10.12μm vs. Control cases 22.42±4.368μm, p less than 0.001). In additon, bone-line distance was significantly longer than in DM cases than Control cases (DM cases 266.72±59.11μm vs. Control cases 239.81±35.56μm p=0.040). On the other hand, joint discus distance was longer in the control group than in DM cases (DM cases 96.84±36.80μm vs. Control cases 113.63±23.81μm, p=0.001). No apparent bone erosion was observed in the auditory ossicles.
Conclusions: This study reveals a notable increase in the hyalinized cartilage layer and bone-line distance accompanied by reducing joint discus distance within the IMJ in DM cases. These findings suggest that DM may influence microjoints, such as the IMJ, and potentially impact auditory function. Furthermore, the distinct

nature of pathological changes in the IMJ associated with DM, as compared to those seen in presbycusis, highlights the need for further investigation into the precise mechanisms underlying these changes. The implications of these subtle cartilage layer differences in IMJ remain uncertain but suggest a potential link between DM and microjoint alterations in the middle ear.

S130. Immune Profiling of Secreted Factors From Human Vestibular Schwannoma Cells and Tumor-Associated Macrophages

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Category: Other, Vestibular schwannoma

Background: This study compared the immune-related secretory capacity of human vestibular schwannoma (VS) and tumor-assisted macrophages (TAMs) with their normal counterparts (Schwann cells [SC] and peripheral blood monocyte-derived macrophages [Mo-MFs], respectively), and examined relationships with presurgical hearing and tumor size.

Methods: VS tumors (n=16), auditory nerve (n=1), blood (n=9), and great auricular nerves (n=3) were used. SCs (S100B+) and TAMs (CD68+) were isolated from VS tissue for culture. The secreted levels of 65 immune-related factors were measured and compared using unpaired t-tests with Welch correction (schwannoma vs. SCs) or Mann-Whitney tests (TAMs and Mo-MFs). Associations between factor concentration and word recognition (WR), pure-tone average (PTA), and tumor size were evaluated with Spearman correlation.

Results: Secreted factors with significantly higher concentrations in schwannoma vs. SC supernatants included IL-2 and BAFF, while MMP-1, IL-6, FGF-2, VEGF-A, MIP-3α, and GRO-α concentrations were significantly higher in TAMs vs. Mo-MFs (all p less than 0.05). Worse WR was significantly associated with higher secretion of fractalkine, eotaxin-3, CD30, and IL-16 by VS cells; IP-10, eotaxin-3, multiple interleukins, GM-CSF, SCF, and CD30 by TAMs; and TNF-α and MIP-1α by Mo-MFs (all p less than 0.05). Worse PTA was significantly correlated with higher secretion of IL-16 by VS cells (p less than 0.05). Larger tumor size was significantly correlated with higher secretion of eotaxin by VS cells, and of IL-7, IL-21, and LIF by TAMs (all p=0.017).

Conclusions: Differential secretion of immune-related factors was observed in schwannoma vs. normal SCs and in TAMs vs. Mo-MFs, some of which were correlated with worse hearing and larger VS tumors.

S131. Identification of Immune-Related Candidate Biomarkers in Plasma of Patients With Sporadic Vestibular Schwannoma

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Category: Other, Vestibular Schwannoma

Background: Vestibular schwannoma (VS) is an intracranial tumor arising from neoplastic Schwann cells, and typically presenting with hearing loss. The traditional belief that hearing deficit is caused by physical expansion of the VS, compressing the auditory nerve, does not explain the common clinical finding that patients with small tumors can have profound hearing loss, suggesting that tumor-secreted factors could influence hearing ability in VS patients.

Methods: More than 170 patients with sporadic VS and 110 healthy controls were organized in discovery and validation cohorts. In the discovery cohort, 159 patients, including 34 with good hearing (GH) and 121 with poor hearing (PH) were compared with 70 healthy controls. The validation cohort comprised 50 patients, including 13 with GH and 32 with PH, all compared with 43 controls. GH was defined as word recognition score (WR) greater than 70% and pure tone average (PTA) less than 30 dB (AAO-HNS class A hearing). Otherwise, patients were classified as having PH (AAO-HNS class B, C, and D hearing). Immuno-profiling

of 66 immune-related factors was performed using ELISA, Luminex, and electrochemiluminescence-based human assays. Generalized linear mixed-effects regression models were used to assess the relationship between candidate biomarkers' levels in the plasma of VS patients and controls vs. clinical variables (WR, PTA, and tumor volume). Receiver-operating characteristic curve (ROC) analysis was used to evaluate the diagnostic power and utility of significantly elevated candidate plasma biomarkers.
Results: Twenty of the 66 profiled factors were detectable in the plasma of greater than 75% of VS patients and considered candidate biomarkers. The levels of 14 candidate biomarkers were found to be significantly different between the VS and control groups in the discovery cohort, and 13 of them were confirmed as significantly different in the validation cohort. In both VS and control patients, the highest mean plasma values were detected for IL-2R, SDF-1α, and TWEAK, while TNF-R2 was the most elevated factor in VS patients compared to controls. We further identified and validated S100B and MCP-3 as candidate biomarkers associated with tumor size and hearing, respectively. S100B levels were associated with tumor size and the likelihood of incomplete tumor resection, while MCP-3 levels were associated with pre-operative hearing preservation and were significantly elevated in patients with serviceable hearing. Furthermore, we demonstrated and validated the diagnostic utility of 9 out of 13 candidate biomarkers and identified a 9-biomarker panel (TNR-R2, MIF, CD30, MCP-3, IL-2R, BLC, TWEAK, eotaxin, S100B) with outstanding discriminatory ability for VS.
Conclusions: The robust immune profiling of blood plasma from a large cohort of patients with sporadic VS revealed possible therapeutic targets for VS, providing a unique diagnostic tool that may predict hearing change and tumor growth in VS patients, and may inform the timing of tumor resection to preserve hearing.

S132. Changes in the Oral and Nasal Microbiota in Pediatric Obstructive Sleep Apnea

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Category: Clinical Otolaryngology and Pathology
Background: Background: A few clinical studies have demonstrated that pediatric obstructive sleep apnea (OSA) is associated with dysbiosis of airway mucosal microbiota. However, how oral and nasal microbial diversity, composition, and structure are altered in pediatric OSA has not been systemically explored.
Methods: Methods: In total, 30 polysomnography (PSG)-confirmed OSA patients with adenoid hypertrophy, and 30 controls who did not snore or have adenoid hypertrophy, were enrolled. Swabs from four surface oral tissue sites (tongue base, soft palate, both palatine tonsils, and the adenoids) and one nasal swab from both anterior nares were collected. The 16S ribosomal RNA (rRNA) V3–V4 region was sequenced to identify the microbial communities.
Results: Results: Alpha diversity were not significantly different between the pediatric OSA patients and controls at the five upper airway sites. However, the beta diversity and microbial profiles were significantly different among different upper airway sites. The abundances of Haemophilus, Fusobacterium, and Porphyromonas were higher at adenoids and tonsils sites of pediatric patients with OSA. Functional analysis revealed that the differential pathway between the pediatric OSA patients and controls involved glycerophospholipids and amino acid metabolism. Several genera in oral sites significantly correlated to PSG-derived sleep variables were identified in pediatric OSA patients, which might shed light on the pathogenesis and diagnosis of pediatric OSA.
Conclusions: Conclusions: In this study, the oral and nasal microbiome of pediatric OSA patients exhibited certain differences in composition compared with the controls. However, the microbiota data could be useful as a reference for studies on the upper airway microbiome or other relevant clinical phenomena.

S133. Effectiveness of the Botulinum Toxin Injection for Facial Nerve Palsy in an Animal Model

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Category: Other, Facial nerve

Background: Botulinum toxin (BTX) has been used for the management of facial palsy (FP) in adjunct to oral steroid medication. However, the mechanism of the faster recovery of the paralyzed facial nerve through contralateral BTX injection has never been identified. Aim of this study is to evaluate the effectiveness of contralateral BTX injection during the process of recovery from peripheral FP in a rat FP model.
Methods: Adult SD rats were divided into 3 groups: (1) control group (CG), (2) facial palsy group (FPG) and (3) FP with contralateral BTX injection group(FPBG). Functional outcomes were measured and evaluated at 3-day, 2-weeks and 4-weeks after inducing FP which was performed by crushing injury to the main trunk of facial nerve near the stylomastoid foramen for 1 minute with mosquito. Twenty mouse unit of BTX was injected at 1 day after the surgery at three points of facial muscles on healthy side in FPBG. Fluorescence histochemistry of target-muscle end plate was performed with longitudinal sections of levator labii superioris muscle on the injection side of the face 2-weeks after FP induction. Qualification of the regenerated neuromuscular junction among 3 groups was performed by calculating SY38/a-bungarotoxin ratio differences.
Results: Facial palsy was successfully induced in all rats after crushing injury. The first functional outcome, measured by vibrissae observation scale, showed significantly lowered movement score in both FPG and FPBG than CG at 3-day and 1-week follow up points. However, at 2-week time point, significant difference was only found between control and FPG. The second functional outcome, measured by angle to indicate facial symmetry, showed significantly more deviated angle only in FPG compared with control group. In FPBG, no significant differences in angle compared to CG from 3-day to 2-weeks follow up points were observed. In fluorescence histochemistry study, significant difference of the SY38/a-bungarotoxin ratio among 3 groups at 2 week-follow up was observed and the ratio differences of CG and FPBG were significantly lower than FPG. There was no significant difference of the SY38/a-bungarotoxin ratio between CG and FPBG.
Conclusions: FP was successfully induced with just one minute of nerve crush in this study, and the contralateral BTX injection facilitated the faster recovery of FP. Neuromuscular junction regeneration has significantly well occurred after contralateral BTX injection. Studies identifying more changes of neuromuscular junction as well as several brain regions at various time points to reveal the mechanism of the contralateral BTX injection for the faster FP recovery are on going.

S134. Ultrasonic Vocalizations in Normal-Hearing ChAT-Cre and CBA/CaJ Mouse Strains

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Category: Other, Acoustic Communication
Background: Modulatory neural circuits utilizing acetylcholine (ACh) impact a broad range of auditory circuits and functions, from adjustment of cochlear sensitivity to enhancement of plasticity in auditory cortex. We propose an additional function: modulation of processing of acoustic communication signals by the basolateral amygdala (BLA) resulting from cholinergic input from the basal forebrain. Further study of this function will benefit from an ability to manipulate cholinergic input to BLA. The recent development of a normal hearing mouse strain in which Cre recombinase is expressed only in cholinergic neurons will enable studies of cholinergic modulation of vocal responses in BLA. These studies will require an understanding of the social vocalizations of this strain and how these may differ from the well understood CBA/CaJ strain.
Methods: A normal hearing ChAT-Cre mouse strain was developed using methods described previously (Beebe et al., Hearing Research 388:107896 (2020)). Recordings of social vocalizations were obtained from adult male and female mice (ages 3-9 months) of ChAT-Cre and CBA/CaJ strains. Two Avisoft ultrasonic-sensitive microphones (CM16/CMPA) were placed in an arena within a sound attenuating chamber. Recordings were obtained under several conditions: isolation, same-sex pairs, and male-female pairs displaying courtship behavior. Recorded signals were analyzed using Avisoft-SASLab Pro and VocalMat (Fonesca et al., eLife 10:e59161) to assess several features of ultrasonic vocalizations within each recording condition: vocalization rate, syllable composition, and features of the major syllables emitted in each condition including duration and minimum/maximum frequency.
Results: In social conditions that included males (male-male, male-female), the calling rate of ChAT-Cre mice was substantially higher than CBA/CaJ mice (means for mating: 78.4 syllables/min vs 25.2

syllables/min) Viewed across recording conditions, both strains utilized the same syllable types most commonly—short, up-FM, and down-FM—but also emitted flat, chevron, and stepped syllables less commonly. Across these syllables, there was a consistent difference in sound frequency of vocalizations between the two strains. On average, ChAT-Cre syllables were almost always higher in minimum frequency: 0 kHz (flat), 2.7 kHz (short), 4.0 kHz (up-FM), 5.8 kHz (stepped), 10.5 kHz (down-FM), and 11.6 kHz (chevron). Average maximum frequencies of these syllables were also higher, by 3.6-23.5 kHz in ChAT-Cre mice. The durations of syllables were not different between the two strains.
Conclusions: These results show similarities in the vocal communication repertoire of the two mouse strains, but important differences in calling rate and sound frequency that may be matched by differences in auditory processing capabilities. These differences require the use of strain-specific vocalizations in behavioral and neural playback studies.

S135. Reduced Auditory Thalamo-Cortical Connectivity (Acoustic Radiations) Due to Blast Exposure in Veterans With Auditory Deficits: A Diffusion Tensor Imaging Study

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Category: Other, Auditory Radiations
Background: Veterans of recent military conflicts have experienced a high rate of mild traumatic brain injuries from exposure to blasts (bTBI), which often result with debilitating auditory disturbances (HD), such as hearing loss and tinnitus. The primary tool for investigating white matter integrity is diffusion tensor imaging (DTI). Unfortunately, prior studies using this tool failed to identify the neuroanatomical correlates of HD in this population. A possible reason for this challenge is that many past DTI studies attempting to identify the neuroanatomical markers of bTBI have ignored the broad range of cumulative blast exposure among Veterans, and therefore potentially reduced sensitivity to associations between bTBI and DTI metrics.
Methods: Herein, we compare commonly used DTI metrics: fractional anisotropy and mean, axial, and radial diffusivity (FA, MD, AD, RD) in U.S. Military Veterans with self reported HD and a history of blast exposure using both the traditional method of dividing the participants into two equally weighted groups, and an alternative method, wherein each participant is weighted by their blast exposure quantity, severity, and recency.
Results: While no differences in FA, MD, and AD (and minimal in RD) were detected using the traditional method, the alternative method revealed diffuse and extensive changes in all four DTI metrics associated with bTBI. These effects were quantified within 80 anatomically-defined white matter tracts as the percentage of voxels with significant changes, which identified the fornix, acoustic radiations, and optic radiations as the pathways most affected by bTBI (with over 50% of the voxels affected). Moreover, additive effects of aging were present in the same tracts suggesting that the neuroanatomical effects of bTBI may compound with age.
Conclusions: The division of study participants into two groups is a commonly employed strategy in neuroscience research. In the present study, however, we demonstrate a shortcoming with this approach, and present an alternative solution. In particular, our approach successfully demonstrated a relationship between HD in bTBI and damage to an auditory structure, the acoustic radiations. In future studies, we plan to further compare Veterans with and without HD to validate this finding.

S136. Prestin as a Biomarker for Sensorineural Hearing Loss in Human Perilymph, Cerebrospinal Fluid, and Blood

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Category: Clinical Otolaryngology and Pathology

Background: While diagnosis of sensorineural hearing loss (SNHL) requires trained personnel, time, and specialized equipment, it only seldomly allows for risk stratification or identification of a subclinical SNHL. Currently, no biomarker is available for diagnosis of SNHL, but finding such a molecule could provide a cheap, safe, and quick alternative to screen for SNHL. Furthermore, a biomarker could mend the shortcomings of current diagnostic means by allowing to objectify and expediently classify degrees of SNHL. Prestin, the outer hair cell motor protein, has been proposed as such a biomarker for SNHL. Hence, this study's goal was to explore the protein's potential by analyzing various different clinical samples. Prestin concentration was determined in human blood, cerebrospinal fluid (CSF), and perilymph (PL) and subsequently correlated to clinical outcome parameters to determine its suitability as a biomarker for SNHL.
Methods: Blood, CSF, and PL samples were collected intraoperatively in patients with tumors of the internal auditory canal during middle fossa or translabyrinthine approaches. Subsequently, prestin concentration was measured using enzyme-linked immunosorbent assay, and then analyzed in correlation with clinical parameters including audiometric and vestibular test results.
Results: Overall, samples were collected from 42 patients (26 women; median age: 53 years). With a mean concentration of 2.15±4.95 ng/mL, prestin levels were lowest in blood samples, while its concentration in PL samples (96.31±91.86 ng/mL) was significantly higher compared to CSF samples (12.31±17.38 ng/mL). Nonetheless, the prestin levels of the three compartments did not correlate with each other. However, a significant association between CSF prestin concentration and preoperative audiometric test results notably in the middle to high frequency hearing range was observed. Translabyrinthine surgeries did not lead to a significant rise of prestin levels in sequential blood samples.
Conclusions: In accordance with previous studies, these findings suggest that prestin could represent a suitable biomarker for SNHL. Therefore, determining prestin concentration in a clinical setting may aid screening for, and following up patients with SNHL, and hence could greatly impact clinical decision-making. Nevertheless, the observed discrepancies in blood, CSF, and PL concentrations and the novel findings of CSF prestin levels' correlation to audiometric results should prompt new investigations into prestin homeostasis, its temporal dynamic after cochlear trauma, and differences between distinct etiologies of SNHL.

S137. Effect of Yap Inhibition on Radiation Response in NF2-Mutant Schwann Cells and Primary Vestibular Schwannoma

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Category: Other, Vestibular Schwannoma Treatment
Background: Vestibular schwannomas (VS) are benign, intracranial tumors that arise from Schwann cells of the eighth cranial nerve. Approximately 93% of cases occur sporadically and involve either the right or left eighth cranial nerve, while the remaining 7% occur bilaterally and are associated with a genetic condition called Neurofibromatosis Type 2 (NF2). It is estimated that 1 in 500 people will be diagnosed with a VS in their lifetime. These tumors may be associated with hearing loss, dizziness, and life-threatening neurological complications related to brainstem compression. Management of these tumors usually involves observation with surveillance imaging, microsurgical resection, and/or radiotherapy. For patients undergoing radiotherapy, 9-12% may exhibit radiation resistance and continue to progress with time. One pathway associated with radiation resistance in cancer is the Hippo/Yes-associated protein (YAP) signaling pathway. Several investigations have demonstrated that inhibition of YAP-related pathways can sensitize certain tumors to radiotherapy. In this study, we evaluate the effectiveness of a YAP inhibitor (YAPi) called YAP-TEAD Inhibitor 1 (Peptide 17) in sensitizing NF2-mutant Schwann cells and primary VS cells to radiation treatment.
Methods: With informed consent, fresh tumors were harvested from patients undergoing VS surgery, and primary VS cells were cultivated. Human NF2-mutant Schwann cells (HS01), mouse NF2-mutant Schwann cells (MTC), and primary human VS cells (N=3) were cultured on 384-well plates (5000 cells/well; n=6 replicates per treatment condition) and pre-treated with YAPi (0, 0.1, 1, or 10 μM concentrations) for 24 hours prior to irradiation (0 Gy or 18 Gy). Cell viability was measured 72 hours post-radiation using a cell-based assay. Immunohistochemistry and fluorescent microscopy were performed to confirm gamma-H2AX (marker of DNA damage) and total YAP expression. Statistical analysis was performed using 95% confidence intervals and two-way analysis of variance.

Results: In HS01 cells, small reductions in viability were seen with 10 μM of YAPi alone and 18 Gy of radiation alone; however, treatment of cells with 10 μM of YAPi alone and 18 Gy of radiation caused large reductions in viability (p less than 0.001), compared to individual treatments alone. The synergistic effect of YAPi and radiation were not observed in MTC cells, suggesting YAPi may be more effective in human NF2-mutant Schwann cells. The effects of YAPi and radiation will be described for individual primary VS cells and for nuclear and cytoplasmic expression of gamma-H2AX and total YAP for all cell types used.
Conclusions: YAP inhibition synergizes with radiation to reduce viability of NF2-mutant human Schwann cells and may be effective as a mode of radiosensitization in primary VS cells. Further investigations into the mechanisms of YAP inhibition and radiation toxicity are warranted to identify effective therapies that can be translated to preclinical models and clinical trials for vestibular schwannoma.

S138. Eargenie - A Clinician-Friendly fNIRS System to Evaluate Infant Speech Detection and Discrimination

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Category: Other, Infant Speech Detection and Discrimination
Background: When a sound is heard, there is a change in blood flow or oxygen concentration in prefrontal and/or temporal regions of the brain. An fNIRS (Functional Near- Infrared Spectroscopy) system contains a noninvasive set of infrared emitters and detectors placed on the surface of these regions and is designed to measure changes in brain activity due to auditory stimuli. Existing fNIRS systems leverage open-source data cleaning and statistical modelling techniques, that infer whether auditory processing is indeed taking place and that the changes are not just arbitrary. However, it is not clear whether such systems can do this consistently at an individual level and specifically for speech discrimination.
Our contributions are the following: (1) We present speech presentation protocols algorithms capturing blood flow changes and enabling measurement of speech discrimination and detection at an individual subject level with high sensitivity. (2) Our method and systems are captured in our patent portfolio. We have developed a new fNIRS system, EarGenie®, that is wireless, portable and hence clinically friendly; we are aiming to be the first such device that is approved by the FDA for clinical use. (3) In this system, our algorithms run online and present results to the clinician as they are gathered, which enables early stop of the test.

Methods: We tested between 16-38 subjects, who had no known hearing loss, with consonant-vowel speech tokens ranging from 35-90 dBSPL. To demonstrate the sensitivity and specificity of the algorithm, we show speech detection results to a “ba” token and speech discrimination results gathered from 3 different speech-token contrasts: “tea/ba”, “ga/ba”, “bee/ba”, where data are gathered from off-the-shelf NIRx NIRScout fNIRS system. The NIRx contains 16 channels with 4 channels each in the prefrontal and temporal regions in both the left and right hemispheres. The wireless EarGenie optodes consist of a similar configuration.
Results: Our results show sensitivity for the discrimination of the “tea/ba” contrast of 94-100%, for “bee/ba” of 88-93% and for “ga/ba” of 78-92% across 4 regions in the temporal and prefrontal cortex at a specificity of 95%. These results were obtained after the algorithm automatically stopped anywhere between 3-7 trials per token which translates to 3 minutes per token.
Similarly, for detection of the “ba” sound, we found a sensitivity of 97-100% at 65 dB SPL and 100% for 35-90 dB SPL across 38 subjects. A 0 dB false positive test was applied separately across 14 subjects with a specificity of 95%.

Conclusions: Results demonstrate that the EarGenie test protocols and analysis algorithms are robust and accurate and our portable prototype device is ready for further clinical validation and assessment across a wider cohort of subjects.

S139. Visually Evoked Potentials in Early and Late-Onset Hearing Loss

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Category: Hearing Loss: Consequences and Adaptation

Background: The loss of a sensory modality triggers a phenomenon referred to as cross-modal plasticity, which involves the reorganization and adaptation of brain areas responsible for the lost sense in favor of the remaining senses. In congenital and early deafness, both humans and cats have demonstrated enhanced visual motion detection abilities, and in cats this phenomenon has been casually attributed to reorganized auditory cortex. This study investigated the electrophysiological responses of hearing, early-deaf, and late-deaf cats to motion-onset stimuli of varying speeds. We hypothesized that greater neural activity would be captured in deaf subjects compared to hearing in response to the motion-onset stimuli due to the cross-modal reorganization of the visual and auditory systems after hearing loss.

Methods: Seven hearing, seven early-deaf, and four late-deaf cats were examined. Deafness was induced by administering ototoxic drugs systemically during the first postnatal month for early-deaf subjects and in maturity (beyond 6 months) for the late-deaf subjects. In adulthood, visually evoked potentials (VEPs) were recorded and analyzed from electroencephalogram (EEG) recordings while the subjects were under light anesthesia. VEPs represent averaged and amplified signals of the gross electrical action potentials generated by the brain in response to visual stimuli. This technique is commonly used in ophthalmology to assess the functional state of the visual system. The stimuli used consisted of 200-ms long leftward-moving dots with coherent motion. These dots (in the same trials) were randomly positioned, and the speed of the dots from different trials varied between 2 and 64 degrees per second. VEP waveforms were derived from averaging 160 trials for each speed. Parameters such as peak amplitudes and root-mean-squared (RMS) values were studied for all VEP waveforms.

Results: Across all subject groups, peak amplitudes increased as stimulus speed rose. Significantly larger peak amplitudes and RMS values were observed in the early-deaf subjects at higher speeds (8 deg/s and above, as determined by Mann-Whitney U tests with p less than 0.05) compared to the hearing controls and 2-way mixed effects ANOVAs demonstrate that the effect of early deafness was significant in both parameters. Furthermore, late-deafened animals demonstrated an increasing trend in VEP amplitudes throughout recordings in a time course investigation after the onset of deafness.

Conclusions: These findings suggest that cross-modal reorganization could contribute to the improved motion detection thresholds observed in deaf subjects, and in turn may also play a role in enhancing their neural response to visual motion stimuli resulting in larger measured VEPs. Overall, our work demonstrates the functional changes in the brain after hearing loss and establishes the evaluation of VEPs as an additional non-invasive tool in clinic to assess cross-modal plasticity in the deaf.

S140. Influence of Visual Speech Cues on Auditory Brainstem and Cortical Evoked Responses in Normal and Impaired Ears

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Category: Hearing Loss: Consequences and Adaptation

Background: The human brain can integrate information from different sensory modalities to produce a unified representation of the external world. Temporally congruent auditory and visual stimulation facilitates multisensory perception and integration, allowing us to better process and understand the information presented to us (1-6), especially in the presence of background noise (7-9) and for individuals with hearing difficulties(10). Interestingly, when auditory and visual inputs are conflicting, the auditory perception may partially or completely be determined by the visual input (11), and this phenomenon has been extensively studied as the McGurk effect (12). Functional magnetic resonance imaging, and electrophysiological studies have mostly evaluated auditory cortical responses. Little is known about the influence of visual speech production cues on the auditory brainstem phase-locked ensemble responses in normal and individuals with sensorineural hearing loss. To address this knowledge gap, we evaluated the effects of congruent- and incongruent-audiovisual (AV) speech on scalp-recorded brainstem frequency following response (FFR) and the cortical acoustic change complex (ACC). For the FFR, both phase locking to envelope periodicity (FFRENV) and to the temporal fine structure (FFRTFS) were evaluated. The concurrent recording of these responses allowed us to evaluate the influence of visual speech cues on neural encoding at two levels of processing along the auditory neuraxis.

Methods: FFR and ACC responses were recorded concurrently from 23 normal-hearing young adults (18-55 years) and 15 hearing-impaired (mild-to-moderate SNHL) adults using CV syllables /ba-da/. Responses were recorded for audio-alone; and AV with congruent visual speech cues; and AV with incongruent visual speech cues. Post-acquisition, EEG responses were filtered 70-2500 Hz to visualize subcortical EFR-FFR and 1-30Hz to visualize cortical ACC responses.
Results: Spectral data for both syllables showed robust f0 (FFRENV data), and the formant-related peaks (FFRTFS data) for both groups in the audio-alone condition. Comparison of the FFR response magnitude in the congruent and incongruent AV conditions, revealed response enhancement for both envelope (f0) and TFS components (F1, F2) for the normal-hearing individuals in the congruent condition only. In contrast, hearing-impaired individuals showed facilitation for only the FFRENV component (f0), with degraded TFS representation (F1, F2) in all conditions. Compared to audio-alone the amplitude of the P1-N1 and N1-P2 of the ACC increased for the congruent-AV conditions and decreased for the incongruent-AV conditions in both groups. Overall amplitudes of the hearing-impaired group were poorer than that of normal-hearing group.
Conclusions: These initial results showing that visual speech cues do influence evoked responses at both cortical and brainstem levels, albeit differently, is encouraging. These results appear to suggest that neural activity relevant to audio-visual integration may be already emerging at the brainstem level. Thus, these measures have the potential to be developed as clinical metrics for evaluation of audio-visual integration, and as measures to evaluate hearing-aid outcomes and prognosis.

S141. Accessibility of Speech Used in Broadcast: Implications for Older Listeners With Hearing Loss

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Category: Hearing Loss: Consequences and Adaptation
Background: The problem of unintelligible speech in television and broadcasting ("broadcast speech") has attracted considerable media coverage. The British Broadcasting Corporation (BBC) previously identified 4 key factors which their audience frequently complain about: lack of speech clarity, unfamiliar or strong accents, background noise and background music (Armstrong, 2011). Here we developed a novel test platform to assess the contribution of these key factors to the comprehension of broadcast speech, and to the listening effort needed to understand it.
Methods: Seventy-five native-English speakers (41 female, aged 51-75 years) took part in this study: 37 participants had 'normal hearing' (NH group) while 38 had sensorineural hearing loss and wore hearing aids (HAs) for greater than 2h/day (HA group). Novel stimuli were created from BBC programmes by mixing excerpts of "foreground" broadcast speech with plausible "background noise" recorded for the same programme. Excerpts of "foreground" broadcast speech were selected based on the subjective diversity (within the UK and Ireland) and strength of the accents used by the actors or presenters. The eSTOI model (Jensen and Taal, 2016) was used to equate predicted intelligibility across stimuli and hence the mixes presented in this test platform were different from the original broadcast mixes. The comprehension of the speech was measured using multiple choice questions designed to assess overall understanding of each excerpt, rather than the intelligibility of individual words. The time taken to answer the multiple-choice comprehension question was used as an objective measure of listening effort. Participants were asked to provide subjective ratings of their listening effort and also to report on relative contributions of the factors "clarity", "accents" and "background noise" to their listening effort.
Results: One-tailed t-tests showed that the HA group performed significantly more poorly than the NH group in terms of comprehension of broadcast speech. Significantly more objective and subjective listening effort was measured for the HA group. The HA group reported that the factors "clarity" and "background noise" contributed to more of their listening effort than the NH group. There was no significant difference between HA and NH groups in the perceived contribution of "accents" to listening effort. Linear regression was used to model the potential predictors (age, hearing thresholds; ratings of "clarity", "accents", "background noise") of comprehension and listening effort. Comprehension of broadcast speech was predicted by hearing thresholds only. Objective listening effort was predicted by age only. Subjective listening effort was predicted by the factors "clarity" and "background noise".

Conclusions: These results suggest that broadcast speech is more difficult to understand and more effortful for older people who wear hearing aids than for older people with normal hearing. Improving the clarity of broadcast speech and permitting personalized foreground-background mixes based on multi-factor demographics may improve the accessibility of broadcast speech.

S142. Cross-Species Investigations of Place and Time Coding of Pitch Using Envelope-Following Responses

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Category: Hearing Loss: Consequences and Adaptation

Background: An elusive empirical neural explanation for pitch perception has sparked a multitude of cochlear place and time-dependent hypotheses. Most experts debate the importance of tonotopy versus temporal coding, but it is still unclear how disruptions of these mechanisms are influenced by sensorineural hearing loss (SNHL). SNHL is complex, but as a diagnosis, it groups variable degrees of inner and outer hair cell (IHC/OHC) and cochlear synapse (CS) damage which likely results in different patterns of pitch perception. With careful stimulus design and electrophysiological data harmonization, a more direct link between neural coding and pitch perception can be drawn. Here, we describe a novel cross-species study framework that leverages Envelope Following Responses (EFRs) to tone complexes collected in animal models of hearing loss and human subjects with diverse hearing status to better understand this link.

Methods: EFRs to six tone complex stimuli (F0 = 103 Hz, six harmonics, 80 dB SPL) varying in harmonic rank have been collected in both human subjects and chinchillas with normal hearing (determined using Pure Tone Audiometry and ABR thresholds, respectively). The harmonics were presented with alternating phases to elicit higher phase-locking value (PLV) energy at the envelope frequency (2*F0) as more harmonics are unresolved by auditory filters. EFRs were recorded using a 32-channel EEG cap with the BioSemi ActiveTwo System in humans (N=7), while subdermal electrodes with custom MATLAB software were used in chinchillas (N=7). Data collection in both chinchillas (IHC/OHC/CS damage) and humans with hearing loss is ongoing.

Results: The expected increase in 2*F0 energy and harmonic rank identifies a resolved to unresolved transition point with increasing harmonic rank in both species. However, findings in normal hearing chinchillas indicate that this transition point occurs at a lower harmonic rank than in humans, and is steeper at high sound levels, both effects likely due to broader cochlear filtering. Tone complex presentations at lower sound levels (65-70 dB SPL) in chinchilla elicit a gradual transition point that is particularly helpful when examining the effects of disrupted tuning in animal models of cochlear hearing loss. Preliminary chinchilla data suggest this transition point does not deviate substantially following selective IHC and CS damage (as expected), despite EFR amplitude and morphological changes.

Conclusions: Collecting identical EFR measures across species serves to not only relate the neural coding of tone complexes to human cortical and perceptual measures of pitch discrimination but also to attribute variability in these measures across individuals to specific cochlear deficits induced in chinchilla hearing loss models. Considering level-dependent effects driven by differences in cochlear filtering will maximize the utility of this framework.

S143. Loudness in the Normal Ear of Single-Sided Deaf Listeners

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Category: Hearing Loss: Consequences and Adaptation

Background: Single-sided deafness (SSD), also when acquired in adulthood, has been shown to lead to a reduction in inhibition and an increase in excitatory responses in inferior colliculus and auditory cortex in the ipsilateral hemisphere when sounds are presented to the normal ear (Mossop et al., Hear. Res., 2000; Tillein et al., Cereb. Cortex, 2016). These increased excitatory responses may lead to an increase in loudness. Therefore, we examined loudness in adult subjects with acquired SSD hypothesizing an increase compared to

normal-hearing (NH) controls. Additionally, we examined the effect of cochlear implantation (CI) in the deaf ear on loudness perception in the normal ear of the SSD patients.
Methods: Narrow-band noises with center frequencies at 0.5, 1, 2 and 4 kHz were presented monaurally to 16 adult NH subjects (age range 22-55 years) and 18 SSD subjects (age range 19 – 73 years). The listeners were seated 1 m from the speaker; the NH subjects were tested at each ear with the other ear plugged and covered by an earmuff. The SSD subjects were tested twice: 1 to 4 months before and 4 to 8 months after cochlear implantation (Peters et al., PLoSOne, 2021). We applied the Oldenburg Adaptive Categorical Loudness Scaling (ACALOS) procedure. Accordingly, sound levels were varied between -10 and 105 dB SPL, and the listeners had to rate the loudness using 11 categorical units from 0 (inaudible) to 50 (too loud). Data were fitted according to Brand and Hohmann (JASA, 2002) yielding 3 outcome measures: slope of the lower segment of the loudness function (mlow) slope of the higher segment (mhigh), and the level at the intersection of the two slopes (Lcut).
Results: Slope mlow was significantly steeper for SSD subjects than for the NH subjects, which can be largely attributed to higher thresholds for the SSD subjects (pure tone averages 13 dB HL versus 4 dB HL). Slope mhigh did not differ between the two groups. The level at the intersection of the two slopes, Lcut, was lower for SSD subjects than for NH subjects by 2 to 8 dB (repeated measures ANOVA, p=0.06), most prominently at 0.5 and 1 kHz, which reflects an increase in loudness in the SSD group. CI of the deaf ear had no significant effect on the three loudness function measures in the normal ear.
Conclusions: Our data confirmed the hypothesis of an increase of loudness, which we assume is caused by an increase in excitatory neural responses in the ipsilateral hemisphere which in its turn is caused by a reduction in inhibition from the contralateral ear.

S144. Evaluation of the Benefits of Replacement Hearing Aids (HAs) and Cochlear Implant (CI) and Hearing Aid in a Bimodal Solution With Hearing in Noise Test (HINT), Pupillometry and the Speech, Spatial and Qualities of Hearing Scale (SSQ12)

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Category: Hearing Loss: Consequences and Adaptation
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. Speech perception in noise can be studied with the Hearing in Noise Test (HINT) and the task engagement during the test can be studied and quantified with pupillometry as there is a correlation between pupil size and complexity of a given task.
The present study investigates changes in signal to nose ratio (SNR) using HINT under constant task engagement measured with pupillometry between existing HAs and replacement HAs as well as HA+CI in the bimodal solution.

Methods: The population consist of 55 bilateral HA users with mean age of 62 years (range 23-83) referred for CI and randomized to one month vs. four months of HA use before cochlear implantation. The hearing aids were replaced with new HAs (Phonak Link M or GN (ReSound LiNXQuattro, ENZOQ) fitted to NAL-NL2 prescription and verified with Real Ear Measurement (REM).
The patients were tested before change of HAs and after use of replacement HAs for one or four months as well as three months after cochlear implantation. SNR was determined at 70% correct word recognition score at 65 dB SPL using HINT where task engagement was controlled with pupillometry measuring peak pupil dilation (PPD). Patients reported hearing difficulties using the Speech, Spatial and Qualities of Hearing Scale (SSQ12) questionnaire at every visit.

Results: Patients with existing HAs (baseline) compared with new replacement HAs after 1 month, showed a non-significant improvement of -0.39 dB in SNR with a mean change in PPD of 0.0082 (0.82%).
When comparing baseline and 4 months follow-up after new HAs there was a mean change in SNR of 1.82 dB and a change in mean PPD of -0.0090 (0.90%) as well as between 1 month follow-up after new replacement HAs and 4 months follow-up after new replacement HAs with a mean change in SNR of -0.09 dB and a mean change in PPD of 0.00058 (0.058%).

HA+CI demonstrates a further mean decrease of -16.2 dB SNR three months after activation in relation to baseline and -11 dB and -10 dB in relation to one- or four- months follow-up of replacement HAs respectively. SSQ12 mean scores increase with 1.3 and 2.6 scale points with replacement HAs and CI+HA respectively in relation to baseline.

Conclusions: Replacement of HAs does not seem to improve SNRs measured with HINT. However, HA+CI in the bimodal solution does seem to improve SNR. Patient reported outcomes using SSQ12 improve when changing HAs and when a CI+HA is used.

S145. The Noise Outcomes in Service Members Epidemiology Study – a 10-Year Cohort Profile Update

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Category: Other, Hearing and tinnitus epidemiology

Background: The Noise Outcomes In Service members Epidemiology (NOISE) Study is a multi-site longitudinal epidemiology study following Service members and Veterans to understand how noise and other military exposures affect hearing and tinnitus over time. Study sites include the Veterans Affairs, National Center for Rehabilitative Auditory Research (NCRAR) in Portland, Oregon and the Defense Health Agency, Hearing Center of Excellence (HCE), in San Antonio, Texas. Recently, data collection expanded to Naval Medical Center San Diego and Camp Pendleton near San Diego, California. The purpose of this update is to describe additions to the data collection and point to new findings.

Methods: Enrollment in the NOISE Study began in 2014 and remains ongoing. Comprehensive audiologic exams are collected at baseline and every five years, which include pure-tone and speech audiometry (in quiet and in noise), distortion-product otoacoustic emissions, and immittance, with some participants completing central auditory processing and auditory brainstem response testing. Participants with tinnitus undergo a comprehensive tinnitus psychoacoustic assessment. Approximately 15-18 questionnaires are collected at baseline and then annually, asking participants about noise, chemical and blast exposures, traumatic brain injury, physical and psychiatric comorbidities, and other military and nonmilitary exposures and outcomes that can affect auditory function. The study will begin collecting 10-year follow-up data in 2024.

Results: To date, 1390 participants have enrolled (NCRAR n=800; HCE n=517; San Diego n=73), of which 48% are Service members and 52% are Veterans. Annual survey response rates are high across sites with 74% of participants returning at least one survey. Among Service members and Veterans due for their five-year in-person exam, 269 have undergone testing. Analysis of data over five years collected with the Tinnitus Screener (n=1114 individuals and n=3260 surveys) suggests that among those with no tinnitus, occasional tinnitus, or intermittent tinnitus at baseline, the probability of transitioning to reporting constant tinnitus was 5.3%, 9.2% and 16.8% year-to-year, respectively. Comparing audiometric results collected at baseline and at five-years, those with hearing loss at baseline had higher odds of experiencing a threshold shift (greater than 15 dB at any frequency 250-8,000 Hz) in five years compared to those with normal hearing (unadjusted odds ratio=3.1, 95% CI: 1.3-6.9).

Conclusions: Understanding changes in hearing and tinnitus status over time is crucial for the prevention of and understanding of factors contributing to developing auditory issues. After almost 10 years of data collection, we have a greater understanding of the causes of auditory health concerns, and how they progress, which can inform future prevention and treatment options. Continued data collection will produce an unprecedented epidemiologic dataset revealing cross-sectional and longitudinal associations between a wide range of exposures, medical and mental health conditions, peripheral auditory function, central auditory function, and tinnitus, knowledge that will assist with conservation and treatment efforts.

S146. Assessing Physiological Indicators of Subtle Auditory Trauma: ABRs and DPOAEs in Rhesus Macaques at 2 and 8 Months After Noise-Induced Temporary Threshold Shifts With Perceptual Changes

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Category: Hearing Loss: Consequences and Adaptation

Background: Cochlear synaptopathy is a subclinical pathology for which there are no well-defined biomarkers in humans. Our studies in macaque monkeys showed that moderate-level noise exposures cause temporary but not permanent threshold shifts, and deficits in masked detection tasks. Here, we describe changes in the auditory brainstem responses (ABRs), a commonly used non-invasive clinical tool, in the same macaques to assess its utility as a biomarker.

Methods: Testing was conducted in anesthetized young adult macaque monkeys (Macaca mulatta, 6-10 years old) before and after noise exposure (120 dB SPL octave-band noise from 2-4kHz for 4 hours) at two time points: 2-months (n= 9 male, n= 4 female) and 8-months post-exposure (n= 6 male, n= 4 female). ABRs (vertex-to-mastoid) were obtained to tone pips (0.5-32 kHz, in ½ to 1 octave steps), clicks (100µs) and chirps (1.6ms) presented at many sound levels (30-90 dB SPL in 5-10 dB steps). ABRs were also obtained to click pairs at varying interclick intervals (ICI: 10 to 1ms), and to clicks at many presentation rates (200/s to 27.7/s). All ABR Waves (I, II, and IV) were analyzed for amplitudes and latencies. Distortion product otoacoustic emissions (DPOAEs) were measured from 1 – 10 kHz.

Results: ABR and DPOAE thresholds were unchanged at 2- and 8-months post-exposure. Sex-specific ABR amplitude and latency changes were observed with high-level (70-90 dB SPL) frequency-specific stimuli near the noise exposure band. In males, Wave I amplitude increased at 2 months post-exposure but decreased at 8 months, whereas Waves II and IV decreased at 2 months and increased, eventually surpassing pre-exposure values, at 8 months. In females, a similar trend was observed. However, Wave II amplitude, further decreased at 8 months post-exposure. In males, latencies for Waves II and IV increased at 2 months post-exposure; Wave II latencies were higher at 8 months, whereas Wave IV latencies decreased below pre-exposure values. In females, Wave I latencies increased at 2 months post-exposure, and at 8 months post-exposure, decreased past pre-exposure values. While initially stable at 2 months post-exposure, Wave IV latencies in females increased at 4 kHz at 8 months. In both sexes, ABR amplitudes to clicks at higher presentation rates decreased post-exposure compared to pre-exposure values when they were normalized (re: amplitudes at 27.7 clicks/s) to reduce within-subject variability.

Conclusions: Frequency-specific compensatory changes were observed in ABR amplitude and latencies in a sex-specific and level-specific manner that likely reflect homeostatic plasticity in the auditory system. Regardless of sex, broadband stimuli presented in rapid succession highlighted trends at the group level suggesting decreased synaptic function. Ongoing work will correlate physiological, behavioral, and histological findings, to better assess the utility of the ABR as a sensitive indicator of hidden hearing loss.

S147. FTRS

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Category: Tinnitus

Background: Tinnitus is highly prevalent in the population, but there are currently few effective therapeutic interventions. Mobile applications (apps) might be helpful in tinnitus diagnosis and treatment by offering sound or music tools as well as questionnaires. We assessed the efficacy of a free, publicly available smartphone app (Fudan Tinnitus Relieving System, FTRS) for self-management of tinnitus and related symptoms.

Methods: Among a total of 3564 participants recruited primarily online, 2744 patients had complete information at baseline and were an average of 37 years old and were 59.84% male. Web-administered self-report measures THI, HADS, AIS, and other multi-dimensional scales were conducted at baseline and at 1 month and 2 months following treatment. Data from 54 participants who completed continuous follow-up were used for the final efficacy analysis and longitudinal analysis.

Results: Following the intent-to-treat principle, t-tests revealed that the distribution of patients and the tinnitus features of patients of different genders were heterogeneous. One-way ANOVA showed that after using the FTRS app, THI scores showed a decreasing trend (p less than 0.001).

Conclusions: FTRS use resulted in significantly greater improvements in tinnitus and other outcomes relative to their baseline condition before treatment. Given the ubiquity of smartphones, FTRS may provide a wide-reaching and convenient public health intervention for individuals with tinnitus symptoms.

S148. Functional MRI Neurofeedback Versus Cognitive Behavioral Therapy for Reducing Tinnitus Distress: A Prospective Randomized Clinical Trial

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Category: Tinnitus

Background: Tinnitus is a phantom auditory perception in the absence of external acoustic stimulation. Chronic, severe forms of tinnitus substantially reduce quality of life. Cognitive behavioral therapy (CBT) is the current standard treatment; however, preliminary evidence suggests that real-time functional MRI (fMRI) neurofeedback therapy may be more effective in downregulating excess activation in the auditory cortex, thereby reducing tinnitus. This study aims to compare the ability of real-time fMRI neurofeedback versus CBT to reduce chronic tinnitus distress.

Methods: In this prospective, controlled trial, 43 patients with chronic, severe tinnitus were randomized to receive either group CBT for 10 weekly sessions of 120 min or real-time fMRI neurofeedback therapy (fMRI group) during 15 weekly sessions of 60 min. Change in the Tinnitus Handicap Inventory (THI) score (range, 0–100) from baseline to 6 or 12 months was assessed. Secondary outcomes included 4 quality of life questionnaires (Beck's Depression Inventory, Pittsburgh Sleep Quality Index, State-Trait Anxiety Inventory, and WHO Disability Assessment Schedule).

Results: The fMRI group included 21 participants (mean age, 49 years ± 11.4 [SD]; 16 males) and the CBT group included 22 participants (mean age, 53.6 years ± 8.8 [SD]; 16 males). The fMRI group showed a greater reduction in THI scores compared to the CBT group at both 6 months (mean score change, -28.21±18.66 versus -12.09±18.86; P = .01) and 12 months (mean score change, -30±25.44 versus -4±17.2; P = .02). Compared to baseline, the fMRI group showed improved sleep (P = .006) and trait-anxiety (P = .02) at 1 month, and improved depression (P = .01) and general functioning (P = .01) at 6 months. No difference in these metrics over time was observed for the CBT group.

Conclusions: The novel real-time fMRI neurofeedback therapy robustly outperformed the established group CBT for the reduction of chronic severe tinnitus and associated morbidity. These findings corroborate the potential of neurofeedback for tinnitus treatment and may inspire the development of more cost-effective versions, including refined real-time fMRI or electroencephalography-based applications.

S149. Open Board

S150. Neural Adaptation Model of Hyperacusis

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Category: Tinnitus

Background: Hyperacusis is a devastating disorder that affects people's lives. The symptom is commonly reported in people after noise exposure or in children with neurological disorders, including Williams syndrome, FoxG1 syndrome and autism spectrum disorders (ASD). Increased neural activities in the central auditory system (CAS) to compensate for the hearing loss, named "central gain", are thought to underlie the cause of hyperacusis. However, as most people who experience hearing loss do not develop hyperacusis, it is unclear whether the "central gain" represents a plasticity change of the CAS to the hearing loss or the cause of hyperacusis. In this study, we used two different animal models of hyperacusis, i.e., the noise exposure model and the FoxG1 gene mutation model, to study the neurological model of hyperacusis.

Methods: CBA mice, wild-type mice, and FoxG1 gene mutation mice (G216S) were used in the experiment. Low-level noise exposure (83 dB SPL, 2 weeks, 12 hrs/per day) was used to induce hyperacusis in the WT mice. Acoustical startle responses (ASR) were used to evaluate sound sensitivity, and gap-induced prepulse inhibition (gap-PPI) was used to evaluate sensory gating. The auditory cortex (AC) response was measured in the control, noise-exposed, and FoxG1 mutant mice. Auditory brainstem response (ABR) was used to evaluate peripheral hearing loss induced by noise exposure or FoxG1 gene mutation.
Results: ABR results showed no significant hearing loss was induced in the noise group or FoxG1 gene mutant group. Enhanced ASR and increased gap-PPI were recorded in the noise group compared to the control group. A significant enhancement of AC onset response was recorded in noise-induced mice, an indication of "central gain". FoxG1 mice showed no significant enhancement of the ASR. However, the ASR showed a lack of habituation for repetitive acoustic stimuli. These mice also showed a lack of gap-PPI suggesting impaired sensory gating caused by FoxG1 gene mutation. The AC recording shows no signs of enhanced response, but longer post-stimuli responses were recorded.
Conclusions: Our study shows two different models of hyperacusis. Noise exposure induced a central gain increase, consistent with previous studies. A neural adaptation model that has been reported in ASD patients has been identified in the FoxG1 mutant mice. Our study suggests that a lack of sound habituation, which is a non-associative learning process reflected by startle magnitude decreasing with stimulus repetition, may affect sound tolerance and loudness perception in children with hyperacusis.

S151. Tinnitus Suppression and Enhancement of Cognitive Function Following High Definition tACS Using Cross Frequency Coupling Electrical Currents

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Category: Tinnitus
Background: Tinnitus refers to the phantom perception of sound in the absence of external sources. Transcranial electrical stimulation has demonstrated a positive effect on tinnitus loudness and annoyance. However, the efficacy of tinnitus treatment still lacks consistency. Cross-frequency coupling (CFC) indicates interactions between oscillations at different frequency bands, which is critical for sensory information processing across wide cortical networks in our brain. Our previous study revealed the absence of CFC in tinnitus subjects. We developed the high definition (HD)-tACS algorithm based on the principle of CFC and hypothesized that a personalized protocol might lead to the entrainment of targeted neuronal activity, which could be beneficial for tinnitus suppression and enhancement of cognitive function.
Methods: A prospective clinical study of 25 patients (3 women) with tinnitus lasting more than six months was conducted. Detailed otologic tests, tinnitus questionnaires, and cognitive function tests including MoCA, TMT, and DST, were obtained before and after tACS stimulation. The tACS was delivered using NG Pistim electrodes coupled with the Starstim 32 device (Neuroelectric, Barcelona, Spain) and generated based on individual alpha frequency phase and gamma amplitude coupling using a current of 2 mA. The protocol consisted of 5 sessions of tACS, with each session lasting 20 minutes, given 1-2 weeks apart.
Results: Four patients dropped out and 20 patients were included in the final analysis. A repeated-measures ANOVA with within-subjects (pre- vs. post-stimulation rating scale) and t-tests was performed using SPSS 22.0. After HD-tACS using personalized CFC, significant reductions for pre- vs. post-stimulation in the THI scale were observed [$F(2.324, 23.243) = 6.889$, $p = 0.003$] for five sessions. While VAS-tinnitus loudness did not significantly affect pre- vs. post-stimulation, the initial and final VAS-loudness score revealed a significant reduction ($t=3.105$, $p=0.011$). In addition, the initial MoCA score was 26.62 ± 2.47, which yielded a significant improvement in cognitive function compared to the final MoCA score (28.06 ± 2.40) ($t=-3.715$, $p=0.002$). Both TMT and DST scores significantly improved between the initial and final session ($p=0.018$ for TMT, $p=0.009$ for DST). Finally, we found enhancement of alpha oscillation in target areas after the final HD-tACS.
Conclusions: Our findings suggest that a personalized HD-tACS protocol using a combination of electrical current with low-frequency phase and high-frequency amplitude coupling might restore maladaptive neuroplasticity in patients with tinnitus and that the stimuli might also benefit cognitive functionality. Additionally, our data provide evidence that our protocol could enhance neuronal activity for inter-regional communication of target areas.

S152. The Size Analysis of Stapedius and Tensor Tympani Muscles in CT Scan in Patients With Middle Ear Myoclonic Tinnitus

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Category: Tinnitus

Background: This study was performed to analyze the sizes of the stapedius and tensor tympani muscles using a temporal bone CT (TBCT) scan in patients with middle ear myoclonic tinnitus (MEMT) and to investigate its clinical value for the diagnosis of this rare cause of objective tinnitus.

Methods: The medical records and TBCT scans of patients with MEMT, and vascular tinnitus at Seoul, St. Mary's Hospital from 2012 to 2022 were retrospectively reviewed. The length and width of the stapedius and tensor tympani muscles were measured and a comparative analysis of the MEMT pathologic side and the same muscles in the patients with vascular tinnitus as a control group (CG) was conducted. A correlation study of the size of the middle ear muscles with their demographic and clinical characteristics was also performed.

Results: A total of 38 patients with unilateral MEMT (63.2% men, 36.8% women) who underwent middle ear tendon resection for the intractable symptom of tinnitus and 39 patients with vascular tinnitus (20.5% men, 79.5% women) were included in the study. The mean age of the MEMT group was 34 years (16-63) while the mean age of the vascular tinnitus group was 44 years (18-75). The MEMT group had 21 (55.3%) right-sided pathology and 17 (44.7%) left-sided pathology. The vascular tinnitus group had 30 (76.9%) right-sided symptoms and 9 (23.1%) left-sided symptoms. The median symptom duration for the MEMT group was 24 months with an interquartile range of 45 months while the median symptom duration for the vascular tinnitus group was 6 months with an interquartile range of 33 months. The average stapedius tendon length and width of the MEMT group were 1.47±0.60mm and 0.89±0.32mm, which were significantly higher values compared to those of the vascular tinnitus group; 0.98±0.24mm, p less than 0.001, 0.71±0.19mm, p less than 0.001, respectively. The average length and width of the tensor tympani tendon for the MEMT group were 3.11±0.51mm and 2.02±0.36mm, which were also significantly higher values compared to those of the vascular tinnitus group; 2.27±0.42mm, p less than 0.001 and 1.75±0.26mm, p less than 0.001, respectively. A moderate correlation between the stapedius tendon width and the duration of symptoms for MEMT was also observed ($r = 0.314$, p less than 0.05).

Conclusions: The length and width of the stapedius and tensor tympani muscles measured in TBCT in the MEMT group were significantly longer and wider than those of the vascular tinnitus group, which suggests a possible value of TBCT scan as a diagnostic tool for MEMT when used in conjunction with other clinical findings. Further studies in a larger study group will be needed to validate our results and investigate its more practical clinical value for the diagnosis and management of MEMT.

S153. Applying an Unequal Variance Signal Detection Theory Model to Patient Decisions in the Tinnitus Patient Journey

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[1]*University Medical Center Utrecht,* [2]*Dutch Foundation for the Deaf and Hard of Hearing Child (NSDSK)*

Category: Tinnitus

Background: Chronic tinnitus management requires a multidisciplinary approach with attention for medical, audiological, psychological, and social-emotional issues. Several Cochrane reviews indicate that psychological intervention is most effective, yet many patients seem to prefer other options. The aim of this study is to apply an unequal variance Signal Detection Theory (SDT) model to map the outcome of patient decisions during the tinnitus patient journey.

Methods: We used the clinical data of 148 tinnitus patients who visited an Audiological Center in The Netherlands during a two-year period. Inclusion criteria were age (18 years and older) and referral for tinnitus care. At baseline and after tinnitus intervention patients were asked to fill in the Tinnitus Handicap Index (THI) questionnaire. All patients received standard of care tinnitus psychoeducation. As follow-up, they were offered to start a trial period with a sound enrichment device (hearing aid, ear worn tinnitus masker or

combination device), and they were offered psychosocial counseling based on elements of cognitive behavioral therapy and mindfulness. The proportion of patients that accepted the different interventions were analyzed as a function of hearing loss and baseline THI-score. Using an improvement in THI-score of more than 7 points as clinically relevant to distinguish between successful intervention outcomes (true positives) and unsuccessful intervention outcomes (false positives), we estimated the probability density functions of false and true positives. With these probability density functions we estimated the likelihood ratio of false and true positives.
Results: For starting a trial period with a sound enrichment device, the likelihood ratio of false positives and true positives is a function of hearing loss. Patients with a hearing loss over 43 dB are more likely to report benefit from a trial period. For device uptake, the likelihood ratio is also a function of hearing loss, with a successful intervention being 1.7 times more probable for a hearing loss above 20 dB. For psychosocial counseling, the likelihood ratio is a function of baseline THI-score only, with successful intervention being more probable than unsuccessful intervention for baseline THI-scores up to 83 points.
Conclusions: This study shows that using an unequal variance SDT model allows mapping of patient decisions during the tinnitus patient journey. The probability ratio of unsuccessful and successful intervention outcomes can be estimated as a function of hearing loss and baseline THI-scores. The results can be used to create patient profiles that allow for more individualized counseling in shared decision making.

S154. Cortical Evoked Activity is Modulated by the Sleep State in a Ferret Model of Tinnitus

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Category: Tinnitus
Background: Subjective tinnitus is a phantom auditory perception in the absence of an actual acoustic stimulus. It affects 15% of the global population and can be associated with poor mental health, reduced quality of life and impairment of sleep, yet the involvement of spontaneous brain dynamics has received little attention in tinnitus research. To date, there is no effective treatment for tinnitus.
Methods: We used adult ferrets exposed to mild noise trauma as an animal model of tinnitus. We assessed their phantom percept using two operant paradigms, silent gap detection and silence detection, as behavioural indicators of tinnitus before and up to six months after the mild acoustic trauma. The integrity of the auditory brainstem was assessed over the same period using auditory brainstem response recordings. To explore the interaction between sleep and tinnitus, we evaluated the sleep–wake pattern and both spontaneous and auditory–evoked EEG activity across vigilance states.
Results: Following noise overexposure, ferrets developed lasting, frequency–specific impairments in operant behaviour and evoked brainstem activity. Behavioural performance and auditory–evoked activity measurements after noise overexposure suggested different degrees of tinnitus and hearing impairment between individuals. Animals that developed signs of tinnitus consistently showed sleep impairments, suggesting a link between the emergence of noise–induced tinnitus and sleep disruption. However, neural markers of tinnitus were reduced during sleep, suggesting that sleep may transiently mitigate tinnitus.
Conclusions: These results reveal the importance of sleep–wake states for understanding tinnitus comorbidities and their potential to inform future treatments. Unravelling the neurophysiological link between sleep and tinnitus may offer a new angle to research into the basis for phantom percepts.

S155. Tinnitus Clinical Research: Challenges with Recruitment, Eligibility, and Symptom Variability

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Category: Tinnitus
Background: Methods of measuring bothersome tinnitus are critical for understanding tinnitus and for assessing potential treatment effects. We recently initiated a pilot human study for assessing a novel device

intervention involving tinnitus participants. We sought to recruit individuals with tinnitus that was bothersome enough to justify an intervention but not associated with high risk, catastrophic psychological distress. We leveraged the Tinnitus Handicap Inventory (THI) using an inclusion criterion of scores between 38 and 76 (i.e., standard range for moderate or severe). During recruitment, we observed many potential participants had measurable differences between their self-administered THI screening and a subsequent in-person test at the time of eligibility determination. We aim to characterize and quantify these challenges encountered in recruitment that may have broader implications for tinnitus research.

Methods: Interested participants were asked to complete an online THI screening. If their THI score met inclusion criteria, participants completed an in-person visit to finalize eligibility, during which they completed a second THI amongst other questionnaires. We analyzed the changes in THI between the online screening and the in-person visit. Per IRB requirements, the trial protocol including THI score for inclusion, was published on clinicaltrials.gov.

Results: In the initial recruitment phase, the study was announced widely with tinnitus user groups and public sites and 22 potential participants were determined to be within the eligible range. Following on site re-testing of the THI of those 22, 9 increased and 13 decreased in score. The score of 7 of those 22 individuals changed by 20 points or more (median decrease 16 points) making some ineligible for the study.

We tightened screening procedures by recruiting from local clinics and advertising with local organizations for persons with tinnitus. An additional 24 potential participants were identified as likely eligible based on online THI screening. The scores from nine participants changed by 20 points or more between the screening and in-person test. Of those 24, 4 increased, 19 decreased in score, and 1 stayed the same. Overall, approximately 70% of THI scores decreased from the online screening to their in-person test, with an average decrease of 16 points. Only 28% of scores did not show a clinically significant change prior to enrollment in the study.

Conclusions: Overall, we observed large differences in scores between a self-administered THI and a subsequent on-site test. Initially we were concerned that recruiting nationally and publication of specific criteria on clinicaltrials.gov resulted in potential participants biasing their THI scores. However, substantial variability in THI scores was still observed after changing recruitment sources. These findings reveal variation and potential unreliability of THI scores during the screening stage of a study and may reflect how transitory tinnitus-related distress is affected by multiple factors, including a desire to enter a treatment trial.

S156. Exploring the Link between Low-Pass Speech in Noise and Tinnitus Distress

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Category: Tinnitus

Background: In a previous study at Ghent University, we observed that patients with tinnitus performed better on a low-pass filtered speech-in-noise (SPIN) test than an age-matched control group. However, audiometry and objective EEG measurements (auditory brainstem response and envelope following response) showed no tinnitus-related differences, suggesting no underlying peripheral hearing damage. Given that peripheral hearing loss could not explain the significant difference found in low-pass SPIN scores, we looked deeper into tinnitus-specific characteristics of the participants and explored the relationship between SPIN understanding and the results on tinnitus questionnaires.

Methods: The study included audiometry, auditory brainstem responses, envelope following responses, filtered speech (in noise) tests, and tinnitus-related questionnaires. In this part of the study, we delved into the link between the latter two. The Flemish 5-word matrix sentence test was administered with or without a stationary speech-shaped background noise at 70 dB SPL. To differentiate between the encoding of temporal envelope (TENV) and temporal fine structure (TFS), we applied low-pass (LP) and high-pass (HP) filtering to the speech and noise. Participants completed the Tinnitus Sample Case History Questionnaire (TSCHQ), Tinnitus Handicap Inventory (THI), Tinnitus Functional Index (TFI), and Hyperacusis Questionnaire (HQ). Based on these THI- and TFI-scores, patients were categorized into subgroups related to tinnitus distress, while the HQ was used to categorize patients into those with and without hyperacusis.

Results: More tinnitus distress (based on TFI and THI scores) correlated with better low-pass SPIN performance in the older test group. Additionally, we observed that patients with both tinnitus and hyperacusis performed better on the low-pass SPIN test, while tinnitus patients without hyperacusis scored similar as the age-matched control group.
Conclusions: These observations suggest that hyperacusis may explain the improved SPIN scores in our study. Since this effect is only observed in low-pass filtered SPIN, improved TFS processing could be a possible underlying mechanism of hyperacusis and/or tinnitus. Based on these findings, future studies on tinnitus and hyperacusis should investigate TFS coding, and apply low and high frequency filtering to speech (in noise) tests. Furthermore, this study highlights the importance of examining hyperacusis complaints in tinnitus research.

S157. The Role of Ion Channels: From Misfiring to Misfolding

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Category: Binaural Hearing and Sound Localization
Background: Spinocerebellar Ataxia Type 13 (SCA-13) is caused by a loss of function mutation in the high-voltage activated K+ channel sub-unit Kv3.3. Alongside cerebellar Purkinje Neuron atrophy, human patients with SCA-13 exhibit impairments in sound localisation due Kv3.3 expression in auditory brainstem nuclei such as the MNTB and LSO. By using a novel CRISPR-Cas9 mouse model of SCA-13, dubbed R420H, we are able to study the role of Kv3.3 in sound localisation processing and Purkinje Neuron degeneration, with a direct clinical correlate in previously characterised human kindred.
Methods: By combining in vitro and in vivo electrophysiology, alongside auditory brainstem responses and behavioural data, we hope to characterise the way in which each of the affected brainstem nuclei in the mouse sound localisation pathway is disrupted by the R420H Kv3.3 mutation. We are also using the same methods to characterise the misfiring in the Cerebellum, identifying mechanisms of neuronal resistance and atrophy underlying SCA-13.
Results: Due to the broad expression of Kv3.3 in the auditory nuclei, multiple points of failure along the sound localisation pathway have been identified to be associated with the R420H mutation. Our previous work on the mouse model has demonstrated an average threefold increase in action potential half-width in affected neurons as a result of Kv3.3 loss of function. Through a disruption to the fast repolarising K+ current in their action potentials, most LSO and MNTB neurons affected by the mutation fail to repolarise fully at spiking intervals higher than 200 Hz. The temporal convergence of ipsilateral excitation and contralateral inhibition likely fails in the LSO due to misfiring of the intermediary Calyx of Held synapse, where Kv3.3 is expressed both pre- and post-synaptically.
Where the Kv3.3 loss of function mutation is sufficient to disrupt network activity in the auditory brainstem, the ataxia in SCA-13 is driven by neuronal atrophy which occurs as a result of intracellular apoptotic signalling cascades. We hypothesise that Purkinje Neuron atrophy in the cerebellum is driven to by a chronic Ca2+ overload occurring as a result of the cell spending a prolonged duration in a depolarised state during each action potential. Chronic Ca2+ elevation should affect MNTB and LSO neurons in a similar manner, yet based on our histological findings, it seems that auditory brainstem nuclei are uniquely resistant to this.

Conclusions: The defect in sound localisation seen in SCA-13 is likely due to a combined effect of misfiring in the MNTB and the LSO. We also hypothesise that due to a differential Ca2+ buffering mechanism, neuronal survival is promoted in auditory brainstem neurons. We aim to understand this mechanism in the auditory brainstem and utilise it in the Cerebellum in order to promote neuronal survival and potentially rescuing the degenerative phenotype in the mouse model.

S158. Effect of Reverberation on Interrupted Spatial Auditory Attention

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Category: Binaural Hearing and Sound Localization

Background: Listeners use features like pitch and location to segregate competing speech streams and selectively focus attention on one. In such settings, reverberation can 1) interfere with speech intelligibility directly, and 2) impede selective attention by smearing out such features. In anechoic simulations, unexpected, salient "interrupters" disrupt spatial selective attention (Liang et al, 2022), but we do not know whether reverberation affects such tasks. Reverberation may degrade performance overall, but also may have a differential impact on interrupters. Specifically, by smearing out features, reverberation may make interrupters less salient and less disruptive. However, by making the task harder, reverberation could exaggerate the effect of an interrupter. Here, we conducted online spatial selective attention experiments to measure whether reverberation impacted the recall of a target stream of syllables that are interrupted by an unexpected event.
Methods: Participants (N=45, self-reported normal hearing) heard two competing, temporally interleaved streams of syllables with and without random interruptions. Both streams consisted of syllables from the same male talker; they differed only in their location, with one stream spatialized to 30 degrees left and the other to 30 degrees right of center. On half of the trials (randomly selected), a novel "interrupter" occurred, mid-target-sequence, from 90 degrees contralateral to the target. All syllables and interrupters were spatialized using binaural room impulse responses (BRIRs) measured using a KEMAR (Shinn-Cunningham et al., 2005). Half of the trials used pseudo-anechoic BRIRs while the other half used reverberant BRIRs measured 1 meter from the center of the KEMAR head.
At the start of each trial, a single spatialized /ba/ syllable played from either 30-degrees left or right, cueing the listener to attend to the stream at that location (the target) and ignore the other (the distractor). Both streams comprised 5 syllables randomly drawn with replacement from [/ba/, /da/, /ga/]. Syllable onsets within each stream were 600 ms apart; the target began 300 ms before the distractor. At the end of the trial, subjects were asked to report the target syllables in order. In 50% of trials, a novel interrupter occurred 125ms before the onset of the 3rd syllable in the target stream. Anechoic and reverberant trials were randomly intermingled. We quantified the effect of the interrupter by comparing target syllable recall with and without the interrupter, separately for anechoic and reverberant trials.

Results: Reverberation had no significant effect on either overall performance or on the disruptions caused by interrupters. In both reverberant and pseudo-anechoic conditions, an interrupter reduced recall accuracy of the syllable immediately after the interrupter by ~20%.
Conclusions: Consistent with previous work, interrupters affect selective spatial attention; however, modest reverberation has no effect on performance. Future work will explore whether greater levels of reverberation impact bottom-up disruptions of selective attention.

S159. Sound Localization in Bimodal CI Users With Various Device Latencies

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Category: Binaural Hearing and Sound Localization
Background: Individuals with a hearing aid servicing one ear and a cochlear implant (CI) servicing the other ear have very poor sound localization abilities. A combination of level, tonotopy, and latency mismatches prevents the exploitation of binaural cues. Compensating for the estimated latency mismatch by adding a fixed delay to the CI stimulation has been shown to improve sound localization (Angermeier et al., Trends in Hearing, 2023). Here, we measure sound localization error and bias as a function of the additional delay.
Methods: 13 loudspeakers were placed semicircular with a 15° spacing at ear level in the frontal azimuthal half-plane with a radius of 1 meter. Bimodal MED-EL CI users that have functional hearing up to at least 4 kHz performed a loudspeaker identification experiment. Each stimulus consisted of three 70-ms long broadband noise bursts each gated with a 5-ms Hanning windows, separated by two 30-ms long silent intervals. Stimuli were presented 5 times from each of the 11 most central speakers and each CI latency (0, 2, 4, 6, 8, and 10 ms). For a subset of CI-latencies and subjects the test was repeated with the German word "Doris" as the stimulus.
Results: In line with Angermeier et al. (2023) the localization bias shifted towards the hearing aid side with increasing CI latency. The amount of bias shift varied from 1° to 9° per millisecond latency. When using speech instead of noise the trends remained similar, but the bias shift was smaller. A bias-free latency could usually be identified within the tested latency interval. This latency also resulted in one of the smallest RMS

localization errors. However, the bias-free CI latency was usually smaller than the latency compensation suggested by Angermeier et al. (2023).
Conclusions: The results emphasize the importance of latency optimization when fitting single-sided deaf and bimodal CI users. The difference between the estimated latency mismatch and the bias-free best localization latency may originate from latency estimation errors or from a level bias towards the hearing aid side that can be compensated for by a CI-favoring latency mismatch. Most likely, however, it originates from an additional tonotopic mismatch: Both acoustic latencies from the traveling wave in the inner ear and MED-EL CI latencies are larger at lower frequency bands than at higher frequency bands. The common tonotopic mismatch results in CI frequency channels being sent to electrodes that stimulate the same tonotopic place as higher acoustic frequency channels. This increases the place-specific CI latency relative to its acoustic counterpart.

S160. A Signal Detection Theory Model of Binaural Cue Reweighting

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Category: Binaural Hearing and Sound Localization
Background: Normal-hearing listeners weight binaural localization cues depending on the sound's frequency content. Interaural time differences (ITDs) dominate at low frequencies, and interaural level differences (ILDs) dominate at high frequencies. However, the relative weighting of the cues used by the listeners can be sub-optimal, resulting in poorer localization in many scenarios, e. g., in reverberation. Previous studies showed that various training protocols can be used to induce a binaural cue reweighting both to increase the ILD weight and to increase the ITD weight. These studies used various measures to determine the ILD/ITD weight, making it difficult to compare the effectiveness of the different training protocols, as well as to compare the weights obtained in these studies to the standard "trading ratio". E.g., in our previous study using discrimination training, the proportion of discrimination responses following the ILD (PILD) was used as the relative weight measure [Singhal et al. (2023) ARO Abstract #SU44]. Here, the goal is to develop a signal-detection-theory (SDT) model, based on which a measure of the ILD/ITD weight can be estimated that is independent of the task and conditions used in each study.
Methods: The model is based on the standard SDT model for the 2-Intervals–2-Alternative-Forced-Choice discrimination task, as used in our previous study. It uses several simplifying assumptions, the main one being that the responses are unbiased. And, it derives wLT, a d-prime-like measure of the relative weight per unit of azimuthal shift in cues.
Results: The derived relative weight estimate wLT, when applied to our previous results, shows that the effectiveness of the training was approximately equal for both ITD training and ILD training. Also, the measure is more robust to noise and less affected by outliers, compared to the PILD measure used originally.
Conclusions: A STD-model-based measure of relative weight provides a reliable and location-independent estimate of the binaural cue weighting. Applied to our experimental data, it shows that a simple adaptive discrimination training without visual signals can induce binaural reweighting in both directions. Next steps are to extend the model to localization-based estimates of relative weight and to studies estimating the "trading ratio". This will allow us to compare the effectiveness of different training procedures, like visually guided training and training in reverberant environments.

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S161. Perceptual Spatial Acuity in a 9th Order Ambisonic Array

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Category: Binaural Hearing and Sound Localization

Background: Spatial hearing is fundamental for the perception of auditory scenes, and various methods are employed to simulate and manipulate auditory scenes in experiments sensitive to spatial hearing experience. Although traditional loudspeaker arrays and Head-Related Transfer Functions (HRTFs) over headphones are commonly used, they have limitations related to source location resolution, dynamic manipulations, and HRTF measurement effort. Higher Order Ambisonics (HOA) loudspeaker arrays represent a growing alternative technique that addresses these limitations while approximating naturalistic auditory presentation. This study aims to identify the potentials and limitations of such a system in simulating perceived sound source locations.
Methods: We utilized a 91-loudspeaker array capable of 9th order HOA rendering arranged in a dome shape with a radius of ~1m (Sonible, Austria), situated within a sound-attenuating room. 6 normally hearing participants were seated in complete darkness with their heads at the center of the dome and ears aligned at 0° elevation, fixating on an LED in front of them (FP). Each trial consisted of two 450ms white noise bursts on the horizontal plane separated by a 200ms interval, followed by 1250ms silence period during which participants indicated the location of the second burst relative to the first (clockwise/counterclockwise).

Minimum Audible Angles (MAAs) were estimated for 17 Reference Points (RPs) spanning from -90° to +90° azimuth angle in a fully randomized method of constant stimuli design (13 test points/RP). In the main condition, the FP was always at 0° at the center of the central loudspeaker. To assess the impact of the loudspeaker positions on perception of HOA rendering, an additional condition involved rotating the participants' bodies with the FP to ±50.40° where neither the RP nor the surrounding test points coincided with a loudspeaker.

Binaural cues under HOA and normal audio rendering conditions were compared using a Head and Torso Simulator.

Results: MAAs increased from 1.03°±0.12° at the front to 2.81°±0.47° at the right and 4.46°±0.66° at the left at ±50.40° RPs. Response curves deviated from a sigmoid shape to a random response curve for the remaining RPs in some participants. Excluding such response curves, the average MAAs ranged from 4.46° to 8.41° for these RPs. These results align with MAAs measured similarly using discrete-loudspeaker sources. (Strybel and Fujimoto, 2000).
Additionally, estimated MAAs did not significantly differ between the central RP and the rotated conditions ($F(2,10)=0.8275$, p less than 0.465), demonstrating the capability of HOA to render focal sound sources between loudspeaker locations.

Conclusions: Using a 9th order ambisonics system, we successfully demonstrated similar spatial acuity to that observed in traditional loudspeaker array designs, with a robust accuracy resilient to sound source location, highlighting the promise of this technique for complex auditory scene designs.

S162. Differences in Sound Localization in Semi-Fossorial and Lab Reared Animals

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Category: Binaural Hearing and Sound Localization
Background: Hearing ability in mammals is influenced by many factors such as, behavior, habitat, and physical morphology. The ability to localize sound is vital to the animal's survival and fitness. It also affects mammal's ability to communicate and avoid predators. Prairie voles are often used to study social behaviors, but their hearing has never been characterized.
Methods: To characterize their hearing, we measured their physical morphology and utilized a custom-built head-related transfer function (HRTF) array to measure pinna contribution to hearing. The external ear (pinna) was measured including both length and width, the distance of pinna from each other, and the distance of the middle of the pinna to the ear. This data was then compared to the contribution of the pinna to capture sound in 180-degree space. An arrangement array of a speaker along 10-degree increments at 19 possible locations on the horizontal axis and 19 possible locations on the vertical axis was used. The animal is located equal

distance from the speaker at all 361 potential measurements. A sweep of noise was presented, and the noise captured by the pinna is measured by a microphone placed through the back of the ear just inside the pinna.
Results: The interaural time difference (ITD), which is the difference of when the sound arrived at the pinnae, and the interaural level difference (ILD), which is the difference in the intensity of the two responses at the pinna were calculated for each animal. Pinna measurements and HRTF measurements were in agreement for the range of ITD and ILD cues available to the animals measured. In the future, we plan to evaluate how habitat affects the prairie vole's ability to localize sound.
Conclusions: This work was done on lab-reared prairie voles so to understand how habitat affects sound localization we plan to compare lab-reared data to wild prairie voles. There is a lot of research on social communication ability but little focus on how the habitat can influence the way that individuals hear. Prairie voles are a great model since they are semi-fossorial, or they have burrows but also forage and communicate above ground. Having to hear both underground and above we would expect to see differences in the way that they localize sound compared to the lab-reared voles. In addition, we expect that semi-fossorial rodents may have different hearing abilities than other solely terrestrial species due to the limited acoustic cues available underground.

S163. Characterizing Hearing Ability Using Acoustic Startle Response and Prepulse Inhibition in Prairie Voles (Microtus Ochrogaster)

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Category: Binaural Hearing and Sound Localization
Background: Prairie voles (Microtus ochrogaster) are a highly social rodent species that live primarily monogamous lifestyles and participate in bi-parental care, making them an excellent model for studying animal behavior in regard to communication, reproduction, and neuroscience. Prairie voles, like many rodents, rely heavily on acoustic cues to effectively communicate with others—especially with mates and pups.
Methods: To communicate via auditory information, animals must be able to decipher where a signal is coming from in the environment. The mammalian brain ultimately uses three cues to do this—interaural time differences (ITDs), interaural level differences (ILDs), and cues provided by the pinna. Acoustic behavior experiments can be used to measure the auditory temporal processing ability of animals through their acoustic startle response (ASR) and prepulse inhibition (PPI) ability. PPI specifically has been used as a measure for how well an individual can integrate and inhibit sensory information from the environment in multiple scientific disciplines such as clinical psychology and neuropsychiatric research, providing valuable information on the physiology of sensorimotor processing.
Results: Here, we measure the hearing ability behaviorally of prairie voles using PPI of the ASR with varying cues that indicate hearing ability.
Conclusions: These studies are important to further utilize the prairie vole as a model organism in acoustic and social behavioral research by increasing understanding of how they respond and integrate acoustic information.

S164. The Influence of Binaural Cues on Auditory Stream Segregation

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Category: Binaural Hearing and Sound Localization
Background: Binaural cues are one of the primary tools used by listeners to separate different sources of auditory information. While substantial research has characterized the ability of listeners to discriminate small differences in interaural time (ITD), level (ILD), and correlation (IAC), less is known about the influence of these cues to promote or inhibit auditory stream segregation.
Methods: Here, we used the ABA auditory stream segregation paradigm (Bregman, 1990) to quantify listener perception of integrated or segregated auditory stream while binaural cues were slowly modulated over time (50 sec period). Stimuli consisted of continuous repetition of ABA triplets, made of 1/3rd octave narrowband

noise with center frequencies separated by 6 semi-tones for the A and B components. Electroencephalography (EEG) data were collected using a 64-channel cap concurrent with continuous listener responses that indicated perception of integrated or segregated auditory stream.
Results: In comparison to the baseline condition that did not have a modulated binaural cue, participant response patterns showed increased segregation when the A-component carried a more lateral binaural cue while the B-component was maintained at the midline (ITD and ILD = 0, IAC = 1). EEG responses quantified via global field potential were larger when triplets were perceived as segregated compared to integrated and for triplets corresponding to a switch in perception, consistent with previous results (Higgins et al., 2020).
Conclusions: These results demonstrate the influence of binaural cues on auditory stream segregation, and represent the initial steps toward characterizing how listeners weigh spatial cues for separating sound sources.

S165. Examining Individual Variability in the Acoustic Effects of Hearing Protectors on Cues for Sound Source Localization

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Category: Binaural Hearing and Sound Localization
Background: Modern hearing protection devices (HPDs) mitigate the risk of noise-induced hearing loss when used as intended, but negative auditory perceptual side-effects continue to limit usability in critical settings. Dozens of studies, including previous studies by our group, have shown that HPDs lead to significant errors in sound source localization, including large errors in source elevation perception and disorienting front-back confusions. Degradation of performance relative to open-ear listening arises due to peripheral disruptions of monaural and binaural acoustic cues for sound localization. For higher-attenuation devices, reduced audibility can also limit performance even if cues are relatively intact. A recent multi-site effort by our group leveraged manikin-based acoustic measurements of HPD-induced cue distortions to predict human localization performance during use of HPDs. Manikin-based acoustic metrics were positively correlated with behavioral performance across a large population of human listeners ($n \geq 120$), enabling discrimination of localization impacts across HPDs. However, significant individual variability was also evident. Behavioral variability may be attributable in part to individual acoustic variability in the form and extent of HPD-induced spatial cue distortions. Here, we sought to quantify such variability using individualized acoustic measurements.
Methods: The heads and pinnae of 45 (22 female) audiometrically normal-hearing subjects, who had previously completed behavioral localization testing, were digitized using a hand-held 3D scanner (Einscan H). Digitized pinnae (right ears only) were extracted and merged with a standard base designed to couple to an acoustic manikin (G.R.A.S. 45-CB). Merged pinnae were printed using a soft (Shore 30A) polymer and mounted on the test fixture. Head-related transfer functions (HRTFs) were then acquired across azimuth and elevation with open (unoccluded) ears and with a variety of HPDs, for which behavioral data had previously been obtained. Measurements were also obtained for standard manikin pinnae. HPD-induced HRTF distortions (HPD re: open ear) were then quantified.
Results: Large variability in the magnitude and form of HPD-induced HRTF disruption was evident, both across HPDs within subjects, and across subjects within HPDs. Notable deviations of individualized HPD acoustic impacts from those quantified with standard manikin pinnae were also evident.
Conclusions: Improved quantification of spatial acoustic cue distortions produced by HPDs, including impacts of individual variability, will support improved prediction of HPD impacts on behavioral performance, toward improved device selection, compliance, and hearing protection in critical settings.

S166. Auditory Competition and Coding of Salience Across Midbrain Space Maps of Barn Owls

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Category: Binaural Hearing and Sound Localization

Background: The rich sensory environment challenges the brain to prioritize the processing of salient stimuli. The barn owl, a sound localization specialist, exhibits a circuit called the midbrain stimulus selection network, dedicated to representing locations of the most salient stimulus in circumstances of concurrent sensory cues. Previous competition studies using unimodal (visual) and bimodal (visual and auditory) stimuli have shown that relative salience is encoded in the strength of spiking rates. However, full understanding of coding effects by competition between concurrent auditory signals remains unknown. Specifically, auditory signals contain complex properties, such as spectrotemporal envelopes, which vary over time, and are vulnerable to corruption from other acoustic signals.
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 the midbrain space maps, the external nucleus of the inferior colliculus (ICx) and optic tectum (OT). As the ICx only encodes auditory information, stimulus competition has not been assessed in the ICx. While both ICx and OT exhibit a topographic map of space, OT integrates auditory and visual inputs and is the central hub of the midbrain stimulus selection network, which may implement coding changes of stimulus salience and induces emergence of brain oscillations within gamma range.
Results: Through comparative investigation of these regions, we show that while increasing strength of a competitor sound decreases spike rates of spatially distant neurons in both regions, relative salience determines firing synchrony of nearby units only in OT, a novel finding. Further testing by manipulation of spectrotemporal envelopes shows that changes in firing synchrony by sound competition in OT are weakly associated to temporal properties of the stimulus but strongly correlated to gamma range oscillations of local field potentials (LFPs).
Conclusions: The results of this investigation suggest that modulations in spiking temporal synchrony between units induced by gamma oscillations is an emergent coding scheme representing relative salience, which may have relevant implications for downstream readout.

S167. Wider Auditory Filters and Greater Overshoot in Children Than Adults for Brief Tones at Noise Onset

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Category: Development: Human Subjects
Background: Children have greater difficulty hearing in noise than adults, but the reasons why are not fully understood. We investigated two potential contributing factors: auditory filter width and processing efficiency. Auditory filter width reflects the frequency-resolution ability of the auditory system. It can be assessed by determining the range of frequency components in a masking noise that affects the detectability of a tonal signal in that noise. Processing efficiency encompasses any factor, other than auditory-filter width, that contributes to signal-detection performance. It can be assessed by determining the signal-to-noise ratio at threshold at the output of the filter used for detection. Wider filter widths and poorer processing efficiency are both associated with hearing-in-noise deficits. Previous work suggests that auditory filter width matures early in development while processing efficiency matures much later, implicating immature processing efficiency in children's difficulty hearing in noise. However, that conclusion is largely based on the detection of long tones in continuous noise, circumstances that are not indicative of listening in everyday environments where signals are often brief and background noise is discontinuous. Additionally, for adults, detection thresholds for brief tones are higher when the tones are presented at the onset rather than later in a noise, but this phenomenon--called overshoot here--has not been investigated in children.
Methods: We examined signal threshold, auditory filter width, and processing efficiency in children (7-9 years old; n=8) and young adults (n=7) for a 20-ms, 1-kHz tonal signal presented 0 ms (onset) or 200 ms (delay) after the onset of a 300-ms masker. The masker was a fixed-level bandpass or notched noise (notch width: 0.4 kHz) centered on the signal frequency. We fit the threshold data using the roex(p) model to estimate filter width (p) and processing efficiency (K).
Results: For both age groups, thresholds were higher in the onset than the delay condition (documenting overshoot), but only for the notched noise. However, the overshoot magnitude was three times larger in the children, due to children's higher thresholds at noise onset. In the delay condition, children had similar filter widths, but somewhat poorer processing efficiency compared to adults, like previous observations. In contrast,

in the onset condition, children had both notably wider filter widths and notably poorer processing efficiency than adults.
Conclusions: Overall, the results suggest that children have greater difficulty hearing in noise than adults in part because children have greater overshoot than adults. The greater overshoot in children appears to be associated with immaturities in both auditory filter width and processing efficiency for brief signals at masker onset. Of note, the developmental changes in filter width and processing efficiency are larger at masker onset than after a delay. Thus, children may also be adversely affected by more extreme temporal fluctuations in those factors.

S168. Eye-Fixation and Speech Perception for Visual and Auditory Words in Age-Related Hearing Loss

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Category: Multisensory Processing/Interactions
Background: Adults with hearing loss appear to show a bias toward visual processing during audiovisual perception, as shown by more frequent fusions of McGurk stimuli (Rosemann and Thiel 2018, Neuroimage 175:425-37) and stronger susceptibility to visual distractors (Puschmann et al. 2014, Hear Res 316: 28-36). Visual bias could result from cross-modal reorganization or from a chronic reliance on the visual modality to resolve degraded auditory signals. Here we test how visual bias in hearing loss is affected by environmental background noise, and how visual attention plays a role. Participants were asked to identify if a visual word preceding an auditory word (or the reverse of this order) matched or mismatched, and eye fixations were recorded for visual words. We predicted that adults with greater age-related hearing loss (ARHL) will (i) benefit more when presented with visual-preceding-auditory speech, and (ii) focus more on the mouth of the speaker when presented with auditory-preceding-visual speech. We also hypothesized that increased fixation to the speaker's mouth would predict better task performance for visual-preceding-auditory words.
Methods: Fifty-nine participants aged 59 to 81 with untreated hearing loss or typical hearing underwent pure-tone audiometry to 8 kHz, and QuickSIN measured speech-in-noise (SIN) listening. Participants were presented with audio and visual monosyllabic words across two manipulations. First, words were presented with audio-only preceding visual-only presentations, or in reverse of this order. Second, words were presented in either quiet, −5 dB SNR, or −10 dB SNR (babble noise). For each trial, participants reported if the audio and visual words were the same or different. Eye-tracking measured fixations to facial features for visual words.
Results: We found that audiometric thresholds did not predict task performance or fixation to facial features during visual-only words. Irrespective of hearing ability, we found that (i) accuracy for identifying whether audio and visual words matched increased when participants heard the audio word before seeing the visual word, and this effect diminished as background noise increased; (ii) proportion of eye-fixation to the mouth, relative to the eyes, of the speaker was larger for auditory-preceding-visual words; and (iii) fixation to the eyes of the speaker predicted better task performance for visual-preceding-auditory words.
Conclusions: The visual bias observed in ARHL may be limited to certain multisensory integration conditions (e.g., McGurk effect) as our findings do not support the notion of enhanced auditory prediction following a visual word when thresholds are elevated. As this study presents words separately and serially, future research should investigate how ARHL may impact audiovisual speech perception when signals are presented simultaneously, similar to day-to-day speech, and how visual attention may play a role.

S169. Open Board

S170. Effects of Electrical Stimulation Rate in Neuromodulating the Auditory System Through Non-Invasive Paired Electrical and Acoustic Stimulation

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Category: Multisensory Processing/Interactions

Background: Paired acoustic and electrical stimulation of the ear regions has been shown to facilitate or suppress neural activity and induce plasticity within auditory brain regions dependent on stimulation parameters. Successful parameters enhancing auditory neural responses or shifting best frequencies of auditory cortical neurons have been demonstrated in animal studies. Recently, transcutaneous stimulation approaches in humans have been used to target both the somatosensory and autonomic nervous system pathways involved in neural plasticity effects. However, there is no consensus on optimal electrical stimulation conditions. Few investigators have initiated a comprehensive and systematic examination of the best electrical stimulation parameters for measurable behavioral outcomes in humans, which can guide developing potential techniques in auditory rehabilitation and hearing health devices. In this study, we sought to investigate the effects of electrical stimulation rate in a paired stimulation (PS) paradigm on changing the perception of simple sounds.
Methods: The study consisted of five days of 30-minutes of electrical and acoustic PS. The PS consisted of either a 25 or 100 Hz burst of electrical pulses delivered to the left cymba concha with a bilateral 250 ms, 1-octave, narrowband noise centered at 6 kHz or 6 kHz pure tone (20 dB SL). There were 600 instances of the PS occurring with an inter-trial interval of 2.75 seconds ± 10% temporal jitter. A music background composed of harps and strings was presented at 15 dB SL in between trials of PS. The level of electrical stimulation was 110% of the daily determined perceptual threshold using the pulse train of the PS paradigm. Five participants with various hearing profiles have completed the study. Recruitment is ongoing to ensure that the expected sample size from the prior power analysis is met. Data was collected at baseline and after the 5 sessions of PS, which includes audiograms, questionnaires, auditory brainstem responses, and cortical auditory evoked potentials. These outcome measures will be analyzed to assess electrophysiologic or behavioral changes in response to PS.
Results: Data collection and analysis are currently ongoing. Analysis of the data will include within-subject comparisons (Day 1 vs. Day 5) on electrophysiological, audiometric, and questionnaire changes. Between-group evaluations will also be performed to determine the effects of the different stimulation rates. The possibility of using electrocardiogram metrics in indexing the activation of the autonomic nervous system will be discussed.
Conclusions: The results of this study can show if the proposed PS can induce neural plasticity in the auditory system. Determining an effective stimulation rate is an important step towards optimizing the parameter space of the proposed neuromodulation method. The protocols and paradigms determined from the study have implications for the development of customizable hearing health devices to meet individual needs, such as potentially improving hearing performance or treating tinnitus.

S171. Extended High Frequency Thresholds, Evoked and Spontaneous Otoacoustic Emissions, and Medial Olivocochlear Reflex

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Category: Otoacoustic Emissions
Background: The purpose of this study was to investigate hearing thresholds at extended high frequency range (EHF) and their possible relationships with otoacoustic emissions (OAEs) of various types: transiently evoked OAEs (TEOAEs), distortion product OAEs (DPOAEs) and spontaneous OAEs (SOAEs). Additionally medial olivocochlear reflex (MOCR) was evaluated by the inhibition of TEOAEs by contralateral noise.
Methods: Study group consisted of 50 adults with normal hearing. EHF was evaluated at: 9, 10, 11.2, 12.5, 14, and 16 kHz. TEOAEs were recorded with a linear protocol (identical stimuli), a constant stimulus level of 65 dB peSPL, and contralateral broadband noise (60 dB SPL) as a suppressor. TEOAE response levels, signal-to-noise ratios (SNRs), raw dB TEOAE MOCR, and normalized TEOAE MOCR were investigated. DPOAEs were evaluated at 1, 1.5, 2, 4, 6, 8, 10, 12, and 16 kHz. Each subject was tested for the presence of SOAEs using the synchronized SOAE technique.
Results: The response levels of TEOAEs correlated significantly with the hearing thresholds from the standard frequency (SF) range and did not correlated with EHF thresholds. DPOAEs, both within the SF range and the EHF range, decrease as thresholds deteriorate. MOCR on the other hand did not correlated with thresholds from the SF range but correlated significantly with EHF thresholds. The better the EHF thresholds

– the higher MOCR. Additionally, the number of SOAEs correlated significantly with EHF thresholds and MOCR. The higher the number of SOAEs the lower EHF thresholds and MOCR.
Conclusions: EHFs influenced DPOAEs but not TEOAEs. However, the MOCR as evaluated by TEOAEs seems to be related to the hearing in EHF. Studies available in the literature indicate that EHF and MOCR are related to similar aspects of auditory processing (e.g., localization or understanding speech in noise). Therefore, the association of these two measures appears not to be coincidental. However, whether indeed the MOCR and EHF portion of the auditory pathway modulate certain auditory processing abilities requires further more detailed research.

S172. Expanding the Objective Audiometric Test Battery for Studying Age-Related Hearing Loss and Novel Therapeutic Candidates in Rats

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Category: Otoacoustic Emissions
Background: Outer hair cell (OHC) dysfunction receives increasing attention in age-related hearing loss and speech discrimination (Wu et al. 2020, Parker 2020) and as early, objective biomarker of subclinical hearing loss and cognitive dysfunction (Medel et al. 2023) potentially foreshadowing hearing loss contribution to early onset dementia (Livingston et al. 2020, Wang et al. 2022). Rats provide a well-established research model of hearing loss onset and progression in the preclinical setting as well as testing of novel candidate therapeutics (Escabi et al. 2019, Holt et al. 2019). Furthermore, rats are broadly used for regulatory general toxicology and safety studies and potentially also best suited for inner ear specific toxicology studies (Shimoji et al. 2019). Age-related hearing loss progression has previously been reported in standard Wistar rats (Petremann et al. 2020), but the characterization of OHC function was limited to stimulus-evoked DPOAEs, but reflection-type otoacoustic emissions that provide information about cochlear tuning (Souter 1995, Abdala et al. 2022) and may be more sensitive to hearing loss onset (Keefe et al. 2019, Blankenship et al. 2019) have not yet been established for this species. We here report initial efforts to broaden the toolbox for objective audiometry in rats to include SFOAE measurements, for use in the study of OHC contribution to age-related hearing loss progression in this species and testing of novel therapeutic candidates.
Methods: Six 13 weeks-old male Wistar rats underwent bilateral audiometric characterization in a soundproof chamber: ABR thresholds (2/4/8/16 kHz, 90-10 dB SPL in 5 dB steps, closed field configuration), DPOAE amplitudes (2/4/8/16 kHz, f2/f1=1.2, L1/L2=80/70 dB SPL, single centered frequencies and upward swept tone stimulus from 2 to 20 kHz (f2), sweep rate 0.0099 kHz/ms) and SFOAEs (2-16 kHz, 10 points/octave, probe/suppressor level 65/75 dB SPL, fs = fp-50 Hz) were determined.
Results: Animals demonstrated normal hearing with ABR thresholds from 20.0±3.0 dB SPL to 30.8±5.1 dB SPL between 2-16 kHz. Single frequency DPOAEs showed mean amplitudes from 10.4±4.5 dB SPL to 36.5±1.9 SPL between 2-16 kHz comparable to amplitudes obtained with swept-tone DPOAEs. Including only emissions with SNR greater than 6 dB (average SNR across all frequencies = 22.5 dB), mean SFOAE amplitudes ranged from 4.9±5.6 dB SPL (2.15 kHz) to 23.9±4.1 dB SPL (6.3 kHz) with some individual maximum SFOAE amplitudes up to 30 dB SPL in the 4.4 to 8.4 kHz range.
Conclusions: These data confirm that SFOAEs can be measured consistently in Wistar rats with fine structure and levels comparable to other species such as guinea pigs (Shera et al. 2010), mice (Cheatham et al. 2011) or chinchillas (Berezina-Green and Guinan, 2015) not commonly accepted for all aspects of translational therapeutic development. Further work aims to demonstrate the evolution of SFOAEs with aging, in comparison to ABR and DPOAE measurements.

S173. Changes in Click Evoked Otoacoustic Emissions Reflect Cochlear Gain Changes That May Underlie Forward Masking

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Category: Otoacoustic Emissions

Background: Forward masking refers to an increase in threshold for detection of a brief probe tone following a masking stimulus. Models for forward masking have typically focused on effects that are hypothesized to interfere with the neural response to the probe tone, such as a prolonged response to the masker or neural adaptation. Another line of studies has suggested that efferent control of cochlear gain, via the medial olivocochlear (MOC) system, plays an important role in forward masking. Click evoked otoacoustic emissions (CEOAEs) can be used to evaluate changes in cochlear gain due to the MOC efferent system (Goodman et al., 2021, JARO 125:1938). Here we measured CEOAEs before and after a tone stimulus to test the hypothesis that the MOC system reduces gain during the masking stimulus, and that recovery of gain mirrors the recovery from forward masking observed in psychoacoustic studies.
Methods: CEOAEs were measured in human subjects with normal or mild hearing loss using an Etymotic 10B+ probe-tube microphone. 100-μs clicks were presented 25 ms before and 25 or 75 ms after 200- or 400-ms duration 65-dB SPL tones. Tones were presented at frequencies for which CEOAEs were measurable in individual listeners, avoiding dips in the fine structure of each listener's CEOAE spectrum. CEOAEs amplitudes at different frequencies were analyzed using a bank of bandpass filters; the amplitude of the long-latency component in each frequency channel was estimated (Goodman et al., 2021). The CEOAEs in response to the clicks before and after the tone were compared to assess changes in cochlear gain due to the tone. CEOAEs based on many repetitions of low-level, single-click stimuli were used to minimize the effect of the clicks on cochlear gain. Click levels were adjusted such that CEOAEs were 10-20 dB above the noise floor, but kept low to maintain sensitivity to the MOC. Long, 1-sec interstimulus intervals were used to allow recovery of the efferent system between trials.
Results: Changes in CEOAE long-latency component amplitudes suggested a reduction in cochlear gain by the MOC efferent system that was greatest at the tone frequency for clicks 25 ms after the tone. The frequency of maximal gain change shifted with tone frequency. The size of the gain change decreased for longer click delays.
Conclusions: The results support the hypothesis that changes in cochlear gain, as evidenced by CEOAE long-latency component amplitudes, could explain changes in threshold associated with forward masking. These changes in cochlear gain would affect detection of a probe tone following a tone masker over a time course consistent with forward-masking effects. Future models of forward masking could incorporate these effects using physiological models that include the MOC efferent system.
Supported by NIH DC001641

S174. Frequency Importance Functions in Simulated Bimodal Hearing

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Category: Psychoacoustics
Background: Compared to the use of a cochlear implant (CI) alone, some bimodal users benefit, while others do not. This variability might be due to the different spectral processing abilities of bimodal users, which are critical for speech perception. The spectral information processed by each user is different due to the extent of their residual hearing in the hearing aid (HA) ear and electrode array insertion depth in their CI ear. This results in different weights for the user in specific frequency bands for speech perception (i.e., the frequency importance functions or FIFs). FIFs of the CI alone (CI FIFs) utilized for bimodal benefit may be different from the FIFs of the CI+HA (bimodal FIFs). This study aims to compare CI FIFs and bimodal FIFs.
Methods: This is an acoustic simulation study of bimodal hearing with twenty adults with normal hearing: 10 for CI FIFs and 10 for bimodal FIFs. Sentence perception was measured in quiet and at 6 dB signal-to-noise ratio, with HA alone, CI alone, and CI+HA as a function of spectral hole. Percent scores were used to derive four sets of FIFs: CI FIFs and bimodal FIFs in quiet and noise. For CI FIFs, acoustic hearing was simulated using low-pass filtering with a cutoff frequency of 500 Hz. Electric hearing was simulated using a six-channel sinewave vocoder with a fixed output frequency ranges (1000-7938 Hz) but with three different input frequency ranges to create frequency maps found in bimodal patients: overlap (188-7938 Hz), meet (500-7938 Hz), and gap (750-7938 Hz), relative to 500 Hz used for the acoustic simulation. A total of 22 spectral hole conditions were created by setting the amplitudes of CI channel(s) to zero: no spectral hole (control), six single-hole, and fifteen two-hole (e.g., 1,2; 1,3; 1,4, ….., and 5,6). For EAS FIFs, the approach is identical to

CI FIFs besides three exceptions: spectral holes on both HA and CI ears, overlap map only, and additional 10 three- and 6 four spectral holes to obtain fine FIFs with the most common bimodal map.
Results: CI FIFs, overlap map weighted channels 4, 5, and 6 (1496.5-7938 Hz), while meet and gap maps weighted channels 5 and 6 (2545-7938 Hz) in both quiet and noise. Bimodal FIFs have gradual increase and decrease patterns both in quiet and noise but were highest at channel 5 in quiet and 4 in noise.
Conclusions: Frequency range of 2647-7938 Hz contributed most to bimodal speech perception when CI alone was considered, but frequency range of 1496.5-4606.3 contributed most when both devices were considered. Frequencies lower than 1500 Hz were weighted less for bimodal speech perception. Bimodal users utilized frequency information differently between quiet and noisy environments.

S175. Spectral Processing in Simulated Electric Acoustic Stimulation Hearing

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Category: Psychoacoustics
Background: Electric acoustic stimulation (EAS) hearing is a monaural hearing, combining electric stimulation through a cochlear implant (CI) and acoustic stimulation through a hearing aid (HA). EAS user may have their own strategy to utilize the spectral information (i.e., the frequency importance functions or FIFs) depending on the extent of their residual hearing and spectral mismatch in electric stimulation. FIFs of the CI alone (i.e., CI FIFs) and the combined use of CI+HA (i.e., EAS FIFs) may differ due to interactions between acoustic and electric stimulations. The goal of this study was to compare CI FIFs and EAS FIFs using simulation of EAS hearing.
Methods: Twenty adults with normal hearing participated: 10 for CI FIFs and 10 for EAS FIFs. Sentence perception was measured in a quiet and at 6 dB signal-to-noise ratio, with acoustic alone, electric alone, and combined stimulations as a function of spectral hole. Percent scores were used to derive four sets of FIFs: CI FIFs and EAS FIFs in quiet and noise. For CI FIFs, acoustic hearing was simulated using low-pass filtering with a cutoff frequency of 500 Hz. Electric hearing was simulated using a six-channel sinewave vocoder with a fixed output frequency ranges (1000-7938 Hz) and with three different input frequency ranges to create typical frequency maps found in EAS patients: overlap (188-7938 Hz), meet (500-7938 Hz), and gap (750-7938 Hz), relative to 500 Hz used for the acoustic simulation. A total of 22 spectral hole conditions were created by setting the amplitudes of channel(s) to zero: no spectral hole (control), six single-hole, and fifteen two-hole (e.g., 1,2; 1,3; 1,4;…5,6). For EAS FIFs, the same approach used for CI FIFs was used with following exceptions: spectral holes on both acoustic and electric sides, overlap map only which is the most common EAS map, and additional 10 three- and 6 four spectral holes to derive fine EAS FIFs.
Results: For CI FIFs, the overlap and meet maps weighed frequency ranges of 188-838 Hz and 2648-7938 Hz most in quiet and 1496.5-7938 Hz in noise, while gap map weighted frequency range of 3737-7938 Hz most in quiet and had a flat shape in noise. EAS FIFs had sharp increase slope both in quiet and noise, but the most weighted the frequency range of 1497-4606 Hz in quiet and 820-2648 Hz in noise.
Conclusions: CI FIFs suggest that the contribution of frequency bands depends on degree of frequency overlap between acoustic and electric stimulation and noise, but higher frequency range (about 2648-7938 Hz) were most weighted regardless of the frequency map. In contrast, EAS FIFs suggest that lower frequency range (820-2648 Hz) contributed to EAS hearing both in quiet and noisy listening condition.

S176. Modulation-Domain Psychophysical Tuning Curves Measured in Normal-Hearing and Cochlear-Implant Listeners

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Category: Psychoacoustics
Background: This study explored the feasibility of measuring amplitude-modulation (AM) domain psychophysical tuning curves (mPTCs) in cochlear implant (CI) listeners, aiming to characterize their

modulation filters, as a basis of AM processing. Despite their presumed importance for CI listeners' auditory performance, their modulation filter properties remain to be explored.
Methods: Two participants, one bilateral CI listener (30 years old) with congenital severe hearing loss and one normal-hearing (NH) listener, participated in the study. The CI listener had been wearing hearing aids until the age of implantation (22 and 29 years old for the left and right ear, respectively). The experimental procedures followed those of Ewert and Dau (JASA, 2002). The stimulus was a broadband low-noise noise (duration 600 ms), modulated in the 1000-4000 Hz range. The signal modulation was a sinusoid with a frequency (Fs) of 4 or 16 Hz, set at 4 dB above each listener's detection threshold. The masker modulation was a 1/2 octave wide noise, and its center frequency (Fm) varied from -2 to +2 octaves around Fs. The stimuli were presented via a single loudspeaker 1.2 m in front of the listener's head at a level of 65 dBA. mPTC is the maximum masker modulation depth at which the signal modulation is detectable (THm), plotted as a function of Fm. Temporal modulation transfer function (TMTF; the detection threshold for signal modulation (THs) as a function of Fs in the absence of the masker) was also obtained.
Results: The TMTFs of both CI and NH listeners showed typical lowpass characteristics, i.e., . THs tended to increase with Fs, but the cutoff frequency for the CI listener was lower than that for the NH listener. The mPTCs also showed a typical "U" shape, i.e., . THm increasing as Fm and Fs separated. The CI listener's mPTC for the 16 Hz Fs showed a marked asymmetry, with the THm for the higher Fm side being higher (i.e., easier to detect) than for the other side, resulting in the minimum of the mPTC being at a frequency lower than 16 Hz. This was likely due to the cutoff AM frequency of the stimulus in the CI processor.
Conclusions: These preliminary results suggest that the auditory system of the CI listener comprises of modulation filters, which can be evaluated by the psychophysical modulation masking paradigm with acoustic stimuli. The measured mPTCs are influenced by the CI processor's parameters. Future studies will compare mPTC characteristics in CI users with varying implantation histories to understand long-term adaptation to CI input in the central modulation processing mechanisms.

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S177. Effects of Salient Interruptions on Auditory Spatial Attention

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Category: Psychoacoustics
Background: Signals received by our auditory systems are always a mixture of multiple different sound sources; selective attention lets us survive the complex acoustic world by enhancing the event of interest and suppressing other competing sounds. However, salient and unexpected events can redirect our attention automatically, disrupting volitional, top-down selective attention. This study explores how bottom-up interruptions interact with top-down auditory spatial attention. In a series of online studies, we investigated the impact of novelty of the interrupters, working memory load of the task, and timing of the interruption. We quantified how unexpected interruptions interfered with the ability to recall a target stream for these different stimulus conditions.
Methods: We used a syllable-recall task design for our spatial attention task. Two competing sound streams made up of random sequences of spoken syllables (BA, DA, and GA) were spatialized to opposite hemifields. Listeners were asked to focus on one of the streams (the target) and report the syllables making up the target. In 50% of the trials, an unexpected interrupting sound occurred, and the performance difference caused by the interruption was computed for each target syllable position.
To investigate the impact of novelty of interrupters, we compared using the same interrupting sound versus using a unique, novel sound for each interrupted trial. To understand the role of working memory load on our ability to resist interruption, we varied the number of syllables in the target sequences. To study the effect of timing of the interruption, we placed the interrupter at different temporal locations. We also contrasted results when the timing of the interrupter was fixed across trials to when it was randomly selected from trial to trial.
Results: There was a robust pattern of interruption effect with novel interrupters. Specifically, recall performance for the target syllable occurring just after an interruption was always significantly worse than when uninterrupted. Depending on the condition, the degradation in recall could extend forward in time to the

3rd syllable after the interruption, or could extend "backward in time," impairing recall of a target syllable that preceded the interruption. As working memory load increased, the effect size of the degradation in recall performance for the syllable just after the interruption tended to increase. Finally, the interrupter tended to have a larger impact when the timing of the interrupter was random rather than predictable.
Conclusions: The consistent impairment to recall of the syllable occurring just after an interruption suggests that a salient enough interruption causes a robust disruption of selective attention, independent of the working memory load and the novelty or timing of the interruption. However, these factors influence how long-lasting the effect of the interruption is and the degree to which it impairs recall of preceding syllables.

S178. A Data-Driven Approach for Rapid Profiling of Loudness Perception

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Category: Psychoacoustics
Background: Rapid estimation of loudness growth across a wide range of frequencies could have important applications in individualized fitting of hearing devices and ensuring listening comfort. Standard procedures for estimating loudness growth typically involve repeating a categorical loudness scaling (CLS) task using pure-tone stimuli over a pre-selected set of test levels and frequencies. The current study explores the feasibility of an adaptive procedure that iteratively selects the test frequency and level.
Methods: A database of CLS data was used to train a data-driven model of loudness perception. The database consisted of data from 148 listeners, including 61 with normal hearing (NH) and 78 with various degrees of hearing loss (HL). Each listener's loudness perception profile was described by a set of multi-category psychometric functions, one for each of the ten frequencies ranging from 250 Hz to 8000 Hz. Each multi-category psychometric function described the levels defining the ten loudness category boundaries that separated the total of 11 loudness categories in the original experiment. These ground-truth category boundaries across frequencies were modeled using a small number of principal components via a principal component analysis, with the listener's loudness perception profile represented in the component scores. Adaptive procedures were developed which iteratively re-fitted the data-driven model after each response was collected and used the interim model for the selection of the subsequent stimulus.
A series of Monte Carlo simulations were conducted to evaluate the feasibility of the adaptive procedures based on the data-driven model. For each listener in the database, the multi-category psychometric function was used to simulate the listener's responses, and the multi-category psychometric functions of the remaining 147 listeners were used to train the data-driven model and formulate the adaptive procedures. Four different adaptive procedures differing in their stimulus-selection strategies were evaluated. Each adaptive procedure consisted of 200 simulated trials and was repeated 10 times for each simulated listener.

Results: All four stimulus selection strategies in the adaptive procedure were able to provide convergent estimates of the loudness category boundaries across frequencies and equal-loudness contours at loudness levels of 20, 40, 60, 80, and 100 phons. The average root-mean-square errors for the estimated equal-loudness contours were typically under 6 dB following 100 simulated trials. Increasing each adaptive procedure to 200 trials only improved the accuracy of the estimates by less than 1 dB. The error was slightly larger for the listeners in the HL group than the NH group. The different stimulus-selection strategies only had very limited effects on the estimated equal-loudness contours, especially for the HL group.
Conclusions: The current study suggests the feasibility of rapid profiling of individual listeners' loudness growth across a wide frequency range using a data-driven approach within approximately five minutes of testing.

S179. Initial Evaluations of a Rapid Psychophysical Procedure for Estimating Loudness Growth Across a Wide Frequency Range

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Category: Psychoacoustics

Background: Comprehensive loudness assessment may be used for individualized hearing-aid fitting and fine-tuning. Traditional approaches based on repeating categorical loudness scaling tasks across frequencies, although with their demonstrated reliability and potential clinical utility, are often too time-consuming to be implemented for routine clinical applications. Bayesian adaptive procedures may present a venue for improving the efficiency of behavioral loudness assessments. The current study performs an initial evaluation of a Bayesian adaptive procedure for estimating loudness growth across a wide frequency range.
Methods: Two cohorts participated in the experiment at two different locations (University of Washington [6 adults 55 years old and above] and Boys Town National Research Hospital [7 adults between 21-35 years old]). The experiment was completed in one test session, which consisted of pure-tone audiometry and two repetitions of loudness assessment. For each repetition of the loudness assessment, a Bayesian adaptive procedure of 100 categorical loudness scaling trials were conducted. During each trial, listeners provided categorical ratings (e.g., "Can't Hear", "Very Soft", "Soft", "Medium", "Loud", "Very Loud", and "Too Loud") to pure-tone stimuli, by selecting one of 11 categories. The pure-tone stimuli were presented monaurally via an insert earphone. The Bayesian adaptive procedure iteratively updated a phenomenological model of the loudness category boundaries and used the interim model estimate for the selection of stimulus parameters (test frequency and level) of the subsequent trial. The selection of stimuli additionally constraint to reduce the probability of "Too Loud" responses and to prevent electroacoustic distortions when driving the earphone at high frequency and level. Following data collection, for each participant and each repetition of the adaptive procedure, the stimulus parameters as well as the participant's responses from the 100 trials were subjected to post hoc model fitting to derive the final estimates of the 10 category boundaries (separating the 11 response categories) between 250 and 6000 Hz.
Results: All participants were able to complete the Bayesian adaptive procedure in approximately five minutes. Eight of the thirteen participants showed satisfactory agreement between the two repetitions of the procedure, with root-mean-square deviations between the two estimates of loudness category boundaries under 6 dB. An analysis of the participants' responses showed that on average less than 5% of the trials led to "Too Loud" responses. Moreover, the median of all responses was close to "Medium" regardless of the degree or configuration of the participant's hearing loss. This suggests that the procedure was able to adaptively prevent presenting disproportionately larger numbers of stimuli near the upper or lower limit of the dynamic range.
Conclusions: The current study provides initial evaluations of using a Bayesian adaptive procedure to rapidly profile older adult listeners' loudness perception.

S180. Acoustic Constraints on Vocal Learning in Songbirds

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Category: Psychoacoustics
Background: Most animals that communicate using hearing and voice produce and perceive species-specific signals that develop without learning. In contrast, songbirds learn their complex courtship vocalizations (songs) by copying the syllables of adult tutors. Prior studies show that juvenile songbirds tutored by adults of a different species are able to learn most of their tutors' syllables. Our experiments on cross-species song learning suggest that syllable copying between species is limited; pupils of one species fail to learn specific syllables from tutors of another species. We hypothesize that syllable learning is constrained by the acoustic similarity of tutor and pupil songs, at the species level. We used cross-species song learning experiments to identify acoustic features of heterospecific tutors' syllables that were and were not copied by pupils, and compared them to the acoustic features of pupil species' syllables. Results indicate that species genetics constrain vocal learning of both spectral and temporal acoustic features.
Methods: We tested the hypothesis that syllable learning is influenced by the acoustic space of conspecific song. We predicted that juveniles would prefer copying heterospecific song syllables that share acoustic features with conspecific syllables. We recorded the songs of adult Bengalese finches (Lonchura striata domestica), long-tailed finches (Poephila acuticauda), and zebra finches (Taeniopygia guttata) that were either tutored by their own species (normal) or tutored by a different species (cross-tutored). From song recordings, we measured 21 acoustic features of each syllable, including duration, mean frequency, and spectral entropy, which differ significantly across species. We used Principal Component Analysis to compare the: 1) acoustics

of tutor syllables that were and were not copied by heterospecific pupils; and 2) acoustics of cross-tutored pupils' syllables and conspecific song syllables. We also compared the acoustic features of normal birds' syllables from tutor generation to pupil generation.
Results: Birds tutored by conspecifics copied tutors' syllables. Cross-tutored birds copied only heterospecific syllables that shared acoustic features with conspecific song. Specifically, the ranges of syllable duration and mean frequency found in conspecific song syllables predicted which heterospecific song syllables were copied.
Conclusions: Results suggest that genetic auditory-vocal mechanisms impose boundaries on vocal learning at the species level. Both spectral and temporal features appear to constrain vocal learning.

S181. What Frequency Was That Tone and When Did it Occur? A Pilot Experiment

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Category: Psychoacoustics
Background: When asked to judge whether a brief clearly audible tone was presented before, during, or after a noise, listeners reliably perceive the tone as having occurred later than it actually did. That is, they frequently perceive tones that were presented before a noise as having been in the noise, and tones that were presented during a noise as having been in the silence after the noise. There is a clear mismatch between perception and reality. We wondered whether this mismatch might affect performance on other auditory tasks. Might there be cases in which performance is more closely tied to the perceived rather than the actual background in which the task is conducted? Here we describe our first, decidedly primitive, attempt to address this question.
Methods: Specifically, on each trial, we randomly presented a 0.9-kHz or 1.1-kHz tone before, during, or after a bandpass noise and asked listeners (n=21) to answer two questions: (1) Was the tone presented inside or outside of the noise? and (2) Was the frequency of the tone high or low? The question order differed between two groups.
Results: Initial analyses suggest that the tone was perceived as having occurred later in relation to the noise than its actual presentation time, replicating previous observations. Further, frequency-identification performance varied across the temporal positions of the tone. Performance was poorest at noise onset, where listeners largely perceived the tone to have occurred during the noise, but improved to near silent-background levels as the tone in noise was increasingly perceived to have occurred in silence. Overall, performance across the tone-presentation times varied approximately inversely for the time and frequency tasks.
Conclusions: This outcome is at least consistent with the idea that performance on a perceptual task can be more closely tied to the perceived rather than the actual background in which the task is conducted. To the extent that this interpretation holds, the results suggest that (1) listeners determine when a stimulus was presented prior to determining exactly what the stimulus was, and that (2) task performance with clearly audible sounds may be more constrained by higher-level (perceptual) than lower-level (sensory) processes.

S182. The Effect of Signal Level and Signal-To-Noise Ratio on Adaptation to Noise in Spectral and Temporal Modulation Detection

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Category: Psychoacoustics
Background: The term 'noise adaptation' refers to an improvement in auditory function as the signal of interest is delayed in the noise. We have previously shown that when the signal is a modulated ripple noise, adaptation occurs for spectral (SM) and temporal modulation (TM) detection, but not for the detection of spectro-temporal modulations combined. Here, we investigate the effect of stimulus level and signal-to-noise ratio (SNR) on noise adaptation for temporal and spectral modulation detection.
Methods: The signal was a 200-ms spectrally (2 cycles/oct) or temporally (10 Hz) modulated ripple noise embedded in white noise. The noise-signal onset delay was 50 ms (early condition) and 800 ms (late

condition). The modulation depth (dB) was varied adaptively to measure a modulation detection threshold. Adaptation was calculated as the threshold improvement in the late relative to the early condition. In Experiment 1, adaptation for SM (N=16) and TM (N=11) detection was measured for signal levels of 45, 60 and 75 dB SPL while the SNR was fixed at 0 dB. In Experiment 2, adaptation was measured for SM (N=10) and TM (N=10) detection for SNRs of -10, -5, 0, 5 and 10 dB while the background noise level was fixed at 60 dB SPL.
Results: SM detection thresholds improved significantly in the late condition at the three levels (1.8 dB at 45 dB SPL; 2.2 dB at 60 dB SPL, and 3.0 dB at 75 dB SPL). For TM detection, significant adaptation was observed at 60 and 75 dB SPL but not at 45 dB SPL (2.9 dB, 2.0 dB, and 1 dB, respectively). The effect of signal level on adaptation was not significant for SM or TM detection. Adaptation for SM detection was significant at all SNRs except 5 dB (1.2 dB at 10 dB SNR; 1.7 dB at -5 dB SNR; 2.2 dB at 0 dB SPL and 2.9 dB at +10 dB SNR). For TM detection, adaptation was significant at - 10 dB SNR (2.4 dB) and at 0 dB SNR (3.0 dB), but not at the other SNRs.
Conclusions: Noise adaptation for SM detection tended to increase with level. For TM detection, adaptation tended to be greater at mid-levels than at lower or higher levels. However, the stimulus level did not affect adaptation significantly for SM or TM detection. Although noise adaptation in SM and TM detection seems to be greater at 0 dB SNR, the effect of SNR on adaptation was not significant. We discuss the implications of the results for elucidating the mechanisms involved in noise adaptation. [Work supported by the University of Salamanca, Banco Santander, and the Spanish Ministry of Science and Innovation (grant PID2019-108985GB-I00)].

S183. The Role of Temporal Coding in Hearing: Evidence From Task Optimization

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Category: Psychoacoustics
Background: Neurons encode information in the timing of their spikes in addition to their firing rates. The fidelity of spike timing is arguably greatest in the auditory nerve, whose action potentials are phase-locked to the fine-grained temporal structure of sound with sub-millisecond precision. The role of this temporal coding in hearing remains controversial because physiological mechanisms for extracting information from spike timing remain unknown. Nonetheless, phase-locked spike timing has been proposed to support hearing in noise, with recognition difficulties of hearing-impaired listeners potentially reflecting an inability to use temporal fine structure. We investigated the perceptual role of auditory nerve phase locking by optimizing models to perform real-world hearing tasks using simulated cochleae, asking whether phase locking in a model's cochlear input was necessary to obtain human-like behavior.
Methods: We trained deep artificial neural networks to recognize and localize words and voices and to make pitch judgments using simulated auditory nerve representations of naturalistic auditory scenes. We manipulated the upper limit of phase locking via the lowpass cutoff in simulated inner hair cells, varying it between 3000 Hz (the presumptive upper limit in humans) and 50 Hz (eliminating virtually all phase locking to fine structure). Models were separately optimized with each cutoff and we measured the extent to which they replicated human behavior in a range of experimental conditions.
Results: Models with high-frequency phase locking replicated human behavior across all tasks and stimulus conditions. Degraded phase locking affected some tasks more than others. Voice recognition and sound localization were most susceptible, with degraded phase locking leading to impaired performance in noise and inhuman responses to pitch and localization cue manipulations. Degraded phase locking left word recognition largely intact, with models exhibiting human-level performance in most real-world noise conditions. Nonetheless, phase locking -- at least up to the fundamental frequency of typical adult speech -- was necessary to account for the fluctuating masker benefit and the effects of tone vocoding seen in normal-hearing humans.
Conclusions: The results suggest that information conveyed by the precise, millisecond-level timing of auditory nerve spikes contributes to perception and therefore must be extracted by the human auditory system. Our modeling approach links neural coding to real-world perception and clarifies conditions in which prostheses that fail to restore high-fidelity temporal coding (e.g., contemporary cochlear implants) could in principle restore near-normal hearing.

S184. Mapping the Timecourse of Adaptation to Noise Statistics During Localisation and Speech Discrimination Tasks

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Category: Psychoacoustics

Background: Adaptation to background noise is an important facilitator for auditory perception, as a way to suppress the deleterious effects of noise and focus on more informative sounds. Previous studies have shown that listeners can rapidly adapt to the onset of background noise when discriminating speech sounds. While it has been shown that auditory cortical neurons adapt to changes in noise statistics, it is unclear whether human listeners perceptually show similar adaptation to background noise statistics. The timecourse of adaptation is also unknown, as is whether adaptation timecourses are task dependent.

Methods: This study aimed to investigate adaptation to changes in noise statistics using two behavioural tasks. The first involved participants reporting the location of a 300ms broadband noise target within an 18-speaker array. The target in each trial was either presented in silence or in one of three types of naturalistic noise located in either the left or right hemifield. The background condition switched in type and/or location for each trial. The delay between the onset of a switch in background and the target stimulus varied on each trial from 0 to 1000ms.

The second task was a four-alternative forced choice speech discrimination task, in which participants identified consonant-vowel (CV) speech stimuli each similarly either presented in silence or in one of three types of naturalistic noise. The delay between the onset of a switch in noise and the target CV stimulus varied on each trial from 0 to 1500ms.

Results: 16 and 22 participants so far have completed the localisation and speech discrimination tasks respectively. In both cases, performance increased with increasing time intervals between the switch in statistics and the target sound, even for the silent condition. To analyse the results, binomial logistic regressions were run to determine how delay, noise type, switch type (i.e., whether the trial switched from silence to noise, noise to silence or noise to noise), noise position (in the localisation task only), and talker (in the speech task only) influenced the odds of a correct response. In both cases, these analyses support rapid adaptation to background statistics that is of a similar time course, regardless of the specific change in background or behavioural task.

Conclusions: Listeners adapt rapidly to shifts in background noise and/or position. The timecourse of this adaptation is similar for sound localisation and speech discrimination on the order of ~100ms, plateauing at ~250ms. The detrimental effect that changes in background noise have on listening includes when background noise ceases, implying that breakdown of previous adaptation has a similar timescale to its build-up.

Insights gathered here may have the potential to be used diagnostically as another measure of "hidden" hearing loss, depending on the extent that adaptation impacts speech-in-noise recognition for hearing impaired listeners.

S185. 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 designs of sound-producing objects in our world, such as machinery and household appliances. Yet it remains unclear why humans have the preferences they do, and whether any such preferences are innate. 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 from the USA, a small rural town in Bolivia, and an indigenous community living in the Bolivian Amazon (the Tsimane') completed experiments in which they rated how much they liked or disliked sounds. 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. from animal vocalizations, machines, running water, etc.).
Results: The results suggest that preference for sounds can vary across cultures. Preferences for loudness and pitch height were opposite between Tsimane' and USA participants: Tsimane' participants preferred high and loud sounds, whereas participants in the USA preferred low and quiet sounds. Participants from the small Bolivian town demonstrated preferences that were intermediate between Tsimane' and USA participants. Control tasks and audiometric tests indicated that differences between groups cannot be explained by differences in task comprehension, stimulus interpretation, or audiometric thresholds. Cross-cultural variation extended to recorded environmental sounds, and was consistent with the variation in preferences for frequency (sounds with more low-frequency energy tended to be preferred by USA participants but not by Tsimane').
Conclusions: The results suggest that preferences for basic acoustic features vary across cultures. Given the gradation in responses – from Tsimane' to the small Bolivian town to USA participants – aversion to high frequencies and loud noises is plausibly related to exposure to the noises that are prevalent in industrialized societies. More broadly, the results suggest that cultural and environmental forces may be strong determinants of acoustic preferences.

S186. In Search of a Psychophysical Correlate of Dynamic Range Adaptation in Human Listeners: Intensity Discrimination within a Context

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Category: Psychoacoustics
Background: Neurons at different stages of the auditory system exhibit different behaviors and adapt their response functions depending on the stimulation history. Several mechanisms related to statistical properties of the stimulus may be involved in these changes. Among them, dynamic range adaptation (DRA) depends on the sound level distribution: the steepest part of the neuron rate-level function shifts towards the range of most encountered levels in the recent stimulation history, hence providing potentially better intensity resolution for the more probable presentation levels. Although some evidence of DRA in humans has been obtained in EEG experiments, few perceptual studies have been undertaken.
Methods: Intensity difference limens (DLs) were measured in three different experiments for standards presented at different "soft" and "loud" levels. These measures were achieved either in silence, or in the presence of a "soft" or "loud" context sound. We expected an interaction between standard level and context level, as well as lower DLs when standard and context levels are matched.
In Experiment 1, DLs were measured in an adaptive two-interval forced choice task for 50-ms, 2-kHz pure tones presented at 55- and 75-dB SPL. The context in this task consisted in sequences of 50-ms narrowband (1-ERB) noise centered on 2 kHz, with a Gaussian level distribution centered either on 55 or on 75 dB SPL. The context, when present, was played continuously during the adaptive run and only stopped when presenting the discrimination trials.

Suspecting off-frequency listening in the loud standard-soft context condition, Experiment 2 repeated the same measure in the presence of notched noise for the loud standard in both context levels.

Experiment 3 was performed similarly, with broadband signals in order to get closer to the animal studies. The context was a broadband noise and the standard a broadband harmonic complex. Both context and standard had the same long-term spectrum but different timbres. The soft and loud levels were 50- and 70-dB SPL.

Results: Exp1 results did partially agree with our hypothesis: the interaction was significant, and DLs were lower in the soft context for the soft standard. For the loud standard however, no difference was found between the conditions.
The notched noise (Exp2) did not show much effect.

In Exp3, results for the soft standard remained similar to Exp1, with lower DLs obtained in the soft than in the loud context. For the loud standard however, significantly lower DLs were obtained in the loud context than in silence.

Conclusions: Exp2 results mean that either off-frequency listening was not suppressed, or it did not play a major role in Exp1.
Although the difference is small in magnitude, Exp3 DLs are intriguing and suggest a possible effect of DRA. Additional experiments are currently being carried out to understand these results.

S187. Perception of Global Properties, Objects, and Settings in Natural Auditory Scenes

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Category: Other, Auditory Scene Perception
Background: Theories of auditory scene analysis suggest our perception of scenes relies on identifying and segregating objects within it, resembling a detail-oriented processing style. However, a more global process may occur while analyzing scenes, which has been evidenced in the visual domain. We evaluated the contributions of high-level global and low-level acoustic information to auditory scene perception. An additional aim was to increase the field's ecological validity by using and making available a new collection of high-quality natural auditory scenes.
Methods: In our first experiment, participants rated scenes on 8 global properties (e.g., open vs. closed) and an acoustic analysis evaluated which low-level features predicted their ratings. In a second experiment, we evaluated participants' accuracy in identifying the setting of—and objects within—scenes across three durations (1, 2, and 4s). Finally, two computational models trained to perform object and setting identification were compared to global property ratings.
Results: Exploratory factor analyses revealed the global properties and acoustic variables were explained by two and seven-factor models, respectively. The acoustic variables linearly predicted the global ratings by different amounts (R-squared = 0.33-0.87), although we also observed nonlinear relationships between acoustical and global variables. The results of our second experiment indicate participants performed better on the object identification task, but this performance was contingent on a longer stimulus duration. These results suggest object identification may require more processing time and/or attention switching than setting identification. Finally, the results of our computational modeling analyses indicate that compared to the object-driven model, the setting-driven model exhibited higher correlations with several global properties. We also found that shorter scene durations led to noisier perceptual judgments for both models.
Conclusions: Taken together, these results provide insight into the mechanisms underlying natural auditory scene perception and suggest representations of auditory objects may be transformed through many stages of processing in the ventral auditory stream, paralleling previous findings on the ventral visual stream.

S188. Language Background and Attention to Speech in Complex Auditory Scenes

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Category: Speech Perception
Background: Every day, we individuate and attend to the multitude of sounds in our environments, but we do not attend to all sounds equally. When we listen to busy auditory scenes, we are biased to attend to speech over other meaningful real-world sounds. However, it is unknown what specific factors contribute to this attentional speech bias. The proposed study will examine whether linguistic experience can influence listeners' attention toward speech in auditory scenes, guiding attention specifically toward speech from native languages over foreign languages.
Methods: Three groups of adult participants (34 each) will complete an auditory change detection task: monolingual English, bilingual English-Mandarin, and bilingual English-Other Language speakers. In this task, participants will hear pairs of auditory scenes. Each scene is composed of 4 sounds played simultaneously and each sound drawn from one of 4 categories: human speech (pseudo-sentences in English and Mandarin), musical instruments, environmental sounds, and animal calls. On each trial, participants will hear Scene 1, a short silence, and then Scene 2. Scene 2 will either be the same as Scene 1 (Same trial), or one sound from

Scene 1 will be replaced by a new sound from a different category in Scene 2 (Different trial). After hearing each trial, participants will be asked whether the two scenes they heard were the same or different. Accuracy rates for participants' responses will be analyzed to characterize which categories of sounds participants are better at detecting changes in than others.
Results: We first aim to replicate the attentional speech bias, with all listeners detecting speech changes more accurately than non-speech changes. If native language experience impacts change detection for speech, accuracy rates should differ between participant groups for Mandarin speech, where the English-Mandarin group would have the highest accuracy. Bilingualism as a type of auditory expertise may also contribute to differences between the English-Other group and the monolingual group in their attention to Mandarin speech changes. These outcomes will allow us to discover how linguistic expertise contributes to the attentional speech bias, to more carefully characterize whether the status of the stimulus as a communicative signal is sufficient to drive attention, or whether it can be modulated by expertise.
Conclusions: This work will incorporate linguistic diversity to come closer to uncovering whether the communicative importance of speech or our specific experiences with language drive attentional biases toward speech. Our findings will allow us to better understand what features of and experiences with speech lead to its prioritization over other sounds in busy environments and what shortcomings may occur in our auditory attention. The results of this research may then allow us to investigate ways to accommodate a more diverse range of listeners from different hearing backgrounds and improve communicative efforts in busy environments.

S189. Listening Effort and Disengagement During Story Listening

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Category: Speech Perception
Background: Listening is often perceived as effortful in complex environments, particularly if the listener has hearing loss. Current measures of listening effort (LE) typically involve brief, isolated sentences that are not very interesting, and thus may not elicit effortful listening as recruited in daily conversations. Speech intelligibility performance using such sentences has previously been linked to an individual's working memory capacity (WMC) but the effects are small. WMC correlates with general cognitive ability, which may explain the relationship between speech intelligibility and WMC. The current study utilizes naturalistic and engaging stories to study individual differences in the signal-to-noise ratio (SNR) at which: 1) listening is first perceived to be effortful (effort point), 2) intelligibility begins to decline (intelligibility point); and 3) participants give up because they cannot understand anything and see no point in listening further (give-up point), and how these points relate to measures of cognition.
Methods: Cognitively healthy adults (N =127, 23 - 71 years) listened to an engaging story, approximately 5 minutes long. A 12-talker babble noise gradually but steadily increased in level as the story progressed. Participants were asked to identify, in any order, the effort, intelligibility, and give-up points by pressing one of three buttons during story listening, and the SNRs corresponding to these button presses were recorded. Working memory was assessed using the Automated Reading Span task (Daneman and Carpenter, 1980) whilst general cognitive ability was indexed using nonverbal matrix reasoning tasks (Raven, 1962; Matzen et al., 2010).
Results: Preliminary results show that, on average, participants identified the effort point at an SNR of + 11.6 dB (SD = 6.8) while the intelligibility and give-up points were reported at SNRs of + 1.7 dB (SD = 4.7) and – 4.1 dB (SD = 3.7), respectively. There were no significant correlations between the effort, intelligibility and give-up points and performance on the working memory and general cognitive ability tasks.
Conclusions: The research takes a new approach to studying LE, by assessing subjective impressions of LE and comprehension success across a range of SNRs and its relation to relevant markers of cognition. While the preliminary results suggest that cognition, at least in the context of this study, may not explain significant variability in listening behaviour, it is essential to recognize the multi-dimensional nature of subjective LE. These subjective points are likely driven by a variety of factors, resulting in variable performance unrelated to cognition. The findings here prompt us to consider additional factors influencing these listening behaviours.

S190. Using Computational Auditory Models to Explore the Impact of the Efferent System on Speech Coding

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Category: Other, Computational modeling (auditory nerve and midbrain)

Background: The auditory system comprises ascending and descending pathways, with the latter collectively referred to as the efferent system. Whereas the ascending auditory pathway has been extensively characterized and modeled, the purpose and mechanisms of the efferent system are less well understood. Efferent activation reduces cochlear gain, but the functional utility of this gain control remains under debate. One prevalent theory posits that, for humans, efferent gain control plays a role in speech-in-noise processing, helping to unmask speech dynamically. To evaluate this hypothesis, we expanded an established model of the mammalian auditory periphery to include the medial olivocochlear (MOC) system, a major subcomponent of the efferent system, and simulated and analyzed responses to speech-like stimuli in noise. Our hypothesis was that efferent activity would dynamically increase the amount of speech information encoded via low-frequency fluctuations in auditory-nerve responses (hereafter "neural fluctuations").

Methods: We modified an auditory-nerve (AN) model (Zilany, Bruce, and Carney, 2014, JASA 135:283) to include the MOC efferent system with two descending inputs: (1) Wide-dynamic-range (WDR) brainstem neurons, converging from a range of nearby characteristic frequencies, regulate cochlear gain based on stimulus sound level such that the set point of the inner-hair-cell (IHC) nonlinearity in each channel maintains contrast in neural fluctuations between spectral peaks and valleys over a wide range of sound levels. (2) Within-channel projections from midbrain inferior colliculus (IC) cells that are excited by modulations further adjust cochlear gain to enhance neural fluctuation contrast across frequency channels. The increased computational cost of these modifications was partially mitigated by replacing the implementation of power-law adaptation associated with the IHC-AN synapse using a flexible and efficient approximate implementation. We then simulated, with and without the efferent system, modulation transfer functions (MTFs) at different sound levels and responses to vowel-like single- and double-formant stimuli embedded in speech-shaped noise at different sound and noise levels.

Results: Our model with the MOC system enhanced modulation coding across sound level. The MOC system also improved neural coding of vowel-like stimuli by dynamically enhancing the contrast in neural fluctuations across channels over the duration of the stimulus, especially at supra-threshold sound levels and signal-to-noise ratios that are ecologically representative of typical speech-in-noise (Smeds et al., J Am Acad Audiol 26:183, 2015).

Conclusions: These findings demonstrate the potential role of MOC efferent activity in enhancing neural coding of speech sounds in noise at ecologically relevant sound levels and signal-to-noise ratios. This model provides a new framework for understanding implications of hearing loss on speech coding in noise.

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S191. Open Board

S192. Open Board

S193. Investigating the Neural Tracking of Speech in Dialogue Listening Scenarios

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Category: Speech Perception

Background: Speech communication is a fundamental aspect of human experience, enabling us to express thoughts and emotions. This study investigates the possibility of measuring the neural processes underlying social speech comprehension with non-invasive electroencephalography (EEG). Past research has contributed

significantly to our understanding of how speech is transformed into meaning by our brain. While previous work typically relied on simplified speech listening tasks, such as listening to sequences of isolated syllables, recent developments have shifted towards employing more naturalistic paradigms in ecologically-valid settings: the perception of continuous speech. In particular, EEG and magnetoencephalography demonstrated a robust relationship between speech inputs and the corresponding neural signal. This enabled researchers to probe the neural encoding of speech and language properties at various levels of abstraction. However, that work focused on how the human brain processes speech monologues, either in quiet or in noise, while ignoring one of the foundational roles of speech: social communication.

Methods: We present an EEG experiment for studying speech for social communication. Participants were presented with dialogues between two interlocutors from conversations in a podcast-style setting. The multivariate Temporal Response Function (mTRF) methodology, which employs a lagged linear regression, was used to measure the EEG encoding of the sound envelope, word onsets, and lexical surprisal.

Results: We present results supporting the hypothesis that the analytical mTRF framework for studying monologue listening also applies to third-person listening of dialogues. Firstly, the EEG encoding of the sound envelope during dialogue listening scenarios was found to be consistent with previous studies that investigated speech monologue scenarios. Secondly, our results demonstrate a relationship between the lexical predictions generated by large language models (LLMs) and the brain: lexical surprisal values generated from GPT-2, which were built by considering both interlocutors as a single linguistic stream, were found to predict EEG signals better than chance (t-test, $p = 0.003$). The TRF weights corresponding to this finding show the expected N400 response (t-test, $p = 0.002$). Finally, we will discuss a creative use of LLMs for investigating how lexical expectations change depending on the particular speaking styles in the dialogue.

Conclusions: TRF analyses can be used to probe lexical prediction mechanisms in dialogue listening scenarios. This social scenario presents challenges that have yet to be addressed in the literature, such as the adaptation of speech and language for their use in social contexts. We will discuss how the outcomes of this study could render a novel avenue for speech neurophysiology, enabling the investigation of social communication in more realistic scenarios involving natural speech listening.

S194. Language-Agnosticism of Linguistics Research Tasks: A Feasibility Study for Application in Hearing Assessment

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Category: Speech Perception

Background: Patients with Limited English Proficiency (LEP) and hearing loss face healthcare disparities in the United States. Most speech perception tests require mutual language understanding between clinician and patient, impacting the ability to measure and treat hearing loss for LEP patients. Thus, there is a clinical need for a validated, standardized speech test that is language-agnostic. The study of linguistics may offer useful insights and experimental techniques to address this gap in clinical need. In this pilot study, we explore the language-agnosticism of using commonly employed tasks from linguistics research as a feasibility for a linguistics-based language-agnostic assessment of speech perception.

Methods: Adult native speakers of English, Spanish, and Mandarin Chinese with normal hearing were included. Subjects were presented with three task protocols, evaluating the impact of voicing onset (die-tie), vowel length (bad-bat), and tonal manipulation (ma-ma-ma). Stimuli were presented in pairs with varying degrees of dissimilarity, and subjects were asked to assess stimulus pairs using a two-alternative forced choice paradigm (same/different). Stimuli were created using Praat audio software. Consent and task protocols were administered in the participants' native language. Results were evaluated for uniformity of responses across languages. Results were analyzed with ANOVA and post-hoc pairwise analysis.

Results: 63 subjects participated in this study, including 36 native English speakers, 14 native Spanish speakers, and 13 native Mandarin speakers, inclusive of 29 males and 34 females. One subject had self-reported hearing loss and their data was excluded from analysis. For the die-tie task, Mandarin and English speakers scored similarly across easy (90.91-94.44%, $p= 0.97$), medium (53.47-60.23%, $p=0.53$), and hard (5.68-6.25%, $p=0.98$) levels of the task. However, there was a statistically significant difference in performance when comparing English and Spanish speakers. For the bad-bat task, all language groups

performed similarly (7.69-11.54%, p= 0.99). For the ma-ma-ma task, all language groups performed similarly (76.92-90.28%, p value= 0.48).
Conclusions: For tasks evaluating voicing onset time, English and Mandarin speakers performed similarly, but Spanish speakers deviated in their response patterns. Tasks evaluating tonal manipulation and vowel length yielded similar performances among all language speakers. A linguistics-based approach shows promise, but continued stimulus development and refinement is required to attain language-agnosticism. Expanded stimulus banks will additionally require a wider distribution of task difficulty and evaluation in additional languages to assess utility in real-world clinical settings.

S195. The Contribution of Spectrum and Modulation Statistics Towards Speech Recognition in Natural Environmental Noise

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Category: Speech Perception
Background: Recognizing speech in noisy environments is a critical human hearing task. While spectrum and modulation cues are known to influence speech recognition in noise, it is less clear how spectrum- and modulation-based summary statistics contribute to differences in speech hearing sensitivity across real-world environmental backgrounds. Here we explore the role of background sound summary statistics and develop a biologically inspired model framework to identify the contribution of spectrum and modulation-based statistics towards speech recognition in real-world noises.
Methods: We assess how the spectrum and modulation statistics of natural sounds mask the recognition of spoken digits (0 to 9). Human listeners (N=18) listened to digits in eleven different environmental background sounds (e.g., water, construction noise, speaker babble; SNR=-9 dB) and their perturbed variants. We perturbed the backgrounds by either 1) phase randomizing (PR) the sound spectrum or 2) spectrum equalizing (SE). PR retains the sound power spectrum but distorts (whitens) the modulation statistics while SE distorts (whitens) the power spectrum and retains modulation statistics. To further quantify this interference between foreground and background spectrum/modulation content, we used texture synthesis (N=16 participants; McDermott and Simoncelli 2011) to manipulate individual modulation statistics from the backgrounds. Finally, in N=9 participants, we also varied the SNR for the original backgrounds (SNR=-18 to 0 dB) to explore how summary statistics contribute to accuracy across noise levels.
Results: Even at a constant noise level (-9 dB SNR), the unique spectrum or modulation statistics of each background could have a beneficial or detrimental effect on speech recognition. Across backgrounds accuracy varied from nearly 0 % to 100 and whitening the sound spectrum (SE manipulation) could either increase or decrease accuracy. For other backgrounds, whitening the modulation statistics (PR manipulation) had a more pronounced effect. Across participants behavioral responses clustered into spectral or modulation dominant masking responses, where either dimension could facilitate or impair digit recognition. In the texture synthesis paradigm, we found that adding texture statistics decreased accuracy for background speech babble as a result the highly similar modulation statistics between the foreground speech and background babble. Interestingly, however, adding statistics in a construction noise background that contains a loud periodic jackhammer increased word recognition accuracy, which is consistent with comodulation masking release. Furthermore, SNR influenced recognition independently from the background sound statistics (spectrum or modulation) and exhibited a logistic relationship that was accurately accounted by our model.
Conclusions: These findings show how the diverse spectrum and modulation statistics of real-world environmental background sounds influence speech recognition, either beneficially or detrimentally. The sound manipulations and feature based modeling using summary statistics to predict perceptual sensitivity allow us to identify the specific acoustic cues contributing to listening in noise. This has critical implications for potential hearing loss intervention and hearing loss diagnostics.

S196. Transmission of Acoustic Cues in Vowel Perception and Its Relationship to Spectral Ripple Discrimination in Listeners With Cochlear Implants

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Category: Speech Perception

Background: Perception of spectrally-modulated noise, as assessed by spectral ripple discrimination (SRD), has been shown to predict performance on speech perception tasks in postlingually-implanted adults and prelingually-implanted children (Horn et al., JASA 2017; Noble et al., 2023). This relationship may be weaker in children suggesting they may be less reliant on spectral cues than their normal hearing (NH) peers (DiNino and Arenberg 2018), and adult CI users (Gifford et al 2018). SRD performance reflects two factors: Frequency resolution (FR), the ability to resolve spectral peaks, matures in infancy whereas spectral modulation sensitivity (SMS), the ability to detect changes in intensity across-spectrum, matures around 9-12 years of age. In our lab, we have examined how these factors individually are related to speech perception in pediatric CI users. We completed a secondary analysis of data already published (Noble et al 2023). We hypothesized 1) vowel cues characterized by their spectral information (F1,F2) would be related to FR 2) no vowel cues would be related to SMS.

Methods: Participants included 15 prelingually-implanted children (5-16 years) and 12 post-lingually implanted adults. SRD was measured at 4 modulation depths (3,7,10,15dB) using a 3-afc-2-down-1-up adaptive method at 70 dBZ (Noble et al. 2023). A spectral modulation transfer function (SMTF) was fit and FR and SMS were derived from slope and intercept respectively (Horn et al., 2017). Vowel identification was measured using 10 target vowels spoken by a male talker in /hVd/ format; presentation was 60 dBA in quiet. All testing was completed via a computer in a sound-booth with stimuli presented at 0 degrees azimuth using the “preferred” CI side. Vowel confusion matrices were analyzed using the sequential information transmission analysis (SINFA) (Miller and Nicely, 1955.) Vowels were analyzed and characterized by duration, F1 and F2 height and were similar to previous studies (Hillenbrand et al. 1995, Xu et al. 2005).

Results: Adults and children showed equivalent mean vowel identification, similar transmission of F1 and duration, but children had stronger F2 transmission. For both groups, transmission of F1 and duration were highly correlated, and transmission of each cue was highly correlated with overall vowel identification. FR was significantly related to F1 and duration transmission in children but not for adults. No cues were correlated to SMS in either group.

Conclusions: These findings do not support the hypothesized relationships between FR and vowel cue transmission, which is in contrast to our findings with consonants. It is not immediately clear why FR would be related to duration transmission. One possibility is that SINFA does not reliably differentiate speech cues for our vowel stimuli. Further work will examine whether FR, SMS, and analogous temporal factors, are related to transmission of cues for consonant and vowel perception with a CI in quiet and noise.

S197. Slow Build-Up of the Acoustic Reflex by Contralateral Noise

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Category: Middle and External Ear

Background: The acoustic reflex attenuates sound transmission through the middle ear involving a short brainstem feedback pathway which activates the stapedius muscle in humans. The bilateral reflex can be activated by stimulating either ear with tones, clicks, or noise. Sustained activation of the reflex by the activator stimulus commonly produces a decline in the strength of the reflex referred to as "decay” (Anderson et al, in Sensorineural Hearing Loss, 1970) This weakening of the reflex seems counterintuitive if its main purpose is to adapt hearing to environments with high levels of background noise. We present here evidence that exposure to sustained or intermittent levels of the activator can produce a significant enhancement of the acoustic reflex that develops slowly over several minutes. This adaptation appears similar to that demonstrated previously for the medial olivocochlear system (Larsen and Liberman, Hearing Research, 2009).

Methods: The Etymotic Research ER-10X probe system was used to measure changes in absorbance by the ear, calculated from the pressure response to wideband chirps caused by presenting broadband or filtered noise to the contralateral ear. The elecitor noise was created by a truly random noise generator that was gated and attenuated electronically under software control.

Results: The change in admittance due to the acoustic reflex was measured as a function of the elicitor level. Then, intermittent noise (typically 80 dB SPL) was repeatedly presented using the same measuring procedure over 4-5 minutes. The growth of admittance change with elicitor level was measured a second time. The admittance change increased typically by a factor of 1.5 to 3 and remained enhanced for at least several minutes.
Conclusions: The enhancement of the acoustic reflex described her may explain a previous result in which we explored reflex decay using exposures to the elicitor with increasing duration before measuring the absorbance change in with the maintained elicitor. While decay of the acoustic reflex is apparent initially, several minutes of repeated stimulation with the elicitor seems to result in a reversal and enhancement of reflex strength. These findings suggest that the acoustic reflex may be strengthened in the presence of environmental noise in a way that is beneficial to hearing in noisy environments. The middle ear reflex in this case may be like the medial olivocochlear system (Larsen and Liberman, 2009).

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S200. Distinct and Simultaneous Neural Encoding of Speech and Music Expectations During Sung Speech Listening

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Category: Other, Speech and music perception
Background: Speech and music are highly structured auditory signals involving regularities at progressively longer time scales. Statistical processing of such structures in our brains has a central role in perception as it allows us to generate expectations about the incoming auditory input for efficient sensory processing and learning. Top-down predictions are confronted with bottom-up sensory inputs to adapt our internal model embodying language knowledge or, for music, musical culture and expertise, generating brain responses whose strength is modulated by expectations and surprises. Recent advances in neurophysiology demonstrated that prediction mechanisms can be probed during natural speech and music listening. Yet, the neural mechanisms supporting the engagement of speech and music predictions remain unclear. Measuring such neural signatures for both speech and music with a comparative framework would allow for interesting insights into non-primary auditory processing and provide evidence for a shared fundamental neural mechanism.
Methods: To achieve this goal while minimising possible confounding factors in the comparison (e.g., differences in response strengths and patterns due to acoustical differences), we propose to use sung speech as a connecting link, a case study that implies simultaneous processing of linguistic and melodic components, and compare its processing to the one of speech and music only. We recorded electroencephalographic data from N=20 adult participants as they listened to continuous hummed melodies, speech, and sung speech, where the spoken and sung versions share linguistic content while the sung and music-only versions share the melody. The data was then analyzed with a framework based on linear modelling techniques, namely Temporal Response Functions (TRFs), for studying the cortical tracking of natural stimuli and measuring the contribution of phonemic and melodic expectations simultaneously.
Results: Our results show a robust and consistent cortical encoding of both phonetic and melodic expectations in sung speech as for speech and music only. Measuring such simultaneous and distinct encoding of high-level linguistic and melodic expectations represents an important result that opens the door for studying neural mechanisms specific to lyrics-tunes integration and how the two types of information are weighted for parallel processing. Interestingly, we observed a lateralized spatial reweighting of the expectations encoding strength

when the melody and lyrics have to be processed in parallel, i.e., in sung speech, as opposed to speech and music only.
Conclusions: To the best of our knowledge, this study represents the first direct comparison of the statistical processing of speech and music in ecologically valid scenarios, providing a benchmark for using sung speech in both auditory and translational research and asses linguistic and music processing in one experiment. Our results provide further evidence for speech and music being processed by the brain under the same fundamental statistical processive principles and give interesting insights into non-primary auditory processing.

S201. The Long-Term Stability of the Speech-To-Song Illusion and the Effects of Individual Differences

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Category: Other, Speech and Music Perception
Background: In the Speech-to-Song (STS) illusion, multiple repetitions of a natural spoken utterance can give rise to a perceptual switch wherein the stimulus begins to sound like song to the listener. Anecdotally, once a speech excerpt transforms to song, listeners report they experience it as song when they reencounter it even after long delays, suggesting the STS illusion is temporally stable. However, to our knowledge, the long-term stability of the STS illusion has not yet been empirically validated.
Methods: In our study, we measured the STS illusion by presenting listeners with excerpts known to elicit the STS illusion and asking them to rate the degree to which each repetition sounded song-like across delays from 0-56 days. At both sessions, we also measured the average increase in rating and how readily stimuli transform to song (i.e., transformation position).
Additionally, to assess the effect of individual differences on STS illusion elicitation and stability, we administered the Goldsmiths Musical Sophistication Index (Gold-MSI), a speech prosody (PEPS-C) and tonal enculturation test (from Corrigall and Trainor, 2015), and a question on attention paid to lyrical content when listening to music.

Results: The current study empirically validated anecdotal reports that when listeners hear excerpts transform to song at an initial session, those same excerpts continue to be heard as more song-like, as indicated by higher song-like ratings for those stimuli at a second session up to 56 days later. Also, ratings of individual excerpts are correlated across sessions for the majority of listeners. These results provide strong support for the claim that once a stimulus transforms to song for a given listener, a stable, music-specific perceptual memory of that stimulus is formed that can be elicited upon future encounters with the same stimulus. Furthermore, an average increase in ratings across delays shows STS stimuli may continue to be heard as increasingly song-like with increased exposure.
Moreover, our individual difference tasks showed STS illusion elicitation was positively predicted by Gold-MSI and tonality task scores; participants who scored higher on these musicality measures provided higher overall ratings and experience a more robust perceptual change from speech to song across trials. Additionally, individual excerpt ratings were positively predicted by performance on our speech prosody task and inversely predicted by Gold-MSI scores. Finally, the change in transformation position across sessions was positively predicted by attention to lyrics, wherein paying more attention to linguistic as opposed to musical aspects of stimuli was shown to offset the speech-to-song effect.

Conclusions: These findings show that individual differences in musical and linguistic ability affect the experience of the STS illusion and hold important implications for understanding auditory processing and memory.

S202. Contribution of Vestibular Hair Cells to Function

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Category: Vestibular: Basic Research and Clinical

Background: Mammalian vestibular organs, including the utricle, the saccule, and semicircular canals, are located in the inner ear. Vestibular HCs in the utricles and saccules can detect linear acceleration and gravity, while crista HCs in semicircular canals are responsible for detecting angular acceleration. These HCs are crucial for maintaining balance and spatial orientation, they are susceptible to damage from various factors. In mammals, the loss of cells in different organs can have varying impacts. We speculated as to whether vestibular function is as sensitive to HC loss as hearing function, and if not then we wanted to know how many HCs are required to maintain basic body balance. To address these questions, we needed to establish a link between HC number and vestibular function, which required a suitable damage model and comprehensive tests for vestibular function of mice. Additionally, further studies were performed to investigate the influence of vestibular HC ciliary bundle, subtypes, and nerve innervation on vestibular function.
Methods: Adult Pou4f3DTR mice received two injections of DT intramuscularly 48 h apart, and the inner ears were harvested on day 14 after the first DT injection. Wild-type mice at 7 weeks of age received a single injection of IDPN and the inner ears were harvested 7 days later.
The air-righting reflex, the open-field test, the swimming test were performed to assess the balance fuction. The HAVOR and OVAR test were used to measure vestibular function precisely.

Results: DT induced dose-dependent HC death in all of the vestibular organs of adult Pou4f3DTR mice. Both subtypes of HCs decreased in a dose-dependent manner after DT treatment in the utricle. Dose-dependent HC loss in the saccule was similar to that in the utricle, whereas the majority of residual HCs in the crista were type I HCs in these groups, suggesting that most type II HCs in the crista died. The stereociliary bundles were damaged does-dependently, while disruption of the innervation of type I HCs was only triggered by high-dose DT treatment.
The gross vestibular function tests indicated that the number of residual HCs in the 15 ng/g DT group was sufficient to maintain normal balance function, in which there were 30.75 ±2.66%, residual HCs in the utricle, 16.92 ±2.44% residual HCs in the saccule, 24.67 ±1.92% residual HCs in the crista. The HAVOR and OVAR test indicated that the number of residual HCs in the10 ng/g DT group was sufficient to maintain the normal vestibulo-ocular reflex originating from vestibular organs, in which there were 46.40 ± 2.51% residual HCs in the utricle, 37.42 ± 2.33% HCs in the saccule, 36.19 ± 4.30% HCs in the crista.

Conclusions: Appropriate proportion of HC subtypes and the integrity of innervation are important factors in the recovery of vestibular function.

S203. Developmental Transcriptomic Profiling of Non-Sensory Vestibular Epithelium Reveals Ionic Contribution to Endolymph Formation

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Category: Vestibular: Basic Research and Clinical
Background: In the inner ear, various ion channels have reported and play a key role in signal transduction, their malfunction could lead to hearing and vestibular dysfunction. Ion channels contribute to formation of endolymph. Endolymph is formed from embryonic day (E) 14.5 of developing murine inner ear. Endolymph secretion leads to lumen formation in developing inner ear. Although it was suggested that osmotic gradient created by changes of ionic composition plays a role in the endolymph formation, still exact molecular mechanism of endolymph formation is unknown. We aimed to investigate the mechanism of endolymph formation in the developing inner ear.
Methods: We collected non-sensory epithelium of vestibule at the age of E16.5, E18.5, and Postnatal day (P) 5, and classified the collected specimens according to the presence of dark cells as follows: non-sensory epithelium of utricle and common crus that contained dark cells and semicircular canals that did not contain dark cells. RNA sequencing was performed with those samples to analyze the changes in ion channels according to the development. After selecting candidate genes for endolymph formation, we measured endolymphatic volume changes using confocal 3D live imaging with the application of candidate ion channel inhibitors for functional study. The localization of the candidate ion channels was examined by immunohistochemistry
Results: We selected 1613 differentially expressed genes (DEGs) by RNA sequencing analysis (P less than 0.001 and |Fold Change| less than 2 at least in one comparison). The genes in each sample formed close

clusters according to the cell types and development period. Most iv genes were related to ion activities such as ion transport, and membrane transport. Four major ions potentially involved in the endolymph formation with high probability were sodium, chloride, calcium, and potassium ions. A functional study using 3D volume change with the application of chloride-free solution and amiloride at E16.5 showed blockage of endolymphatic fluid secretion. The calcium-free solution did not show any decrease in secretion rate. Potassium ions only worked at P5, confirmed by XE991 treatment.
Conclusions: During inner ear development, sodium and chloride ions, but not potassium ions, are strongly associated with endolymphatic fluid secretion. These findings may help elucidate the mechanism of inner ear formation as well as congenital hearing loss and vestibular disorders.

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S205. Fast-Phase Nystagmus Enables Precise Evaluation on Vestibular Deficits

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Category: Vestibular: Basic Research and Clinical
Background: During the vestibular ocular reflex (VOR) test, both slow-phase and fast-phase eye movement components are observed. In the past, the focus has primarily been on evaluating the vestibular function based on the slow phases (gain and phase), while the fast-phase nystagmus (FPN) has been systematically ignored. To further understand the potential of FPN in evaluating vestibular function, we conducted vestibular function tests on various animal models with typical vestibular deficits using two VOR test modes: trapezoidal angular velocity (TAV) and pseudo off-vertical axis rotation (p-OVAR), which were chosen as they promote the production of FPN.
Methods: The TAV and p-OVAR VOR testing methods involve the use of a motor with two centrifugal rotating arms, each holding a satellite rotation table. These centrifugal arms produce rotational movement to simulate the otolith organ, while the satellite rotating tables generate stimulus for the semicircular canal. Four typical vestibular deficit models are introduced: (1) Periphery injury by injecting 3, 3'-Iminodipropionitrile (IDPN); (2) Central manipulation by injecting Baclofen; (3) Unilateral vestibular lesion (UVL) created by opening a fenestra on the semicircular canal (SCC); (4) Genetic defects of Zpld1-/- mouse with SCC dysfunction and Otop1tlt/tlt mouse with otolith dysfunction.
Results: (1) Injecting IDPN at different doses (2mg/g and 4mg/g) led to varying levels of slow-phase amplitude, observed during the p-OVAR test. This was accompanied by correspondent reduction in FPN. (2) Administering Baclofen at different doses (5mg/kg and 10mg/kg) resulted in varying degrees of suppression of FPN in the p-OVAR test, but no significant reduction observed in slow-phase components. (3) UVL mice exhibited asymmetrical FPN with clear directional preponderance in the p-OVAR test. (4) Zpld1-/- mice had no FPN in either p-OVAR or TAV tests, while Otop1tlt/tlt mice displayed normal FPN in the TAV and no FPN in p-OVAR test.
Conclusions: Results in this study demonstrated the superior capability of FPN in evaluating various vestibular deficit. For example, both UVL side and effect of central manipulation with Baclofen were identified by FPN but not slow-phase counterpart. It is necessary to include FPN in vestibular function tests. Studies on mice with defects in the Zpld1 and Otop1 genes suggest that the integrity of SCCs and otolith organs functions is necessary for the production of fast phases in p-OVAR. This could lead to better understanding of the generation of FPN.

S206. Hyper-Response of Cervical Vestibular Evoked Myogenic Potential in Patients With Meniere Disease

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Category: Vestibular: Basic Research and Clinical

Background: The purpose of this study is to investigate the hyper-responsiveness of cervical vestibular-evoked myogenic potential (cVEMP) in patients with Meniere disease (MD), and to compare the result of cVEMP between probable and definite MD group.
Methods: A total of 110 patients satisfied with probable MD and definite MD criteria, which is recently formulated by the Classification Committee of the Bárány Society, were included. An interpeak amplitude and interaural amplitude difference (IAD) ratio of both ears was measured. The abnormal response of ipsi-lesional cVEMP was categorized into 2 groups; hyper-response and hypo-response. Chi-square test and Mann-Whitney U-test were used for statistical analysis.
Results: In the probable MD and definite MD group, the mean IAD was 25.24%±17.79% and 53.82%±34.98%, respectively ($p < 0.01$). The abnormal response of cVEMP at the affected ear was more frequent in the definite MD group, compared to the probable MD group (32/40 vs. 13/36, $p < 0.01$). However, hyper-response was more frequently observed in the patients with probable MD, compared to the patients with definite MD (13/36 vs. 3/40, $p < 0.01$).
Conclusions: Hyper-response of cVEMP was more frequently observed in the early probable MD patients. It might be an early sign of MD, related with the saccular hydrops, which can help the early detection and treatment.

S207. Accelerated Degeneration in the Aging Utricle of an Alzheimer's Mouse Model

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Category: Vestibular: Basic Research and Clinical
Background: The vestibular system plays a crucial role in our sense of balance, detecting head motions, and regulating gaze and posture. One important organ in this system, the utricle, is crucial in sensing movements of the head and static balance. As seen with other systems, aging in the vestibular system results in degeneration and dysfunction, 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 AD at an accelerated rate which begins prior to the onset of cognitive decline. To investigate this question, we used the APP^NL-F/NL-F mouse model which is a knock-in line where amyloid precursor protein (APP) is altered to incorporate two mutations found in human Alzheimer's patients. APP^NL-F/NL-F mice exhibit overproduction of amyloid beta without overexpression of APP making it a good model for sporadic AD. Here we investigated age-related changes in the morphology of utricles from APP^NL-F/NL-F mice.
Methods: Temporal bones were collected from male and female APP^NL-F/NL-F and C57Bl/6J mice between 2 and 18 months of age. Immunofluorescent staining was used to observe changes in the morphology of the utricular macula. Hair cells were quantified using the pan hair cell marker, myosin VIIa, with type II hair cells identified by Sox2 labeling. Stereocilia bundles were quantified using phalloidin labeling.
Results: Preliminary examination of the APP^NL-F/NL-F utricles shows possible signs of degeneration. The most notable are non-nuclear, cytoplasmic expression of Sox2 in some hair cells and elongation and splaying of stereocilia bundles. Both of these phenotypes were present in the APP^NL-F/NL-F mice as early as 5 months of age while this phenotype did not appear in the C57Bl/6J mice until 14 months of age. At 14 months of age in the APP^NL-F/NL-F utricles, we also observed extracellular spaces around some hair cells which may be the evidence of possible neuronal swelling or amyloid-beta plaque deposition. This phenotype was not observed in the C57Bl/6J utricles at any of the ages examined. Lastly, preliminary quantification results suggest a decreased number of type I hair cells in the striolar and extrastriolar regions of APP^NL-F/NL-F utricles beginning at 8 months of age compared to C57Bl/6J utricles.
Conclusions: Signs of degeneration in aging utricles of the APP^NL-F/NL-F mouse line appear to precede the onset of cognitive decline, which is detectable at 12 months of age.

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S209. Impact of Impaired Calcium Metabolism on Otolith Development in Zebrafish

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Category: Vestibular: Basic Research and Clinical

Background: Benign paroxysmal positional vertigo (BPPV) is the most prevalent peripheral vertigo disorder, primarily affecting the elderly and women. The higher incidence in women is believed to be linked to hormonal changes during menopause, which impact bone and calcium metabolism, leading to osteoporosis. Similar effects may extend to the formation of otoliths, which consist of calcium carbonate, fibrous materials, and proteins. Consequently, it is hypothesized that individuals with osteoporosis may exhibit underdeveloped otoliths, potentially making them more susceptible to detachment from the macula. Previous reports have noted alterations in otolith density and size in osteoporosis model mice. In this study, we investigated the influence of drugs that disrupt calcium metabolism on otolith formation in zebrafish embryos, employing dexamethasone and cadmium. Zebrafish were chosen for their ease of breeding, drug exposure, and transparent larvae, enabling direct observation of otoliths. Additionally, zebrafish possess a semicircular canal structure akin to humans, with two otolith organs, the saccule and utricle. Unlike mammals with numerous otoliths, zebrafish have only one for each organ. Lacking a cochlea, auditory perception is thought to occur in the saccule, while postural equilibrium perception occurs in the utricle.

Methods: Drug exposure commenced at 2 days post-fertilization (dpf) since hair cells and otoliths can be morphologically identified at this stage. Otoliths were assessed at 5dpf and 8dpf. Furthermore, rather than measuring the larval bone mass, we analyzed the calcium concentration by dissolving whole zebrafish larvae.

Results: Larvae exposed to dexamethasone exhibited significantly larger utricle otoliths than the control group, whereas cadmium-exposed larvae had significantly smaller otoliths in both the saccule and utricle. Calcium levels were 82% of the control in the dexamethasone group and 41% of the control in the cadmium group.

Conclusions: These findings indicate that abnormalities in bone and calcium metabolism pathology affect otolith formation, along with bone loss. Further investigations are necessary to elucidate the connection between immature otolith formation and increased susceptibility to detachment from the macula.

S210. Characterizing Postnatally Added Hair Cell Bundles in Adult Mouse Utricle

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Category: Vestibular: Basic Research and Clinical

Background: Sensory hair cells are mechanoreceptors required for hearing and balance functions. As stereociliary bundles play a critical role in mechanoelectrical transduction (MET), many prior studies examining developing and regenerated hair cells have assessed bundle morphology to determine cell maturity. About half of hair cells in the mouse utricle are added in the early postnatal period. Previous research has suggested the presence of short and immature hair bundles in the postnatal mouse utricle based on qualitative analyses of bundles. However, only a few studies that quantitatively and systematically assess hair bundle dimensions, and it remains unclear if and how hair bundles mature postnatally. In this study, we have developed techniques to quantitatively measure hair bundle dimensions in 3D and applied these techniques to assess hair bundle maturity in the postnatal mouse utricle.

Methods: Plp1CreERT/+; Rosa26RtdTomato/+ mice were treated with tamoxifen at P3 to fate-map supporting cells that give rise to new hair cells. Then utricles were dissected at 1, 2 and 6 months, stained with the kinocilia marker α-Tubulin, stereocilia marker phalloidin, and bundle protein marker FSCN2. Tissues were imaged with confocal microscopy and processed using Imaris 3D imaging software. Bundle lengths, including kinocilia height, tallest and shortest stereocilia, and array length were measured, and bundle protein FSCN2 expression was also evaluated to characterize the maturity of fate-mapped hair bundles and compared to hair bundles observed at embryonic (E) 13.5, 15.5, E18.5, P0, P37 and P180 wild type utricles. Additionally, SUB-

immunogold-scanning electron microscopy (SEM) is used to examine the morphology of fate-mapped hair bundles.
Results: In one-month-old Plp1CreERT/+; Rosa26RtdTomato/+ mice, most traced hair bundles of newly formed hair cells exhibited short, curled or bent kinocilia without stereocilia. By 2 and 6 months, traced bundles appeared relatively shorter than untraced ones, and continue to display short, curled or bent kinocilia. At 6 months, only 46% of traced hair bundles expressed the mature hair bundle protein FSCN2, compared to 97% of the untraced hair bundles. In the wild-type embryonic utricle, we observed short kinocilia and stereocilia, both of which elongate during development. However, some hair cells with short kinocilia and stereocilia remain detectable in the adult utricle. In P180 Plp1CreERT/+; Rosa26RtdTomato/+ mice, the dimensions of kinocilia and stereocilia of traced hair bundles resembled those in the early embryonic stages and the short, immature kinocilia and bundles found in the P180 wild-type utricles, suggesting that they do not fully mature over time. Finally, SUB-immunogold-SEM will be used to further characterize the traced hair bundles at P180.
Conclusions: Hair cell bundles of postnatally added hair cells appear immature, characterized by the presence of short, curled or bent kinocilia and relatively shorter stereocilia. Furthermore, the expression of mature bundle protein suggests a partial maturation process occurring over months.

S211. Long-Term Effects of Repeated Noise Exposure on the Rat Vestibular Periphery

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Category: Vestibular: Basic Research and Clinical
Background: We have previously demonstrated acute noise-induced changes in vestibular sensory epithelia, evoked potentials, and motor performance after a single noise exposure. Prolonged effects of repeated noise exposure have not been explored. Although it is expected that a second noise exposure will increase vestibular injury, effects of noise may worsen with advancing age. We hypothesize that noise exposure is a source of vestibular damage that leads to decreased vestibular-mediated agility and that noise exposure history may contribute to greater age-related vestibular dysfunction and motor impairment. The goal of this work is to evaluate the relationship between noise exposure and aging in a rat model of noise-induced vestibular injury using vestibular short-latency evoked potential (VsEP) measurements and assessment of motor performance in a balance beam crossing task.
Methods: Rats are trained to cross a balance beam and their performance is recorded and analyzed as described by Bartikofsky et al. (2023). When rats demonstrate stable and proficient crossing behavior, they are exposed to noise (120 dB SPL, 1.5kHz 3-octave band noise). Following noise exposure, rats continue to perform the balance beam crossing task for five weeks before being re-exposed to noise. An aging control group was assessed in the same way to observe age-related vestibular dysfunction. Rats were tracked for up to three months after noise exposure. ABR and VsEP measurements were collected immediately prior to ear collection.
Results: At baseline, control and noise-exposed rats had similar crossing times. After the first noise exposure, crossing times were significantly elevated for four weeks. Five weeks after noise exposure, crossing times were elevated, but statistically similar to baseline. The second noise exposure led to a significantly greater increase in balance beam crossing time in the first noise exposure week, versus the first week after the first noise exposure. After the second noise exposure, crossing times improved after week one, but remained significantly elevated from baseline and control. Notably, the final week of noise exposure data, collected from rats at approximately 12-14 months of age was significantly elevated above all prior measurements and versus age-matched controls. Likewise, VsEP responses were reduced as a result of natural aging, but responses were more reduced in rats that had two noise exposures.
Conclusions: These findings show the impact noise has on the vestibular system, not only at cellular and physiological levels, but at a functional level. These results have implications for aging adults that have experienced noisy environments.

DISCLAIMER: The views expressed do not necessarily reflect the official policies of the Department of Health and Human Services, nor does mention of trade names, commercial practices, or organizations imply endorsement by the U.S. Government. This work was supported by R01 DC018003-01 (King), 1IK2RX003271-01A1 (Stewart), I01RX003250-02 (Altschuler), R01 AG073157 (Stewart).

S212. Influence of Varying Positions and Orientations of Vestibular Implant Electrode Contacts on Simulated Neural Stimulation Outcome

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Category: Vestibular: Basic Research and Clinical

Background: During the implantation of vestibular implants, the exact final position and orientation of the electrodes in the ampullae of the semicircular canals is unknown to the surgeon. Small differences in orientation and distances to targeted and non-targeted nerves might already have a considerable influence regarding selective stimulation of vestibular nerve branches and thresholds for dynamic ranges. Computer simulations considering different positions and orientations of electrode contacts can be used to objectively analyze characteristic influences on neural activation of ampullary nerves and, based on the activation of fibers, on alterations in targeted neural stimulation.

Methods: Volume conductor models of human inner ear anatomies were extended by realistic electrode contact geometries positioned in the ampullae of the semicircular canals. Neuron models were applied to sets of generated neuron trajectories along vestibular nerve branches, the cochlear nerve, the facial nerve and the inner auditory canal. Stimulation was considered in neuron models by time-dependent extracellular potential fields originating from modeled current sources in the electrode contacts. Stimulation scenarios were tested with varying distances between targeted ampullary nerves and electrodes positioned within the corresponding ampullae, electrode orientations relative to targeted and non-targeted ampullary nerves, and positions along a path within the semicircular canals and the vestibule. Nerve fiber activation percentages were compared between different stimulation scenarios over a range of stimulation amplitudes.

Results: Increased distances between targeted ampullary nerves and electrode contacts within the ampullae led in all simulated scenarios to a considerable decrease in percentages of activated fibers in the targeted ampullary nerves, while the activation of non-targeted nerves was only slightly affected by distance alone. Changing the orientation of electrodes positioned in the center of the ampullae led only to barely discernible impacts on neural activation for both targeted and non-targeted nerves. However, when positioning electrodes in the anterior and lateral ampullae close to neighboring non-targeted nerves and also orient the electrodes towards these neighboring non-targeted nerves, increased activation was registered for these non-targeted nerve branches, including also the facial nerve. Electrode contacts positioned deeper in the vestibule (overinsertion) resulted in decreased activation of the targeted nerves and higher activation of most non-targeted nerves, while electrode contacts positioned farther away from the vestibule in the semicircular canals (underinsertion) resulted in lower activation of most non-targeted nerve branches.

Conclusions: Simulated stimulation scenarios with different electrode positions and orientations showed that changes of electrode positions even within one millimeter can already considerably influence the stimulation outcome. Simulations also indicate that underinsertion of electrode contacts is preferable over overinsertion with respect to selective stimulation. Although the translation of simulated effects to the perception of the patient is unclear yet, computer simulations can be used to investigate potential strategies for improved stimulation outcomes of vestibular implants.

S213. Characterizing Human Vestibular Sensory Epithelia from Vestibular Schwannoma Surgeries

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Category: Vestibular: Basic Research and Clinical

Background: The vast majority of what is known about vestibular function has been inferred from animal models due to the inaccessibility of human inner ear organs. There are a few reports of anatomical studies that quantify human vestibular hair cells at various ages (Lopez et al., 2005; Severinsen et al., 2009; Merchant et al., 2000) but detailed investigations of freshly harvested adult vestibular cells are lacking. Here, we obtained vestibular neuroepithelia from adult translabyrinthine surgical patients and performed immunohistochemical studies.
Methods: Colorado Multiple Institution Review Board approval was obtained (COMIRB# 19-1340) for these studies. The surgical team recovered ampullae and utricles from consenting patients undergoing translabyrinthine surgical approaches. The vestibular organs were transferred in-ice cold sterile saline to the laboratory within one hour of harvest. The tissue was fixed in 4% Paraformaldehyde and transferred to phosphate buffered saline for storage at 4°C for 12 to 48 hours before processing. For immunohistochemistry, end organs were fixed and stained with a combination of markers to identify hair cells (Myosin7a), afferent fibers (Tubulin β3), and actin filaments (phalloidin). Imaging was performed with Zeiss LSM 780 and Olympus FV1000 confocal microscopes.
Results: Six patients (ages 51-76 years, 4M, 2F) with vestibular schwannoma and non-serviceable ipsilateral hearing loss prior to surgery were studied. None reported vertigo. Koos grading of the tumor severity ranged from 2-3. Immunohistochemistry was performed on 5 utricles, and one crista. Cells uniformly labeled with myosin 7a with distinct nucleus area (a non-stain hole) were identified as hair cells and counted manually. Average hair cell density was low at 0.26 cells/100 μm2 of neuroepithelium in comparison to abundance of afferent calyces labeled with Tubulin β3. In utricles we observed a lower number of hair cells in older patients suggesting age-related hair cell loss. Phalloidin staining revealed highly disorganized hair bundles sparsely present among the mostly stereocilia free hair cells. Additionally, we quantified phalloidin-labeled actin rods at the average frequency of 1 rod in every ninth hair cell. These actin rods were observed crossing diagonally through hair cells, their average length was 11.5 ± 3.4 μm.
Conclusions: We performed fluorescent immunostaining on vestibular epithelia extracted during human surgical inner ear procedures. Counts of myosin-labelled hair cells suggested age-related hair cell loss. We also observed numerous actin rods which have previously been linked to hair cell elimination mechanisms in rodents (Bucks et al., 2017). Our findings suggest that an age-dependent hair cell decline in the utricle may be a dominating factor contributing to hair cell loss if tumor parameters are similar between patients. In addition, rapid access to human vestibular epithelia allows translational studies crucial for better understanding human peripheral vestibular function.

S214. Body Balance in Isolated Otolithic Vertigo - Differences Between Utricular Disorder and Saccular Disorder

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Category: Vestibular: Basic Research and Clinical
Background: The establishment of vestibular evoked myogenic potentials (VEMPs) as a test for otolith function has provided insights into the pathophysiology of isolated otolith vertigo. However, their impacts on body balance are unknown. The aim of this study is to elucidate the differences in the impact on body balance between saccular and utricular dysfunctions.
Methods: We examined 24 cases diagnosed with isolated otolithic vertigo. The lesion site was determined by the VEMP results. Saccular dysfunction was defined by abnormal results in cervical VEMP (cVEMP), while utricular dysfunction was defined by abnormal results in ocular VEMP (oVEMP). We analyzed the results of stabilometry with eyes closed to eliminate visual influences. The results were assessed using standardized values (Z-scores), which are independent of age and gender.
Results: Among the cases, 13 exhibited utricular dysfunction, 17 had saccular dysfunction, and 6 showed both types of dysfunctions. The sway area of utricular and saccular lesions was 1.9 (SD: 1.9) and 0.5 (SD: 1.6), respectively, while the sway path length was 1.0 (SD: 1.1) and 0.1 (SD: 1.3), respectively.
Conclusions: Utricular disorders had a more pronounced impact on body balance compared to saccular disorders. This difference can be attributed to two factors: Firstly, it is well-established that the utricle, positioned in the horizontal plane, is more easily stimulated when the head is upright. Secondly, neurons

originating from the utricle within the lateral vestibulospinal tract are known to extend further down the pathway than those originating from the saccule within the lateral vestibulospinal tract.

Symposium 1 - Good Data In, Big Data Out

2:00 p.m. - 4:00 p.m.

Platinum Salon 5

Good Data In, Big Data Out

Chair: Katharine Fernandez, *NIDCD*
Co-Chair: Laura Dreisbach*, School of Speech, Language, and Hearing Sciences, San Diego State University*

Session Description: Big Data in healthcare encompasses high volume, high diversity clinical, biological, environmental, and lifestyle information collected from single individuals to large cohorts, in relation to their health and wellness status, at one or several time points (Directorate-General of the EU Commission). Despite the availability of data from electronic healthcare records, patient reports, genomics, pharmaceuticals, clinical trials, and telemedicine, the audiology field has not fully utilized Big Data to address global concerns surrounding hearing loss. Adopting a comprehensive data sharing approach on a large scale holds significant potential for gaining insights into the causes and outcomes of diseases, identifying precise drug targets for personalized medicine, and improving disease prediction and prevention. By systematically collecting, storing, processing, and analyzing vast datasets that surpass what a single clinic can gather, we can enable evidence-based decision-making and enhance patient care. Numerous challenges stand in the way of generating big data. Acquiring access and linking health records, tackling challenges related to the data harmonization process (e.g., consistent diagnosis and evaluation practices and using standard outcome measures) is necessary before data harmonization. Finally, for maximal utility, Big Data needs to meet the FAIR and TRUST principles of being findable, accessible, interoperable, and reusable, as well as transparent, responsible, user-focused, sustainable and technology accessible. This session will address the scope of data-informed audiology clinical practice and provide considerations for integrating Big Data and navigating barriers. This symposium will highlight the advances in health care made possible by large-scale data analysis, propose potential solutions to barriers standing in the way of data harmonization, identify sources of variance in clinical practice and emphasize the need for standard outcome measures in clinical trials.

Standardization of Threshold and Supra-Threshold Measures in Clinical Trials Assessing Investigational Inner Ear Medicines

Colleen Le Prell

University of Texas at Dallas

Individual Abstract: Standardization of clinical trial outcome measures for various disease states can help researchers and healthcare providers compare and observe trends across institutions and within different patient populations. However, widespread adoption of such standardized measures is not common in the auditory research field nor is there consensus on recommended outcome sets to be used in the clinical trial setting. Variability in diagnostic test type, inconsistency in evaluation practices, and the irregularity of assessment timepoints prevents direct comparisons and the ability to perform appropriate meta-analyses. Together, these issues limit learning and our ability to use longitudinal data to address intervention effects in our target population. The focus of this presentation will be threshold and supra-threshold tests used in current and recently completed clinical trials. The selection of endpoint measures relevant to patient clinical outcomes will be discussed in detail, with the results of several systematic reviews presented to illustrate the heterogeneity in clinical trial endpoints to date. Hearing loss and suprathreshold hearing disorders are major public health issues, impacting quality of life for millions of Americans and others worldwide. Although supra-threshold functional deficits such as hearing-in-noise difficulties, tinnitus, and hyperacusis have been termed “hidden” because they are not captured by the audiogram, these are common patient complaints. The

identification of specific physiological processes underlying diverse hearing difficulties using animal models has opened the door for development of experimental medicines that may prevent or ameliorate hearing loss and suprathreshold hearing deficits, importantly enabling a growing body of human clinical trials. In parallel to pharmaceutical prevention of injury to the inner ear, efforts to understand developmental sequences have provided insights into the potential for gene and small molecule therapies that might re-activate developmental sequences in the inner ear. Successful regeneration of damaged cell populations might one day allow restoration of auditory function or alleviation of supra-threshold deficits that occur with cell or neuron loss. Supra-threshold testing has been included in most regeneration trials, in contrast to the very low inclusion rate of suprathreshold testing within otoprotection trials. Successful development of prevention and restoration therapies would positively impact patients, and progress is being made despite numerous barriers to the development of inner ear medicines. Given the active drug discovery pipeline and major efforts across academic labs and pharmaceutical and biotechnology companies to identify and develop medicines that effectively treat auditory complaints, standardization of clinical test measures within clinical trials is urgently needed.

The Role of Consistent Clinical Practice Approaches for Data-Informed Hearing Care

Gayla Poling

National Institute on Deafness and Other Communication Disorders

Individual Abstract: Growing demands for audiologic care related to earliest detection of hearing loss and prevention require defining new care pathways and clinical approaches that promote quality and timely accessibility to care. Detection of hearing loss at the initial signs of dysfunction leads to earliest intervention, prevention, and improved quality of life over the long term. Furthermore, detecting and monitoring hearing loss through serial measurements in order to guide preventative actions such as avoidance of predisposing factors that contribute to hearing loss, including noise or ototoxic chemical and medication exposures, is an important consideration and value-added to the practitioner and patient. There is a need for consistency in clinical practices that leads toward data harmonization to leverage the full potential of Big Data from clinical audiology to meet the needs of the individual patient. While national and international standards in addition to best-practice guidelines are available for some core clinical approaches (e.g., hearing thresholds), there are differences/modified approaches in "real world" clinical applications. These include lack of specifications for universal adoption for some tests (e.g., speech audiometry), and differences in diagnostic criteria that add to the complexity of practice convergence. Moreover, differences in serial monitoring practices (e.g., ototoxicity) and definitions of significant change criteria can limit the potential of integrated clinical decision-making tools and complicate "risk" prioritization. Standardizing data collection and integration practices where possible can improve processes and improve patient care delivery. Enhancements in clinical guidelines, triage systems, practice workflows, auditory risk assessment, patient education, and outcomes reporting are just a few of the ways data may inform care. This session will convey the scope of opportunities for data-informed clinical audiology approaches, describe the need for consistent clinical practice guidance to support data harmonization, and provide considerations for navigating barriers while contributing to and integrating Big Data in the clinic.

Here Hear Share!

Ronna Hertzano

National Institutes of Health

Individual Abstract: Despite the extensive collection of clinical audiometric data worldwide, our understanding of hearing health remains limited. Unlike other medical fields, audiology has yet to establish a unified approach for harmonizing, sharing, analyzing, and visualizing data, resulting in a lack of comprehensive databases that include hearing function data for both adults and children. This stands in stark contrast to the substantial benefits that other fields have gained from the development of large clinical databases that lend themselves to the application of data science approaches including machine learning algorithms that enable standardization and discovery. In this context, we present an innovative and secure cloud-based platform designed to host hearing data and tools. This platform is built upon the Probing

Outcomes Data with Visual Analytics (POD-Vis) software and is tailored for use by hearing-related clinicians and researchers. The development of this tool, and its application to hearing health data, is led by the HearShare consortium, currently comprising seven prominent research institutions: Duke University, the National Institute on Deafness and Other Communication Disorders, Massachusetts Eye and Ear Infirmary, Mayo Clinic, Medical University of South Carolina, University of Maryland College Park, and the University of Maryland Baltimore. These institutions have amassed a vast dataset that includes hearing thresholds, tympanometry, acoustic reflex thresholds, and speech audiometry (recognition scores in quiet and noise) from over 500,000 unique adult patients aged 18 years and above. By leveraging this rich dataset, we demonstrate the immense potential of a centralized audiometric data repository that incorporates robust tools for data analysis and hypothesis generation.

From Club Good to Pure Public Good: Some Barriers and Solutions to Sharing Health-Related Data

Michael Cummings

University of Maryland

Individual Abstract: Health sciences and healthcare have undoubtedly benefited from aggregation of data from disparate sources to form shared data resources with little-to-no access restrictions. A recent example are the many advances in understanding SARS-CoV-2 (hCoV-19) and associated epidemic because of data available through Global Initiative on Sharing All Influenza Data (GISAID). Treating audiological and health data more broadly enables better analyses using machine learning and other approaches. Here I will describe some of the common barriers that exclude access to health-related data, thus keeping these data a club good, rather than the pure public good when viewed from an economics perspective and propose solutions. Among the key concerns related to sharing of health-related data are real and perceived legal restrictions related to the Health Insurance Portability and Accountability Act of 1996 (HIPAA) and institutional policies. These can be addressed through better understanding of applicable laws and policies, and eliminating concerns via de-identification, homomorphic encryption, and other technical solutions. Sociological issues, including using control of data as a means of power, dynamics of the academic reward system and intellectual property, can also be barriers to data sharing. Solutions to these problems include better understanding of the individual and institutional rewards associated with data sharing, better understanding of data sharing requirements of funding agencies and publishers, peer pressure, and deliberate data release. Additionally, ethical and moral concerns need to be considered in relation to questions such as the following: Whose data is it really? Who or what are we protecting or possibly harming? What is the greater good? Here, as elsewhere, satisfactorily addressing the problems requires first articulating the specifics of the problems and understanding the underlying concerns and issues. Currently, the field of audiology neither utilizes a common data repository nor are there an established set of standards for data collection in the clinic or within formal clinical trials, thus imposing obvious restrictions on machine learning applications. We need to embrace data diversity, describe it, analyze subsets of the data and look for shared patterns across subsets, use the data diversity as points of discussion to set standards for data collection and sharing. Independent of the barrier category and intentionality, not sharing data imposes excludability on data access, thus rendering data as a club good.

Big Data, Big Possibilities

Kelly Reavis

VA RR and D, National Center for Rehabilitative Auditory Research

Individual Abstract: Big data has become increasingly important in various domains, including healthcare, with the potential to improve patient outcomes. While there are different definitions of big data, its essence lies in vast volumes of data that exceed human comprehension and standard computing capabilities. However, its status as a buzzword has led to ambiguity and uncertainty among professionals. Nevertheless, big data has enabled research that generates valuable insights, which would be challenging to obtain otherwise. This talk explores the interconnectedness of epidemiology and big data and their potential for advancing hearing healthcare. Epidemiology aims to understand disease patterns, risk factors, and health outcomes. Big data, with its vast and diverse datasets, offers unique opportunities to analyze and derive insights from large-scale information. Epidemiology and big data intersect as they leverage comprehensive datasets to uncover

associations and detect patterns that may not be apparent through traditional approaches. Big data sources, such as electronic health records, health registries, wearables, and social media, provide valuable information for epidemiological studies. The integration of big data analytics, including machine learning and data mining, enhances the capabilities of epidemiology. These techniques enable researchers to identify complex associations, generate hypotheses, and improve disease prediction models. By harnessing big data, epidemiologists can gain deeper insights into hearing health, uncover new risk factors, and develop more targeted interventions. While epidemiology and big data are often complementary, there are also challenges and potential conflicts. Epidemiology relies on carefully designed studies with controlled data collection, ensuring validity and generalizability. In contrast, big data includes diverse and extensive datasets, which may introduce biases. Careful study design and bias control are necessary to ensure accurate interpretation of big data findings. To illustrate the practical application of big data in hearing healthcare, this talk will also highlight diverse research studies that utilize big data methodologies. These studies include the prediction of drug-induced hearing damage, understanding genetic foundations of hearing loss, and improving patient outcomes in the context of hearing aids. The talk will also highlight the volume, trends, and patterns of big data research in audiology through bibliometric analysis. The integration of epidemiology and big data offers immense potential for advancing hearing healthcare. Leveraging comprehensive datasets and utilizing advanced analytics techniques, researchers can gain valuable insights, improve disease prediction, and enhance patient care.

Podium Session 1 - Cochlear Mechanics: Physiology

2:00 p.m. - 4:00 p.m.

Platinum Salon 6

Suppression of Basilar Membrane and Organ-of-Corti Responses Towards the Apex of the Cochlea

Sebastiaan Meenderink*[1], Wei Dong[1]

[1]*VA Loma Linda Healthcare System*

Category: Inner Ear: Cochlear Mechanics

Background: Three major roles of the cochlea are transduction, spectral decomposition, and compression of sound. Certainly, the latter process is nonlinear, and causes strong interactions between stimulus components. One manifestation of this is suppression, where responses to a (probe) tone are reduced by the addition of a second (suppressor) tone. This phenomenon has been studied in psychoacoustics and neural responses, and likely originates within the cochlea, where it has also been observed from the basilar membrane (BM) and reticular lamina (i.e., surfaces of the organ of Corti or ooC). Here we measure suppression to study the (nonlinear) mechanical responses to sound not only from the BM, but also within the ooC using optical coherence tomography (OCT).

Methods: We systematically mapped suppression in full ooC's cross-sections to create "suppression-maps" that covered all aspects of the ooC (e.g., BM, outer hair cells (OHC), Deiters' and pillar cells, lateral compartment) in the 2nd turn (tuned to 2–3 kHz) of the alive gerbil cochlea. By using tone complexes as stimuli, in which one component acted as the suppressor, maps for multiple (suppressor, probe)-combinations were created. This extends the two most commonly used stimulus paradigms in which either the probe or the suppressor is at the recording's location best frequency.

Results: On the BM, it was found that suppression was tuned in that near-BF probes were the easiest to suppress. This phenomenon of tuning was independent of suppressor frequency, although near-BF probes were more efficient. Suppression in response magnitude was accompanied by systematic phase changes, where higher suppressor levels caused less phase accumulation of the probe tones. These observations were similar to the level-dependent growth of BM responses we also obtained. Data also showed that suppression was not simply a "local" effect, it built up during traveling wave propagation. The suppression maps allow us to extend the analysis of the cochlear nonlinearities to non-BM regions within the ooC.

Conclusions: Initial analysis indicates that suppression in ooC regions is very similar to the BM response: thus far we have found no indication that suppression in, for example the OHC region, is more prominent or differently tuned. The reason for this apparent invariance of responses across the ooC may be related to the

relatively wide frequency extent of nonlinear responses and tuning of all ooC structures in this frequency regions of the gerbil's cochlea.

We thank our lab technicians, Ana K Anchondo and Laura M Anchondo, for their help with collecting intracochlear responses. The research is supported by NIDCD R21DC019998 and VA RRD Merit 1 I01 RX003491.

Cochlear Vibrations in Response to Frequency Sweeps

Karolina Charaziak*[1]

[1]*USC Keck School of Medicine*

Category: Inner Ear: Cochlear Mechanics

Background: Perceptually, tones that are rapidly swept up in frequency (up-sweeps) tend to be better maskers than tones swept in the opposite direction (down-sweeps). It has been hypothesized that this perceptual sensitivity to the direction of the swept-tone maskers derives from the dispersive properties of the traveling wave (TW) in the cochlea. Our previous results in mice corroborate this hypothesis. At the 9-kHz tonotopic location, cochlear vibrations in response to a tone were more strongly suppressed by up-frequency-sweep maskers as compared to down-sweep maskers when the rate of frequency change approached the TW velocity near its peak. However, when the sweep rate was slow compared to the TW velocity, the direction of the frequency change did not affect the strength of suppression.
When behavioral masking and otoacoustic emission suppression experiments using similar stimuli were conducted in humans, it was discovered that the difference in the strength of masking/suppression between up and down sweeps varied systematically with the sweep rate. The direction of the sweep had a significant effect when the sweep rate approached the estimated TW velocity in humans, but the effects were insignificant for both slower and faster rates. In this study, we are seeking to determine whether similar behavior is observed in the suppression of cochlear vibrations for sweep rates exceeding the TW velocity.

Methods: The estimated TW velocity at its peak in humans is considerably slower compared to the velocities observed in intracochlear vibrations in rodents. This creates a practical problem when generating sweeps with frequency-change rates that match or exceed the TW velocity in rodents, as it can introduce acoustic distortions at the stimulus onset/offset. To overcome this technical difficulty, we designed a sweep where the instantaneous frequency varies with time following a sigmoid function. This means that the instantaneous sweep rate varies with time, with the frequency changing relatively slowly at the edges, allowing for a gradual tapering of the stimulus envelope. The sweep rate at the instantaneous frequency of the probe tone was used as an estimate of the effective rate.
Results: The cochlear vibrations were collected in anesthetized gerbils using optical coherence tomography at locations corresponding to either apical or mid cochlear turns. Preliminary analyses revealed that when sweep rates reached the local TW velocity, there was no considerable difference between responses measured with either up or down sweeps, as expected.
Conclusions: Here, we build upon our previous research in humans and mice to assess the dependence of cochlear responses on rapid sweeps of varying directions in gerbils. We demonstrate that challenges associated with designing sweep with rates comparable to the TW velocity in rodents can be overcome by modifying the trajectory of the instantaneous frequency of the sweep over time.

Organ of Corti Vibrations in the Basal Turn of the Chinchilla

Eric Verschooten[1], Marcel Van Der Heijden*[2], Philip X Joris[1]

[1]*Laboratory of Auditory Neurophysiology, Medical School, Campus Gasthuisberg, University of Leuven,* [2]*Erasmus MC*

Category: Inner Ear: Cochlear Mechanics

Background: Optical coherence tomography (OCT) has brought enormous improvements over previous methods for the measurement of cochlear vibrations. In particular, it is now possible to map vibration patterns

inside the organ of Corti, and to study the relative motion of its constituents. Especially in the basal turns, where the structures can be viewed through the semitransparent round window membrane, the OCT images are crisp and have good contrast, so that the vibrations can be unambiguously attributed to the various anatomical structures. Apart from yielding many new observations, this has also been instrumental in validating the new methods against decades of basilar membrane data obtained with previous techniques.
The majority of the basal-turn OCT data have been obtained in the gerbil cochlea. For several reasons it is important to extend the basal measurements to other species. Apart from the obvious need to test how observations in one species generalize to other, there are methodological advantages, too. The different orientation of the cochlea allows different viewing angles, which may help finding out the direction of the vibrations – something that cannot be assessed directly with a Doppler technique like OCT. Structures that are often poorly visible in one species, e.g., the tectorial membrane (TM), may be better accessible in other.

Methods: We measured organ of Corti vibrations in the basal turns of the chinchilla using two approaches. In the first, we measured through the intact round window membrane, accessing the 15-22 kHz region. In the second, we made a narrow slit in the bony wall of the basal turn, which we then sealed with a piece of cover glass. This exposed the 6-11 kHz region. Apart from accessing different frequency regions, the two approaches also differed markedly in the viewing angle relative to the anatomical axes of the organ of Corti.
Results: With both approaches we found excellent sensitivity (significant responses down to 0 dB SPL), sharp tuning and compressive growth at intensities below 20 dB SPL. We report vibration maps of the entire organ of Corti, including the tectorial membrane, individual tuning curves, I/O functions, and spatial magnitude and phase profiles along the length of the cochlea.
Conclusions: We discuss the similarities and differences of our chinchilla data with data obtained in the basal turn of the gerbil.

Resting Calcium Dependence of the Outer Hair Cell Hair Bundle

Rayan Chatterjee*[1], Daibhid O Maoileidigh[2]

[1]*Stanford University School of Medicine,* [2]*Stanford University*

Category: Inner Ear: Cochlear Mechanics
Background: The hair-bundles of outer hair cells (OHCs) are required for the high sensitivity, sharp-tuning and large dynamic range of hearing. Hair bundles comprise rod-like projections, called stereocilia, which pivot on the hair-cell surface and are connected by filamentous gating-springs that regulate the opening of ion-channels at the stereocilium tips. Sound-induced forces deflect the stereocilia, extend the gating-springs, and open the ion-channels, permitting ionic currents to flow into the cell, which sum to generate the receptor current. These ion-channels are, however, not fully closed at rest (i.e., in the absence of stimulus forces) - there is experimental evidence that the receptor current at rest is around 50 percent of its maximum value in endolymphatic conditions (0.02 mM calcium), whereas in perilymphatic conditions (1.5 mM calcium) it is around 8 percent of its maximum value. Along with this change in resting receptor current, the resting bundle position shifts. The mechanisms responsible for the calcium dependence of the resting receptor current and the bundle position remain unclear.
Intriguingly, additional experiments have shown that stereocilia possessing mechanoelectrical transduction ion channels decrease in height when the calcium concentration is lowered. We hypothesize that stereocilium height reductions owing decreasing calcium concentration raises gating-spring tension. The rise in tension increases the resting current and decreases the resting bundle position.

Methods: We test this hypothesis by building a computational model that accounts for the morphology (stereocilium heights, widths, and pivot positions) of the OHC hair bundle, which are assigned from published experimental data. We calculate the pivot stiffness of each stereocilium by fitting our model to experimental data for deflection of the hair-bundle without gating-springs versus applied force. Fitting our model to force-deflection data of the hair bundle with gating-springs enables us to calculate the gating-spring stiffness. Additional published experimental data enables us to calculate the values of all the parameters in our hair-bundle model.
Results: We show that a decrease in stereocilium heights increases the resting receptor current from perilymph to endolymph. Along with the increase in receptor current, our model also predicts a decrease in the bundle

resting position. We also find that the tension in the gating-springs at rest in endolymph is higher than in perilymph.
Conclusions: We show that a change in the heights of the ion-channel containing stereocilia can explain the difference in resting receptor currents and resting bundle position at high and low calcium concentrations. How these changes might be related to receptor current adaptation remains to be determined.

Sound-Evoked Tonic Outer Hair Cell Length Changes in Mice With Impaired Mechanotransduction

James Dewey*[1]

[1]*University of Southern California*

Category: Inner Ear: Cochlear Mechanics
Background: Outer hair cells (OHCs) are thought to amplify sound-induced cochlear vibrations by changing length and generating force at the frequency of stimulation. Though sound also elicits tonic OHC length changes, the origin and role of these responses remain incompletely understood. One interpretation of such tonic length changes is that they are the electromotile response to a tonic component in the OHC receptor potential, which can arise when mechanotransduction currents are asymmetric. Disruption of OHC mechanotransduction should therefore strongly reduce or alter both cycle-by-cycle and tonic OHC length changes. To explore this, cochlear motions were examined in mice with stereociliary defects that severely impair mechanotransduction and OHC-mediated amplification.
Methods: Using optical coherence tomography, sound-evoked organ of Corti displacements were measured from the ~9 kHz region in adult wild-type (WT) C57BL/6J mice and in mice with mutations affecting mechanotransduction. These included salsa mice, which progressively lose the stereocilia tip links that gate mechanotransduction channels, and TriobpΔex8/Δex8 mice, which lack stereocilia rootlets and experience degeneration of the stereociliary bundle by adulthood.
Results: In WT mice, sound-evoked tonic displacements were consistent with sustained OHC contractions and could exceed 20 nm at high stimulus levels. Surprisingly, tonic displacements were also observed in a significant fraction of mice with impaired mechanotransduction, with magnitudes approaching those observed in WT mice. In impaired mice, tonic displacements could be present despite little to no evidence of cochlear amplification. Tonic displacements in impaired mice also exhibited variable kinetics and were occasionally consistent with OHC elongation rather than contraction. In both WT and impaired mice, tonic displacements could be larger than the response at the stimulus frequency, indicating that they did not arise from an asymmetry in the organ of Corti's passive mechanics. Tonic displacements were significantly reduced or absent after application of salicylate, which inhibits electromotility, and after death, which reduces mechanotransduction currents. Tonic displacements in impaired mice were therefore susceptible to the same manipulations that reduce cycle-by-cycle electromotility and amplification.
Conclusions: Sound-evoked tonic OHC length changes do not require absolutely normal stereociliary mechanotransduction. Nevertheless, it is possible that tonic OHC length changes in impaired mice are just electromotile responses driven by residual, conventional mechanotransduction currents. Altered stereociliary mechanics -- and possibly changes to organ of Corti stiffness -- may lead to responses that are more low-pass in nature, explaining the unexpectedly large tonic displacements observed in some mice. Alternatively, other stretch-sensitive channels or strain-induced mechano-electrical effects could be involved. Regardless, the functional significance of tonic OHC length changes in normal and impaired ears remains to be determined.

Work supported by NIH/NIDCD R21 DC019209, the Hearing Health Foundation, and the University of Southern California's Keck School of Medicine.

Variation of Cochlear Mechanical Responses at Micro- And Macro-Levels Post Acoustic Trauma

Wei Dong*[1], Sebastiaan Meenderink[1]

[1]*VA Loma Linda Healthcare System*

Category: Inner Ear: Cochlear Mechanics

Background: Knowledge of cochlear mechanics in "normal ears" is quickly advancing using optical coherence tomography or OCT. The beauty of OCT is that it not limited to the measurement of vibrations from the surface of the cochlear partition, but also collects information on the motion of within the cochlear partition. Observations within intact cochleae have demonstrated that individual cells/structures do not operate in isolation; their bidirectional (mechanical) 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.

Acoustic trauma leads to the disruption of the cochlear partition and results in sensory hearing loss. The underlying cochlear mechanics, both at the macro- and micro-level, have never been systematically characterized. It is therefore poorly understood how damage within the cochlea leads to changes in the mechanical responses and subsequent activity of the auditory neural system. Sustained damage not only results in elevated hearing thresholds but can also cause hidden hearing loss or form the trigger for chronic tinnitus.

Methods: To investigate the effect of acoustic trauma on cochlear function, we use OCT to "map" the sound induced vibrations within the entire cross-section of the organ of Corti complex (OCC) in the 2nd turn of intact gerbil cochlea in vivo, both under normal and following exposure to an intense pure tone.

Results: Comparison of pre- and post-damage vibrometry maps (obtained from the same cochlear OCC cross-section) allow us to identify "functional changes" in OCC vibrometry. We found that throughout the OCC, acoustic trauma had the largest effects on the amplitudes of low-intensity and high-frequency responses. In addition, we found that the phase of the responses was a more sensitive indicator for acoustic trauma than their magnitude: systematic phase variations were observed for (high-level) stimuli for which the response amplitude did not change.

Conclusions: Our results shed light on the intricate workings of both in normal and impaired auditory systems. It also highlights the connection between local mechanical processes and the overall processing of sound within the cochlea. These insights have the potential to inform the development of diagnoses and treatments for hearing-related disorders and enhance our understanding of auditory perception.

We thank for the help of Ana K Anchondo and Laura M Anchondo with collecting intracochlear responses. The research is supported by NIDCD R21DC019998 and VA RRD Merit 1 I01 RX003491.

Robot-Assisted Multidimensional OCT Measurements of Cochlear Mechanics

Gabriel Alberts*[1], Wiam Lahlou[2], Sunil Puria[3]

[1]*Harvard University,* [2]*International University of Rabat,* [3]*MEE*

Category: Inner Ear: Cochlear Mechanics

Background: Cochlear mechanics measurements involving optical coherence tomography (OCT) show tremendous promise but, with some exceptions (Lee et al., 2016; Zhou et al., 2022), have been limited to one-dimensional motion. It is becoming clear that two- and three-dimensional motion measurements are needed to determine transverse, radial and longitudinal motions of organ of Corti structures. Progress has been made towards developing 3D OCT measurement systems using three co-located beams (Kim et al., 2022). However, at this stage there are tradeoffs that reduce performance. We took an alternate approach to making multiple angle measurements by using our existing high-resolution single-beam system to measure from multiple directions. This was facilitated by mounting a ThorLabs commercial OCT head on a high spatial resolution tabletop robot arm. The Meca500-R4 Robot Arm by Mecademic is a six-axis industrial robot arm with position repeatability of 5 um—comparable to the resolution of our OCT system and less than the width of an outer hair cell. The small footprint of the robot occupied less than 1.5 cubic feet and fit on a small vibration isolation measurement table. We used the RoboDK software to simulate the robot and our experimental setup virtually, which was essential for calibration and control of the OCT optical head.

Methods: The robot was calibrated in RoboDK by marking points in our experimental setup with small reflective beads and moving the robot to multiple configurations such that the beads were positioned at the same depths in our OCT B-scans. After exposing the cochlea for vibration measurements, we oriented the

robot such that the B-scan aligned with calibrated x-z axis. Planar rotations then allowed for vibration measurements to be taken at multiple angles from the same longitudinal location. 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: Two-dimensional vibration measurements were taken through the bony capsule of living, anesthetized mice with intact cochleae and normal hearing near the 10 kHz best frequency region. Measurements spanned roughly 20 degrees and were taken at angles that resulted in a combination of radial and transverse motion.
Conclusions: The ThorLabs OCT system together with the Meca500 Robot Arm are a powerful combination of tools to collect two-dimensional, in-vivo measurements of cochlear motion. The robot-OCT merger along with vector decomposition show promise for extending data collection and analysis to motion in three dimensions. [Supported by the Amelia Peabody Charitable Fund and NIDCD R01DC07910, F31DC021079, and T32 DC000038.]

The Localized Action of OHC Activity in the Base of the Gerbil Cochlea

Elizabeth Olson*[1], Clark Strimbu[2], Lauren Chiriboga[3], Brian Frost[1]

[1]*Columbia University,* [2]*College of Physicians and Surgeons of Columbia University,* [3]*Columbia University*

Category: Inner Ear: Cochlear Mechanics
Background: Auditory sensation is based in the nanoscale vibration of the sensory tissue of the cochlea, the organ of Corti complex (OCC). In the cochlear base, in response to sound stimulation, the region that includes the body of the electro-motile outer hair cells (OHC) moves with larger amplitude than the basilar membrane (BM) and surrounding regions. The intense motion is based in active OHC mechanics, and the region was termed the "hotspot" (Cooper et al., 2018, Nature comm). In addition to this quantitative distinction, the hotspot moves qualitatively differently than the BM, in that its motion scales nonlinearly with stimulus level at all frequencies, evincing sub-BF activity. Sub-BF activity enhances non-BF motion; thus the frequency tuning of the hotspot is reduced relative to the BM.
Methods: Vibrations within the organ of Corti complex (OCC) were measured with a ThorLabs Telesto III OCT in the base of the gerbil cochlea, through the round window membrane. The measurements were made either near the 25 kHz BF place with a longitudinal+transverse viewing axis, or in the hook region where the BF was between 40 and 50 kHz, with a transverse viewing axis. Each OCT Motion-scan (M-scan) allows for motion to be measured at many locations in depth. Multiple M-scans were made radially across the OCC, to allow for sub-BF nonlinearity (activity) to be evaluated at many locations in radial/transverse or radial/(transverse+longitudinal) cross-sections.
Results: Most of the OCC -- including the BM, the reticular lamina (RL) and much of the medial and lateral OCC -- did not exhibit sub-BF activity. These regions were tuned approximately similarly, with amplification only at frequencies near BF. With the transverse viewing angle, the hotspot motion was nearly half a cycle out of phase with the BM and RL through a wide frequency range including BF. This caused near cancellation of the measured motion of the hotspot at some frequencies and sound levels. A complex difference analysis revealed the internal hotspot motion relative to the frame.
Conclusions: Outside of the hotspot, the OCC moves together as a loosely tethered frame-like structure. The OHC hotspot works within this frame, amplifying the frame motion at frequencies close to the BF, while in a healthy cochlea the frame-like structure is shielded from the sub-BF activity of the hotspot. The pillar cells and the Deiters stalk, composed of structural proteins (microtubules, actin and intermediate filaments) anchor the RL to the BM. Intracellular structures, as well as the extracellular collagenous structures of BM and TM are likely key to holding the frame together.

Podium Session 2 - Primary Auditory Cortex

2:00 p.m. - 4:00 p.m.

Grand Ballroom Salon E

Voice Patches in the Marmoset Auditory Cortex Revealed by Wide-Field Calcium Imaging

YANG ZHANG*[1], Xingdong Song[1], Yueqi Guo[1], Chenggang Chen[1], Xiaoqin Wang[1]

[1]*Johns Hopkins University*

Category: Primary Auditory Cortex

Background: Species-specific vocalizations are behaviorally critical sounds. Recognizing the vocalizations is important for the survival and social interactions of the vocal animals. In humans, a voice patch system has been identified on the lateral superior temporal gurus (STG) that is selective to human voices. In non-human primates, vocalization selective regions are found on the rostral portion of the temporal lobe, which are outside of the auditory cortex, both in macaques and marmosets using functional magnetic resonance imaging (fMRI). It is yet unclear whether vocalizations are uniquely processed in the auditory cortex.

Methods: Wide-field calcium imaging, a technique with both high temporal and high spatial resolution

Results: We discovered two voice patches in the marmoset auditory cortex that prefer species-specific vocalizations over other vocalizations and sounds. One patch is located on the posterior A1 (primary auditory cortex), and the other one is located on the anterior non-core regions. These voice patches are hierarchically organized based on latency and selectivity analyses. In addition, call types and identity information are carried by population responses from the voice patches. Furthermore, we found that the voice patches are functionally connected.

Conclusions: These results reveal the existence of voice patches in the auditory cortex of marmosets and support the notion that, for different primate species, similar cortical architectures are adapted for recognizing communication signals both for vocalizations and faces.

A Top-Down, Cingulate-to-Auditory Cortex Projection Facilitates Effortful Listening

Kelsey Anbuhl*[1], Marielisa Diez Castro[1], Nikki A. Lee[1], Vivian S. Lee[1], Dan H. Sanes[1]

[1]*Center for Neural Science, New York University*

Category: Primary Auditory Cortex

Background: We often exert greater cognitive resources (i.e., listening effort) to understand speech under challenging acoustic conditions. This mechanism can be overwhelmed in those with hearing loss, resulting in cognitive fatigue in adults, and potentially impeding language acquisition in children. However, the neural mechanisms that support listening effort are uncertain. Evidence from human studies suggest that the cingulate cortex is engaged under difficult listening conditions and may exert top-down modulation of the auditory cortex (AC). Indeed, previous anatomical tracing work in gerbils reveal a strong, descending projection from the cingulate cortex (Cg1) to dorsal and primary AC. Here, we asked whether this top-down projection facilitates effortful listening.

Methods: To assess auditory effort, an amplitude modulation (AM) rate discrimination task was used, and stimulus parameters (AM rate, sound duration) were varied to adjust the difficulty of listening conditions. Using an appetitive Go-Nogo paradigm, gerbils were trained to discriminate between “Go” stimuli consisting of a range of AM rates (4.5-12 Hz, broadband noise carrier, 100% depth) and a “Nogo” AM stimulus (4 Hz). Trials were clustered into ‘Easy’ or ‘Hard’ blocks, where the sound duration was 1s or 0.25s, respectively. AM rate discrimination thresholds were determined from psychometric functions. A chemogenetic approach (i.e., DREADDs) was used to determine whether the Cg projection to AC is required for optimal psychometric performance. Behaviorally-trained gerbils (n=8) received bilateral injections of a retrograde pAAV-hSyn-hM4D(Gi)-mCherry virus into auditory cortex (AC and AuD), followed by bilateral cannulae implants above Cg1. Animals recovered for one week. Following re-establishment of baseline performance, infusions of Compound 21 (C21; chemogenetic actuator of hM4D) or saline (control) were delivered via cannulae on alternate days, just prior to each behavioral testing session.

Results: We found that inactivating Cg-to-AC specific projections disrupted performance only for Hard trial blocks (Saline threshold: 5.0±0.4 Hz AM; C21 threshold: 7.4±0.7 Hz AM; p less than 0.001) whereas performance for Easy blocks remained unaffected (Saline threshold: 5.2±0.2 Hz AM; C21 threshold: 5.6±0.6

Hz AM; p=0.9). Furthermore, these findings were consistent with a second procedure used to pharmacologically inactivate Cg1 alone (via direct bilateral infusions of muscimol).
Conclusions: Taken together, the results reveal a descending cortical pathway from Cg to AC that facilitates perceptual performance during difficult stimulus conditions. This pathway is a plausible circuit that may be undermined by hearing loss.

Orbitofrontal Cortex Modulates Auditory Cortical and Perceptual Sensitivity

Matheus Macedo-Lima*[1], Lashaka Sierra Hamlette[1], Melissa Caras[1]

[1]*University of Maryland - College Park*

Category: Primary Auditory Cortex
Background: Sensory abilities are not fixed but can quickly adapt to changes in context. Auditory perception, for instance, is strongly affected by whether stimuli important to solve a task. Auditory cortical neurons react to task engagement by changing their receptive fields, firing rates or timing precision, culminating with enhanced neural stimulus detection. However, the brain regions involved in providing task engagement information to auditory cortex are only beginning to be understood. The orbitofrontal cortex (OFC) is a prefrontal cortical region with established roles in signaling reward and in transmitting state-dependent feedback via direct projections to sensory (including auditory) cortices and is therefore a promising candidate to explore. We hypothesized that OFC neurons provide contextual information to the auditory cortex that enhance auditory perception during task engagement.
Methods: If OFC transmits state-dependent feedback to the auditory cortex, then we should observe a neural signature of task engagement in OFC neurons. To test this prediction, we recorded activity of OFC neurons in freely moving Mongolian gerbils using chronically implanted electrode arrays as they performed an amplitude modulation (AM) detection task and compared neural activity during the task with passive sound exposure periods just before and just after the task.
Task engagement enhances the detection or discrimination of behaviorally-relevant stimuli in auditory cortical neurons. If OFC mediates task-dependent changes auditory cortical activity, then inactivating OFC should impair auditory cortical detection both neuronally and behaviorally. To test this prediction, we used muscimol (GABAa agonist) to inactivate bilateral OFC, and simultaneously recorded extracellular responses from auditory cortical neurons in freely moving Mongolian gerbils as they performed an AM detection task.

Results: We found that the firing rates of OFC neurons are strongly modulated by task engagement. Neurons exhibited phasic increases or decreases in firing that were time-locked to various task parameters, such as trial onset, behavioral response, and trial outcome. The majority (77%) of OFC neurons also exhibited tonic increases or decreases in baseline firing rates during the task, compared to passive exposure sessions.
Inactivation of bilateral OFC significantly diminished task-dependent increases of auditory neural sensitivity, by preventing firing rate changes, without affecting firing variability or timing reliability in auditory cortical neurons. Behavioral AM detection was also significantly impaired by OFC inactivation and was strongly correlated with auditory cortical AM sensitivity on a day-to-day basis.

Conclusions: Our findings suggest that the OFC is the source of a top-down signal that supports task-dependent activation of the auditory cortex and the perception of behaviorally-relevant sounds.

Dysfunction of Specific Auditory Fibers Impact Cortical Oscillations, Driving an Autism Phenotype Despite Near-Normal Hearing

Morgan Hess*[1], Philine Marchetta[1], Konrad Dapper[1], Dila Calis[1], Wibke Singer[1], Jakob Wertz[1], Stefan Fink[1], Steffen Hage[2], Mesbah Alam[3], Kerstin Schwabe[3], Robert Lukowski[4], Jerome Bourien[5], Jean-Luc Puel[5], Michele Jacob[6], Matthias Munk[7], Rüdiger Land[8], Lukas Rüttiger[1], Marlies Knipper[1]

[1]*University of Tuebingen,* [2]*Hearing Research Center, University of Tübingen, Medical Center,* [3]*Hannover Medical School,* [4]*Institute of Pharmacy, Pharmacology, Toxicology and Clinical Pharmacy, University of Tübingen,* [5]*Institute for Neurosciences Montpellier, University of Montpellier, Institut National de la Santé et*

de la Recherche Médical, [6]*Tufts University School of Medicine, Sackler School of Biomedical Sciences,* [7]*University of Tübingen,,* [8]*Institute of Audioneurotechnology, Hannover Medical School*

Category: Primary Auditory Cortex

Background: Autism spectrum disorder is discussed in the context of altered neural oscillations and imbalanced cortical excitation-inhibition of cortical origin. We studied here whether developmental changes in peripheral auditory processing, while preserving basic hearing function, leads to altered cortical oscillations.
Methods: Local field potentials (LFPs) were recorded from auditory-, visual-, and prefrontal cortices and the hippocampus of BdnfPax2 KO mice.
Results: These mice develop an autism-like behavioral phenotype through deletion of BDNF in Pax2+ interneuron precursors, affecting lower brainstem functions, but not frontal brain regions directly. Evoked LFP responses to behaviorally relevant auditory stimuli were weaker in the auditory cortex of BdnfPax2 KOs, connected to maturation deficits of high-spontaneous rate auditory nerve fibers. This was correlated with enhanced spontaneous and induced LFP power, excitation-inhibition imbalance, and dendritic spine immaturity, mirroring autistic phenotypes.
Conclusions: Thus, impairments in peripheral high spontaneous rate fibers alter spike synchrony and subsequently cortical processing relevant for normal communication and behavior.

Active vs. Passive Listening Reveals Neural Modulation Frequency Selectivity and Top-Down Control Over Entrainment

Sam Watson*[1], Jens Hjortkjær[1], Torsten Dau[1]

[1]*Technical University of Denmark (DTU)*

Category: Primary Auditory Cortex

Background: Amplitude modulations (AMs) within the envelope of speech signals are known to carry the majority of lexical meaning, and humans have been shown to have both AM frequency selective neural networks and behavioural selectivity. This has led to speculation that behavioural envelope processing models such as the Envelope Power Spectrum Model (EPSM) might have a physical neural basis for a similar signal decomposition within the auditory pathway. Furthermore, attention dependent neural entrainment of cortical evoked potentials makes clear the role of cognisant intent on the representation of auditory envelopes in entrained neural activity.
Methods: This study sought to establish the presence of frequency selective envelope domain (non-energetic) masking in scalp recorded envelope following responses (EFRs) and interrogate the consequence of active or passive listening on EFR power and masking patterns. 14 young participants with normal hearing listened to white noise, the envelope of which contained a target 4 Hz sinusoidal AM and a 3-octave wide band of random modulations at 3 various proximities to the target frequency. The participant's cognisant attention was controlled using two oddball (OB) tasks; one embedded in the audio stimulus and the other an on-screen visual task, whereby participants were required to exclusively detect oddballs in one of the modalities per-trial and ignore the other modality.
Results: Results show that scalp recorded EEG EFR responses are significantly suppressed (4 dB on average) with increasing proximity of the masking modulations to the target, but only during passive listening. While participants actively attended the stimulus, EFR power was significantly increased overall by on average 4 dB, but also unaffected by increasing modulation masking and poorer behavioural detection in the most masked conditions. Results suggest that EFR responses during passive listening are stimulus parameter driven with the neural envelope synchronisation being perturbed by similar random masking modulations. Active listening however shows no such perturbation, despite worse OB detection performance, implying that conscious tracking of the 4 Hz target modulation over time enables entrainment despite the masking. This integration or streaming of the target over time through the masking may be an example of auditory object formation to which the response entrains.
Conclusions: This study finds evidence for the existence of modulation frequency selectivity and masking at the neural level, as recorded with scalp EEG. Furthermore, results demonstrate the role of top-down control over neural entrainment to auditory objects in complex sound environments, despite degraded acoustic presentations, and suggests the role of active listening should be examined in studies seeking to relate evoked potentials with behaviour.

Spatial Tuning of Neurons in Marmoset Auditory Cortex is Dynamically Modulated by Spatial and Temporal Features of Background Noise

Yi Zhou*[1], Katherine Doxey[1], Sharon Crook[1]

[1]*Arizona State University*

Category: Primary Auditory Cortex

Background: In marmoset auditory cortex, location-sensitive neurons respond to both contralateral and ipsilateral directions, exhibiting either narrow or broad tuning. However, whether the apparent spatial heterogeneity is an incidental characteristic of a vast neural network or an integral aspect of auditory spatial processing remains elusive. This study investigates whether spatial tuning is an inherent attribute of cortical neurons by testing the effects of background noise on the stability of tuning features.

Methods: We recorded single-unit activity in the auditory cortex of two adult marmoset monkeys using tungsten microelectrodes and Neuropixel linear probes. The spatial tuning of a neuron was measured in the 360o horizontal plane using best frequency tones or broadband noises. The changes in neuron's preferred sound direction and sharpness of tuning were evaluated when additional broadband noise stimuli was presented, following the Target (random location)+Masker (fixed location) paradigm. Two temporal arrangements of Masker were considered: (1) Masker simultaneously presented with Target and (2) Masker preceding Target by 200-ms.

Results: Our findings reveal that cortical neurons exhibit varied spatial selectivity across different cortical layers. The two fundamental features of spatial response—peak direction and tuning sharpness— were correlated, with sharply tuned neurons predominantly clustering around a peak direction at -90 degrees in the contralateral field. When evaluated in the presence of a contralateral Masker, contralateral neurons broaden their spatial selectivity and ipsilateral neurons sharpen their spatial selectivity. An inverse trend is observed with the presence of an ipsilateral Masker. Notably, these alterations are more pronounced when the Masker precedes the Target.

Conclusions: Similar to frequency tuning of cortical neurons, spatial tuning in auditory cortex can be dynamically modulated by other sounds. Changes in spatial selectivity suggest the employment of a neural strategy for spatial unmasking, whereby selectivity is augmented at locations distanced from a Masker sound. These results suggest that spatial heterogeneity is a likely integral feature of spatial processing in the auditory cortex.

In Memoriam Symposium - Celebrating Arnold Starr: A Legacy of Discoveries in Basic and Clinical Auditory Research

4:15 p.m. - 6:15 p.m.

Grand Ballroom Salon E

Celebrating Arnold Starr: A Legacy of Discoveries in Basic and Clinical Auditory Research

Chair: Fan-Gang Zeng*, University of California Irvine*
Co-Chair: Leonard Kitzes*, University of California at Irvine*
Co-Chair: Nina Kraus*, Northwestern University*

Arnold Starr's Life and Achievements
Leonard Kitzes

University of California at Irvine

Individual Abstract: Arnold Starr grew up in New York City and started his studies at Kenyon College in Ohio when he was only 16. He then interrupted his medical education at New York University spending a year in Scandinavia doing research. Following his residency at Harvard University, he furthered his focus on research as a research associate for two years at the NIH. After a stint on the faculty of Stanford University, he was invited in 1971 to the University of California Irvine to be the Director of the Division of Neurology within the Department of Medicine. The independent Department of Neurology at UCI was established in 1977, he was its founding Chair and Professor Emeritus after he retired from the university. As an academic clinician Dr. Starr often referred to the clinical setting, in which he saw a full complement of patients, as "my second laboratory". Among his many contributions to basic and clinical science, the most direct outcomes of his perspective of the Academic Clinician are evident in his development of the Auditory Brainstem Response (ABR) and his identification of Auditory Neuropathy. The ABR is used both clinically and in basic science research to assess the functionality of the cochlea and lower brainstem, most commonly to assess hearing in newborn humans and in human and animal subjects in clinical and basic science studies. Throughout his career, he thrived on thinking and was most rewarded by understanding. This was manifest in his many original contributions to both science and medicine. His motto in combining both was "outcome may be better when we understand." On the personal level, Dr. Starr was an original thinker and an artist. He was an avid traveler who enjoyed bridging cultural differences, learning, and teaching in all corners of the globe. He traveled to the Soviet Union 30 years before the fall of the Iron Curtain, and his first published paper was on medical education in the Soviet Union. He studied whales in Norway and acupuncture in China, and wherever he traveled, he made personal friendships with the colleagues and students he met. He thrived on unexpected and surprising events and often enjoyed creating them himself. He was also an accomplished painter and his paintings featured in some of his publications and the cover of journals.

Cerebral Responses to the Speech Envelope

Terence Picton

University of Toronto

Individual Abstract: Arne Starr was fascinated by how the brain understands speech and how this ability can be disrupted in disorders such as auditory neuropathy and dementia. Timing is one of the essential cues for analyzing speech sounds: following the vocal pitch facilitates the identification of the vowel, and following the envelope of ongoing speech can help to parse it into meaningful units. In recent years multiple studies have shown that magnetic or electrical recordings from the human brain as it listens to speech can be correlated to the ongoing speech envelope or its derivative. The analysis yields a temporal response function that is similar in morphology and scalp distribution to the normal response to the onset of a simple sound, albeit with a delayed latency. The response to the speech envelope has a P1-N1-P2 waveform with peak latencies near 70, 120 and 200 ms. The measurement of this waveform might provide a way to assess the brain's ability to process speech. Using human speech as the stimulus for evoking brain responses promises to be more meaningful than the clicks, beeps and noises we have become accustomed to. Were he still with us, Arne would enjoy this movement toward natural stimuli.

Site-of-Lesion in Auditory Neuropathy

Fan-Gang Zeng

University of California Irvine

Individual Abstract: The term "Auditory neuropathy" (AN) was coined in the 1990's by Professor Arnold Starr and colleagues, to describe a form of hearing impairment in which afferent neural transmission through the auditory nerve and brainstem is disrupted, but cochlear (outer) hair cell function is normal. There are a number of pathologic mechanisms (involving both pre- and post-synaptic sites) capable of producing the AN result pattern. This presentation includes a review of the current AN literature, exploring the relationships between site-of-lesion, functional hearing ability and intervention outcomes in affected patients. Furthermore, we investigate the capacity of Diffusion-weighted MRI (dMRI) to localize and quantify auditory neural changes in different forms of auditory neuropathy. This presentation summarizes recent dMRI findings in patients with pathology affecting different regions in the auditory pathway. The correlation between

anatomical features (such as neural fibre density) and auditory perception in these individuals are also explored. Different patterns of auditory neural fibre loss were demonstrated for aetiologies known to affect different regions in the auditory pathway. In addition, we found significant correlations between fibre density and the perceptual abilities of the participants. The material summarized in this presentation suggests that dMRI analysis methods have the potential to predict hearing capacity in infants with auditory neuropathy and form an objective basis for early intervention. This is particularly relevant to cochlear implantation candidature decisions where site-of-lesion is a significant predictor of post-operative outcome. In addition, these techniques may provide a biomarker for neurodegenerative diseases (Charcot-Marie-Tooth disease, Friedreich ataxia etc) which involve progressive damage to the auditory neural system.

Neural and Receptor Cochlear Potentials in Patients With Genetic Auditory Neuropathy
Rosamaria Santarelli

University of Padova

Individual Abstract: In the first paper we wrote together, Arnold Starr (Santarelli, Starr A, Michalewski HJ, Arslan A. Clin Neurophysiol, 119: 1028-41, 2008) defined auditory neuropathy (AN) as a "disorder of auditory nerve function characterized by hearing deficits affecting auditory perceptions dependent on temporal cues". The most well-known forms of AN are due to gene mutations and the mechanisms believed to be involved are functional alterations at pre- and post-synaptic sites, including neurotransmitter release from ribbon synapses, spike initiation in auditory nerve terminals and the neural dys-synchrony resulting from demyelination and axonal loss. ABRs are limited in providing detailed information of cochlear nerve and hair cell activities since the recording electrodes are placed at a distance from these generators. Details of cochlear potentials including both receptor (summating potential, SP; cochlear microphonic, CM) and auditory nerve activities (compound action potential, CAP) can be more effectively evaluated through transtympanic electrocochleography (ECochG). ECochG findings help to define objectively the sites of auditory neural dysfunction as affecting inner hair cell receptor SP or neural CAP potential, the latter reflecting disorders of ribbon synapses and auditory nerve fibers. The identification of specific gene mutations combined with typical electrophysiological patterns may be the key-factor in revealing how the failure of different molecular processes underlies the varieties of AN. Moreover, elucidation of the physio-pathological mechanisms and site of lesion may help to predict the outcome of cochlear implantation or hearing aid use in patients with AN related to different gene mutations.

A Synaptic Perspective on a Neurologist's Journey in Hearing Research
Tobias Moser

University Medical Center Goettingen

Individual Abstract: This presentation focuses on the work of the late Arnold Starr dedicated to synaptic sound encoding and its disturbances. Arnold, a clinical neurologist and auditory neuroscientist, had started his journey into hearing research before I was born. I met Arnold in the early 2000's when auditory neuropathy had become an emerging research focus largely triggered by the eminent 1994 publication in Brain by Arnold and his colleagues. Not only were we of different generations and nations, but also our perspective on sound encoding was very complementary. Comprehensive neurological and neurophysiological expertise on his end and molecular and synaptic neuroscience on mine. But we had in common curiosity about disease mechanisms, interest in audiological assessment and understanding the impact of impaired temporal processing for hearing. Arnold appreciated the emerging insights into afferent synapse function and dysfunction. He made seminal contributions to deep phenotyping of otoferlin-related synaptopathy. Arnold helped in a major way to build a network of scientists working on auditory neuropathy, to work towards consensus regarding audiological assessment and the nosology of hearing impairment. On a personal level, I am grateful to Arnold for the great discussions about science and life and his steady encouragement.

Cochlear Synaptopathy: Auditory Neuropathy for the Rest of Us
Charles Liberman

Eaton-Peabody Laboratories, Mass. Eye and Ear

Individual Abstract: Arne Starr's seminal studies of patients with intact otoacoustic emissions and absent auditory brainstem responses first highlighted the existence of hearing impairments arising from primary degeneration of the auditory nerve. This condition, which he termed auditory neuropathy, is rare, is often congenital and it severely reduces speech comprehension. Over the past 15 years, our lab has been studying a kind of acquired auditory neuropathy we called cochlear synaptopathy, because its first manifestation is the loss of synaptic connection between inner hair cell and auditory nerve terminal. The loss of peripheral axons, cell bodies and central axons of the auditory nerve is delayed for months to years. Synaptopathy can be elicited in animals by overexposure to sound, even when threshold shift and hair cell damage is transient, and it appears in aging animals before there is threshold elevation or hair cell loss. Our work on autopsy specimens from normal-aging adults suggests that surviving inner hair cells have lost ~50% of their synaptic connections by age 65, and that this loss is exacerbated by a history of acoustic overexposure. This degree of primary neural degeneration does not affect speech comprehension in quiet, but likely adds to the well-known difficulties understanding speech in a noisy environment. This talk will briefly summarize some of the major conclusions of our studies of cochlear synaptopathy in humans and on the efforts to repair the damage in animal models.

Research supported by grants from the NIDCD: R01 DC 00188 and P50 DC 015857.

Crossmodal Plasticity in Hearing Loss

Andrej Kral

Medical University Hannover

Individual Abstract: Dr. A. Starr had a profound interest in large variety of medical topics, including the relation between hearing and the brain, but also the relation between sensory systems and their contribution to behavior. He studied localization of objects and their relation to saccades, suppression of visual responses during saccades, the cellular brainstem mechanisms of visual and auditory localization, and the effects of anaesthesia and alcohol on sensory responses. One of his early papers on crossmodal plasticity in cats (Stewart and Starr, 1970, Exp Neurology) was a reference for our first study showing absence of cross-modal plasticity in primary auditory cortex of congenitally deaf cats (CDCs, Kral et al., 2003, Exp Brain Res). Subsequent collaboration with several labs could demonstrate that while there are supranormal visual abilities in CDCs (Lomber et al., 2010, Nat Neurosci), these are due to very specific cortical areas (ibid.) that showed only moderate anatomical reorganization (Barone et al., 2013, PLoS One; Buttler et al., 2017, Hear Res). Bottom-up reorganization was of moderate extent, too (Land et al., 2016, J Neurosci). Comparing the literature on multimodal interactions with crossmodal effects following deprivation revealed that crossmodal plasticity is observed in areas normally showing multimodal responses (Kral and Sharma, 2023, Trends Neurosci). Recently, evidence of reversibility of some crossmodal effects was provided (Glick and Sharma, 2020, Front Neurosci), suggesting that it is a dynamic and flexible process. The substrate of crossmodal effects consists of feedback projections from multimodal areas and/or inputs from several sensory systems to the given areas. Crossmodal plasticity does not hinder successful hearing restoration and given its dynamic and versatile nature, it can be exploited for improving clinical outcomes after neurosensory restoration (Strelnikov et al., 2013, Brain; Barone et al., 2016, Cortex). Mechanistically, crossmodal plasticity can be explained by homeostatic excitability changes in the deprived neurons combined with top-down influenced functional connectivity changes (Kral and Sharma, 2023, Trends Neurosci). As in many other subjects, Arne Starr has been ahead of its time and set directions also in crossmodal consequences of congenital deafness.

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Clinical Implications From Arnie Starr's Work in Electrophysiology

Andrew Dimitrijevic

Sunnybrook Hospital ENT University of Toronto

Individual Abstract: Arnie Starr's work on the electrophysiology from brainstem to cortex has had a tremendous impact on clinical studies and clinical interpretation. His insights to the workings of the auditory and cognitive systems have helped shaped how we design experiments and experimental paradigms that have direct clinical implications. In this talk I will cover how his work in both hearing and dementia have shaped the respective fields. This will include studies arising from electrically evoked brainstem potentials for cochlear implant testing to neural adaptation in dementia. Work from our lab relating electrophysiology and behaviour outcomes in people with cochlear implants stemming from Arnie's insights will be presented.

Research supported by the Mason Scientific Discovery Fund and CIHR (Canadian Institutes of Health Research).

Starr's Legacy- Inspiring Research Beyond Otolaryngology
Nina Kraus

Northwestern University

Individual Abstract: As a neurologist, Arne Starr often justified his research by saying: "I use the ears to study the brain." True to his word, he published many of his hearing studies in general-audience journals. For example, he published a series of papers on auditory neuropathy and the use of auditory evoked potentials to detect dementia in Brain. A paper published in Nature (Carmel and Starr 1964) studied "non-acoustic factors influencing activity of middle ear muscles in waking cats". A paper in Science (Squires et al. 1978) showed "acute effects of alcohol on auditory brainstem potentials in humans". His appeal to audiences beyond otolaryngology is instantiated in how he viewed the hearing brain. He saw it as a vast system engaging how we feel, move, what we know, our memories and all our senses. Arne's holistic perspective helps us remember that patients are people, not a collection of facts to be entered into a computer - especially in this moment when the patient is often forgotten. Arne's personality reminds us that science is done by humans. Finally, his viewpoint as an artist helps us remember that science is an art.

Sunday, February 4, 2024

Young Investigator Symposium 2 - Emerging Perspectives on Misophonia

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

Grand Ballroom Salon E

Emerging Perspectives on Misophonia

Chair: Emily Coffey*, Concordia University*
Co-Chair: Marie-Anick Savard*, Concordia University*

Clinical Validation of a New Diagnostic Test for Misophonia and Hyperacusis
Philippe Fournier

Universite Laval Faculty of Medicine

Individual Abstract: Reduced sound tolerance is a pathological condition for which tolerance to sounds is so reduced that tolerable normal everyday sounds induce discomfort. This general term encompasses different

forms of reactions and responses to sounds including hypersensitivity to loud sounds (loudness hyperacusis) and aversion to specific sounds (misophonia). According to epidemiological studies, 10 to 15% of the general population suffers from loudness hyperacusis and 12 to 15% from misophonia. Currently, hearing healthcare professionals rely on semi-structured interviews, standardized questionnaires, and their clinical judgments to assess the presence of hyperacusis and misophonia. There is no objective diagnostic measure or biomarker for these disorders. A new clinical tablet application that uses natural sounds for the diagnosis of hyperacusis and misophonia designed for clinical use was developed. The test consists in presenting pleasant and unpleasant natural sounds at three intensities (60-, 70-, 80- dB SPL) through headphones and asking the patient to rate the pleasantness of the sound on a visual analog scale (VAS) from 0 (Very pleasant) to 100 (Very unpleasant). "Misophonic" trigger sounds such as chewing, sniffling, and slurping sounds were included in the list of sounds presented. The first studies on this test performed with a laboratory version compared the pleasantness rating of hyperacusis and misophonia patients to those of matched controls and identified the most discriminant sounds, that is, the sounds that provided the best sensitivity (SEN) and specificity (SPE) for each disorder. Seven sounds were identified for hyperacusis (SEN: 81%, SPE: 88%) and ten sounds for misophonia (SEN: 95%, SPE: 87%). Since the most discriminant sounds for misophonia were all different from the ones for hyperacusis, it is possible to test both conditions within the same test session and differentiate them. The project aimed at validating the sensitivity and specificity of this clinical application for the diagnosis of hyperacusis and misophonia in a clinical setting when both conditions are assessed simultaneously using the most discriminant sounds. Other objectives included acquiring control normative data for different age groups representative of the life cycle and exploring the contribution of loudness in the pleasantness ratings. The addition of a new VAS designed to assess the loudness of each natural sound will provide complementary information to the pleasantness ratings. The results of this project will be presented and discussed.

Effect of Misophonia on Cognitive and Social Processing

Heather Hansen

Concordia University

Individual Abstract: Misophonia, an abnormal aversion to certain sounds, turns normal cognitive and social exercises (e.g., paying attention during a lecture near a pen-clicking classmate, coexisting at the dinner table with a food-chomping relative) into massive struggles. How does exposure to triggering sounds impact cognitive and social processing? We investigated this question in a sample of 65 participants (26 misophonia, 39 control) from the general population. In Phase 1, participants saw faces paired with auditory stimuli while completing a gender judgment task, then reported sound discomfort and identification. In Phase 2, participants saw these same faces with novel ones and reported face likeability and memory. For both oral and non-oral triggers, misophonic participants gave higher discomfort ratings than controls did – especially when identification was correct – and performed worse on the gender judgment. Misophonic participants rated lower likeability than controls did for faces they associated with high discomfort sounds, and face memory was worse overall for faces originally paired with high discomfort sounds. Altogether, these results suggest that misophonic individuals suffer impairments on social and cognitive tasks if they have to endure discomforting sounds. This experiment helps us better understand the day-to-day impact of misophonia and encourages usage of individualized triggers in future studies.

Selective Attention as a Means of Modulating Misophonic Responses

Marie-Anick Savard

Concordia University

Individual Abstract: Misophonia is a disorder whose sufferers experience significant distress (exaggerated negative emotional responses accompanied with heightened autonomic arousal) when exposed to specific sounds. Although recent findings show that higher-level cognitive processes are important contributors to misophonic reactions, we have little insight into how these processes could be used to mitigate them. In a set of two studies, we investigated the role of selective attention in modulating responses to sounds in people with and without misophonia. In the first study, participants with and without misophonia completed a task in which they were asked to focus their attention on one of two sound streams played simultaneously through

headphones. In one ear, they were presented with triggering, neutral, or generally aversive sounds; in the other, they heard unfamiliar musical excerpts. Physiological measures of emotional reactivity (pupillometry, skin conductance response, heart-rate-variability) were recorded. We will present results concerning the ability of attention-based strategies to modulate physiological aspects of misophonic responses and determine contributing factors to individual differences in these modulations. In a second study, we used functional magnetic resonance imaging (fMRI) to investigate the effects of selective attention on brain activity within emotional and auditory networks, in people with misophonia relative to controls. Participants with and without misophonia performed the same selective attention task while we recorded fMRI measures of brain activity. Preliminary results will be presented. By combining behavioural, physiological, and neuroimaging approaches, we aim to enhance our understanding of misophonic reactions and explore potential strategies for coping with trigger sounds. This work has the potential to to reduce distress and improve the quality of life for individuals with misophonia.

Misophonia Reactions Increase During Childhood and are Associated With Musicality and Other Auditory Affective Experiences

Solena Mednicoff

University of Nevada, Las Vegas

Individual Abstract: Hearing plays a critical role in human affective experience, yet different people can have varying emotional reactions to the same sound. For example, people with misophonia can experience extreme distress and anger to sounds that others would consider pleasurable, such as in Auditory Sensory Meridian Response (ASMR). The expression of misophonia varies widely in the general population, ranging from subclinical misophonic reactions to extreme disruptions in quality of life. Anecdotal evidence suggests that misophonia may emerge during childhood and worsen with age. Despite this, relatively little is known about the potential relationship between misophonia and negative and positive auditory affective experiences during development. In the present study, we examined whether misophonia experiences are associated with greater emotional reactions to sounds with both positive and negative affect, as well as with other high-level auditory perceptual skills, like musicality and speech prosody. Adults and 6-15-year-old children were asked about their self-reported misophonia, ASMR, and musical chills and were tested for their misophonia severity, music training, self-reported musicality, and auditory perception skills (e.g., rhythm, tonality, and speech prosody). Real-time emotional reactions to stimuli designed to elicit misophonia, ASMR, or musical chills were also measured. Participants were asked to press a button for any momentary emotional reaction they had while watching each video, whether it was negative like disgust or positive like tingles/goosebumps, and to give an overall valence and arousal rating to each video. Our measures of misophonia severity were correlated with each other and with real-time emotional reactions to misophonia videos, however of the misophonia measures, only real-time reactions increased with age. Misophonia severity was correlated with self-reported musical chills, and real-time reactions to misophonia videos correlated with real-time reactions to both music and ASMR videos. Additionally, misophonia severity and misophonic reactions were predicted by music training, musical perceptual skills (especially recognition of musical emotions) and sensitivity to speech prosody, particularly in the child sample. These findings suggest that musicality, auditory perceptual skills and overall auditory affective responsiveness may predict the likelihood of someone experiencing misophonia.

Altering the Perceived Cause of Unpleasant and Misophonic Sounds With Videos and Words

Urszula Oszczapinska

Carnegie Mellon University

Individual Abstract: Everyday sounds can elicit a range of emotional and physiological responses in people. For individuals with Misophonia, some everyday sounds can produce strong feelings of irritation, rage, and disgust. Promising studies show that emotional reactions to a sound can be attenuated by manipulating the interpretation of the sound's source using sound-swapped videos (Samermit et al., 2019; 2022), or written descriptions (Edelstein et al., 2020). Although these studies suggest that context is a contributing factor in evoking an emotional reaction to a sound, the mechanism of this effect is not well understood. In the present study, we ask how integral semantic reassignment is to the effect of causal reinterpretation of the sound. We

predict that the effect of causal reinterpretation is greater when there is high plausibility that the sound was produced by an alternative source. We combined unpleasant and misophonic trigger sounds with neutral attributable stimuli that suggest a more positive interpretation of the sound's cause. In Experiment 1, the sounds were combined with neutral, dynamic visual events. For example, the sound of utensils scraping was combined with a video of birds chirping. Experiment 2 was identical to Experiment 1 except the sounds were combined with neutral written descriptions invoking semantic representations. In Experiment 3, we measured pupil diameter as listeners viewed stimuli from Experiment 1. Listeners across each of the studies rated the sounds' pleasantness (11-point scale) and how well the sound and neutral source matched (5-point scale). Ratings were compared to counterparts that depicted the sound's true cause. Sounds were rated as significantly more pleasant when combined with alternative causes. The greatest change in sound pleasantness occurred when the sound was combined with a neutral, dynamic visual event (avg: 1.19-points, Cohen's d = 0.98) compared to description (avg: 0.64-points, Cohen's d = 0.46). Further, sound pleasantness ratings increased by 0.81-points for dynamic events with 1-point increases in match quality. Approximately half of the effect of the neutral visual event can be explained by semantic representations, leaving the remainder of the effect to be explained by the visual nature of the input. Lastly, we found pupil responses that were distinguishable between the true sources and alternative sources. Altering the perceived cause of a sound is a viable option to attenuate emotional responses to unpleasant and misophonic sounds, which is evident behaviorally and physiologically. [Funded by REAM]

Reactions to Misophonic Trigger Sounds are Reduced by Visual Stimuli
Ghazaleh Mahzouni

UC Santa Cruz

Individual Abstract: Misophonia (literally meaning hatred of sound) is a relatively new term that has been used to describe extreme psychological and physiological reactions to specific types of trigger sounds and associated stimuli (e.g., chewing, slurping, snoring). An important characteristic of the misophonic experience is the inordinate negative reaction such as anger, verbal aggression, and violence toward the person generating the sounds, which can severely compromise day-to-day activities and healthy social interaction. While traditionally, misophonia has been characterized as an auditory disorder, recent findings suggest that high-level multisensory factors also play a role. Our lab's previous work with neurotypical populations showed that negative reactions to general aversive sounds (e.g., nails on a chalkboard) and trigger sounds (e.g., chewing) are significantly reduced when the sounds are synchronized with a positive attributable visual source (PAVS) (e.g., tearing a piece of paper or walking on leaves; Samermit et al., 2019; 2022). In our current work, we investigated the role of PAVS in modulating misophonic reactions in people with misophonia. We recruited 26 misophonia and 31 healthy controls and presented them with 26 short video clips: 13 misophonia trigger sounds (e.g., crunchy chewing) paired with the 13 original video sources (OVS; e.g., video of crunchy chewing) and the same trigger sounds paired with 13 positive attributable visual sources (PAVS; e.g., video of tearing a piece of paper). After each video clip, participants rated the pleasantness and the intensity of bodily sensations felt and described the nature of these sensations. Our results show that PAVS-paired sounds significantly increased ratings of pleasantness and reduced the intensity of bodily sensations in both misophonia and control participants, compared to OVS-paired sounds. Importantly, participants with misophonia showed significantly more reduction in the intensity of bodily sensations compared to the control participants. An analysis of self-reported bodily sensation descriptions revealed that PAVS-paired sounds led to qualitative changes in these descriptions among misophonia participants, resulting in fewer words pertaining to body parts. We also found that participants who scored higher on the Duke Misophonia Questionnaire (DMQ) also reported higher clarity of auditory imagery, yet visual imagery was not associated with the DMQ. Overall, our results show that the negative emotional and physical reactions to misophonic trigger sounds can be attenuated by presenting them alongside positive attributable visual sources which can potentially provide an avenue for developing treatments that include PAVS and auditory imagery for misophonia sufferers.

Podium Session 3 - Hair Cell Spiral Ganglia Synaptic Specializations in Normal and Pathological States

8:00 a.m. - 10:00 a.m.
Platinum Salon 5

Dynamics of Noise-Induced Dopamine Upregulation in Lateral Olivocochlear Neurons

Jane Mondul*[1], Catherine Graham[1], Megan Wood[2], Amanda Lauer[1], Elisabeth Glowatzki[2]

[1]*Johns Hopkins University,* [2]*Johns Hopkins University School of Medicine*

Category: Inner Ear: Anatomy and Physiology

Background: Noise exposure can damage the inner ear, eliciting downstream compensation or protection mechanisms in the central auditory system. The lateral olivocochlear (LOC) efferent system may help prevent noise-induced excitotoxicity at the synapse between inner hair cells and auditory nerve fibers. Moderate environmental noise and high-level acute exposures can increase expression of tyrosine hydroxylase (TH), an enzyme involved in dopamine synthesis, in cholinergic LOC neurons. However, the time course and dose-dependence of this LOC plasticity is not well-described. We investigated changes in LOC neurotransmitter expression in mice following single, short-duration noise exposures of different intensities at different post-exposure time points.

Methods: Young adult mice (C57BL/6J, 4 weeks old, male and female) were exposed to broadband noise (2-20kHz) for 15 minutes at a level of 94, 97, 100, or 103 dB SPL. Auditory brainstem responses were measured in anesthetized animals to clicks and tonebursts (4, 8, 12, 16, 24, 32kHz) at one day, one week, and three weeks post-exposure. Cochlea whole mounts and cleared brain sections containing the lateral superior olive (LSO) were labeled using fluorescent immunohistochemical markers for cholinergic (choline acetyltransferase, ChAT), dopaminergic (TH), and other neurotransmitter pathways. Cochleas were also labeled for hair cells (myosin, Myo7a) and ribbon synapse markers (c-terminal binding protein, CtBP2; glutamate receptor, GluR2). Confocal microscopic images were analyzed to quantify TH expression and co-labeling with ChAT, hair cell loss, and synapse counts.

Results: The 94 dB SPL exposure caused no significant threshold shifts. The 97, 100, and 103 dB SPL exposures caused 15-50 dB temporary threshold shifts, which resolved within 1-3 weeks post-exposure. Sham and 94 dB SPL exposed subjects had variable TH expression in cholinergic LOC neurons that was usually minimal and restricted to high frequency regions (cochlear base and medial LSO). Following the 97, 100, and 103 dB SPL noise exposures, LOC TH expression in the cochlea and LSO core increased with exposure intensity. TH upregulation was greatest at 1 week post-exposure and resolved by 3 weeks post-exposure.

Conclusions: Noise exposure induces a dose-dependent increase in TH expression in cholinergic LOC neurons that peaks at one week following exposure, after resolution of temporary threshold shifts. Dopaminergic transmission by LOC neurons may support inner ear recovery following acoustic injury and may represent a therapeutic target for noise-induced hearing loss. This work was supported by NIH NIDCD DC006476 (PI: EG), NIH T32 DC000023 (JM, PI: Cullen, Fridman), and the David M. Rubenstein Fund for Hearing Research.

Ageing of Cochlear Hair Cells in Mice

Jing-Yi Jeng*[1], Adam Carlton[1], Piece Yen[1], Walter Marcotti[1]

[1]*The University of Sheffield*

Category: Inner Ear: Anatomy and Physiology

Background: Age-related hearing loss (ARHL) is the most common sensory deficit and one of the most common health conditions experienced by older adults. AHRL is a heterogeneous disorder resulting from the cumulative effects of environmental factors, genetic predisposition and cellular senescence. With age, the hair cells of the mammalian cochlea are known to degenerate, which ultimately leads to deafness. However, there is evidence suggesting that age-related changes in the morphological and physiological properties of hair cells occur well before their degeneration. Here we compared mouse strains with different onset of AHRL to investigate changes in the hair cells properties with age.

Methods: We performed patch clamp recordings from IHCs in acutely dissected organs of Corti from mouse strains that are widely used in hearing research: CBA/CaJ, C3H/HeJ and C57BL/6N mice. Depolarizing and hyperpolarizing voltage steps were applied in voltage clamp to elicit potassium currents. Mechanoelectrical transducer currents were elicited by displacing the hair bundles of the IHCs with a fluid jet. Voltage responses were recorded in current clamp. Immunostaining was used to investigate the innervation pattern of the IHCs. Auditory brainstem responses (ABRs) were used to investigate the hearing function of the different mouse strains.
Results: C57BL/6N mice showed an earlier onset of hearing loss compared to CBA/CaJ or C3H/HeJ mice, which was already evident by 6 months of age. CBA/CaJ and C3H/HeJ have similar hearing thresholds, although the former performed better at higher frequencies up to at least 22 months. A common ageing feature in all mice, was the reduction in the surface area of the hair cells. However, we found that the size of the mechanoelectrical transducer current and the efferent re-innervation in the inner hair cells was correlated to the degree of hearing phenotype of the different mouse strains.
Conclusions: We found that the inner hair cells gradually lose their ability to process acoustic information optimally with age, which was associated with the degree of progression of ARHL. These changes occurred before inner hair cell degeneration.

Synaptic Ribbon Dynamics After Noise Exposure in the Hearing Cochlea

Peu Santra*[1], Noura Ismail Mohamad[1], Yesai Park[1], Ian R. Matthews[1], Emily Taketa[1], Dylan K. Chan[1]
[1]*UCSF*

Category: Inner Ear: Anatomy and Physiology
Background: Moderate noise exposure induces the loss of afferent ribbon synapses between cochlear inner hair cells (IHCs) and spiral ganglion neurons (SGNs), also known as cochlear synaptopathy. This is associated with decline in functional hearing, or “hidden hearing loss”. Prior studies have shown synaptic changes after acoustic overstimulation in fixed specimens. Here, we describe the use of a live imaging model using RIBEYE-tagRFP, to directly visualize pre-synaptic ribbons in neonatal as well as noise- exposed, mature hearing juvenile cochlear inner hair cells.
Methods: RIBEYE-tagRFP mice constitutively express RIBEYE conjugated to RFP tag. P4-P6 neonatal RIBEYE-tagRFP mice were dissected and cultured overnight, then imaged the next day, whereas the juvenile mice were immediately imaged after the dissection. For glutamate excitotoxicity induction, neonatal cultures were incubated with 0.4mM kainic acid for two hours or media as control. For cochlear synaptopathy model, 8-week-old RIBEYE-tagRFP mice were exposed to 8-16kHz octave-band white noise for 2 hours at 98dB SPL and unexposed littermate mice were used as negative controls. Neonatal and juvenile cochlear explants were loaded with FM1-43, a vital dye, to delineate the hair cells. Live imaging was performed using Nikon A1R upright scanning confocal microscope with 60x water objective and OKOlab stagetop incubator. Z-stacks of the mid portion of the neonatal and apical turn of the juvenile cochlea were recorded and processed using Imaris, version 9.9.1. Additionally, immunohistochemistry was performed after tissue fixation for pre-synaptic ribbons, post-synapses and spiral ganglion neurons using anti-CTBP2, anti-GluR2 and anti-NF respectively.
Results: Co-labelling for RIBEYE-tagRFP and antibody staining with anti-CTBP2, anti-GluR2 and anti-NF for pre-synaptic ribbon, post-synaptic terminal and SGN showed proper colocalization and conservation of IHC-SGN ribbon synapses. Kainic acid treatment did not change RIBEYE-tagRFP puncta number or size but induced random movement of ribbons in neonatal cultures. Compared with neonatal cochleae, juvenile cochleae had pre-synaptic ribbons that were larger in volume and less mobile. Noise exposure induced an increase in size and mobility of pre-synaptic ribbons in the mature, hearing juvenile cochlea. These changes persisted at 2 weeks post noise exposure. Finally, we observed that the basal-most pre-synaptic ribbons moved towards the nucleus immediately after noise exposure, with subsequent migration back to the basal pole.
Conclusions: Live imaging of pre-synaptic ribbons in neonatal and mature hearing cochlea demonstrates that drug-induced glutamate excitotoxicity in neonates and noise exposure in juvenile cochlea induce random and, for a subpopulation of basal ribbons, directed movement. This novel model will enable investigation of the early, dynamic events in cochlear synaptopathy.

BAI1 Localises AMPA Receptors at the Cochlear Afferent Postsynaptic Density and is Essential for Hearing

Adam Carlton*[1], Jing-Yi Jeng[1], Fiorella Grandi[2], Francesca De Faveri[1], Ana Amariutei[1], Lara De Tomasi[1], Andrew O'Connor[1], Stuart Johnson[1], David Furness[3], Steve Brown[4], Federico Ceriani[1], Michael Bowl[5], Mirna Mustapha[1], Walter Marcotti[1]

[1]*University of Sheffield,* [2]*Sorbonne Université,* [3]*Keele University,* [4]*MRC Harwell Institute,* [5]*UCL Ear Institute, University College London*

Category: Inner Ear: Anatomy and Physiology

Background: Hearing is dependent on the cochlear inner hair cells (IHCs) transducing sound into changes in receptor potential and relaying this information to the central auditory pathway via the spiral ganglion neurons (SGNs). Recent single-cell RNA sequencing experiments have identified several new genes that are selectively expressed in SGNs, including brain specific angiogenesis inhibitor 1 (BAI1). BAI1 is a large (200kDa) G-protein coupled receptor encoded by the ADGRB1 (adhesion g-protein coupled receptor B1) gene, and has been implicated in multiple cellular processes including suppressing angiogenesis and facilitating synaptogenesis. BAI1 deficient mice show memory and learning defects, susceptibility to seizures, and thinning of the post-synaptic density (PSD) at excitatory synapses. BAI1 was also recently identified as a potential hearing loss gene by the international mouse phenotyping consortium (IMPC), though the mechanism of this hearing loss was unknown.

Methods: To investigate the role of BAI1 in the cochlea, the ADGRB1 gene was disrupted via the replacement of exons 3 and 4 with a LacZ cassette (Bai1 tm2b/tm2b), which also facilitated the visualisation of Bai1 expression in the cochlea via X-Gal staining. The role of BAI1 in hearing function was investigated using in vivo auditory brainstem response (ABR) and distortion product oto-acoustic emission (DPOAE). The function of Bai1 in the cochlea was investigated with single-cell electrophysiology, immunolabelling, ex vivo calcium imaging and whole-cochlear RNAseq.

Results: Bulk whole-cochlear RNAseq revealed that Bai1 tm2b/tm2b selectively disrupted the long isoform of the protein, and X-Gal staining identified Bai1 expression as specific to the SGNs. ABR recordings revealed substantial hearing loss from post-natal day (P) 15, whereas DPOAE thresholds, and thus the function of the outer hair cells, were unaffected. Electrophysiology data indicated no change in mechanotransduction, potassium currents, calcium currents, or synaptic release in Bai1 tm2b/tm2b hair cells. However, immunolabelling experiments revealed the near complete absence of GluA2-4 glutamate receptors at the SGN afferent terminals of Bai1 tm2b/tm2b mice. RNA sequencing has also revealed that the absence of functional Bai1 transcriptionally phenocopies many changes found in the VGlut3 knockout mice.

Conclusions: BAI1 is expressed in SGNs within the cochlea and the long isoform is required for the localisation of GluA2-4 to the afferent terminal postsynaptic density, resulting in early onset hearing loss when disrupted.

Functional Development of Embryonic IHCs and SGNs in the Mouse Cochlea

Jingjing Zhao[1], Yige Li[1], Fang Wang[1], Geng-Lin Li*[1]

[1]*Eye and ENT Hospital, Fudan University*

Category: Inner Ear: Anatomy and Physiology

Background: In the cochlea, inner hair cells (IHCs) connect to axonal terminals of type I spiral ganglion neurons (SGNs) through ribbon synapses where auditory signals are encoded and processed with extraordinary precision. It is well established these ribbon synapses undergo dramatic changes from birth to adulthood, but how they emerge over the embryonic stage of development is poorly understood.

Methods: To study cochlear IHCs and SGNs in the late embryonic stage, we performed patch-clamp recording in both cell types in the mouse cochlea in vitro, before (embryonic, E16-E19) and after birth (neonatal, P0-P3). Patch-clamp recordings on IHCs were made in the whole-mounted organ of Corti, and exocytosis from IHCs was monitored with whole-cell capacitance measurement. Separately, patch-clamp

recordings on SGNs were performed in cochlear slices prepared from a vibrotome, and step currents were injected under the current-clamp mode to probe their excitability and spiking pattern.
Results: Compared to their neonatal counterparts, embryonic IHCs had a significantly smaller Ca2+ current (~ 60%) and released significantly less synaptic vesicles (~ 25%). Furthermore, we found exocytosis from embryonic IHCs was Ca2+- and Otof-dependent, suggesting that it could occur physiologically in the developing cochlea. For SGNs, while those at the embryonic stage showed homogeneity in that they fired only a single spike upon current injection, neonatal ones exhibited diversity with two additional types that fired multiple spikes. Importantly, we found that compared to their neonatal counterparts embryonic SGNs were significantly more excitable, owing to a more depolarized resting membrane potential (~ 10 mV) and a higher input resistance (~ 30%). Lastly, we found that embryonic SGNs had a significantly smaller Na+ (~ 20%) and K+ current (~ 50%), when compared to their neonatal counterparts.
Conclusions: Taken together, we conclude that IHCs and SGNs in the embryonic cochlea are co-developed in a highly coordinated manner that small and sporadic exocytosis from IHCs can drive highly excitable SGNs to fire spikes and therefore finely tune the ribbon synapses to acquire exquisite functions over development.

The Biophysical Differences Between Murine Type I and Type II Spiral Ganglion Neurons

Nathaniel Nowak*[1], Radha Kalluri[1]

[1]*University of Southern California*

Category: Inner Ear: Anatomy and Physiology
Background: Spiral ganglion neurons (SGNs) relay auditory information transduced by cochlear hair cells to the central nervous system. 95% of SGNs are type I SGNs which send an unbranched dendrite to a single inner hair cell; whereas, the remaining 5% (type II SGNs) have long dendrites that contact several outer hair cells. In vivo recordings of type I SGNs show that they encode specific frequencies of sound at wide ranges of sound intensity. Contrastingly, due to their scarcity and small caliber fibers, there has only been one in vivo recording of a putative type II SGN which did not respond to sound. Instead, type II SGNs are hypothesized to detect cochlear damage. More characterization of the biophysical properties of type II SGNs, however, is required to further assess type II SGN function.
Methods: To identify type II SGNs we used two mouse lines in which a fluorescent reporter, tdTomato, is either preferentially expressed in type II SGNs but not type I SGNs (SERTCre;Ai14) or where expression is mixed but the tdTomato-positive neurons have an increased probability of being type II SGNs and the tdTomato- negative neurons are exclusively type I SGNs (Tac1Cre;Ai14). Therefore, SERTCre;Ai14+ SGN were considered putative type II SGNs and SERTCre.Ai14-and Tac1Cre;Ai14- SGNs pooled together were considered putative type I SGNs. Spiral ganglia from SERTCre;Ai14 and Tac1Cre;Ai14 mice were dissected, enzymatically treated, and triturated to isolate their somata. Perforated patch-clamp recordings were carried out on these isolated somata to characterize ionic currents and firing properties while alleviating space-clamp issues. Multivariate discriminant analysis was applied to evaluate if differences in electrophysiological properties could classify putative type I and II SGNs.
Results: Putative type II SGNs (n=10) had smaller whole-cell conductances that inactivated as compared to putative type I SGNs (n=21). Putative type II SGNs were also significantly more depolarized and less likely to fire action potentials in response to current pulses at baseline. Moreover, putative type II SGNs could not phase lock to trains of fast current pulses as well as putative type I SGNs. Lastly, the observed differences in whole-cell conductances, resting membrane potential, and number of action potentials induced by a current pulse, between putative type I and type II SGNs were sufficient to classify SGNs with a 97% success rate (R2=0.83) using multivariate discriminant analysis.
Conclusions: Recordings from type II SGNs have previously relied on random chance or by targeting their thin dendrites. By exploiting a fluorescent reporter in type II SGNs, we demonstrate the utility of molecularly defined methods to identify and target SGN types. These results demonstrate clear differences in intrinsic excitability and whole-cell currents between type I and II SGNs. Additionally, similar fluorescence strategies can be employed to select specific subsections of SGNs in future studies.

Objective Thresholding of the Auditory Brainstem Response Using Cross-Correlation Across Subaverages

Luke Shaheen*[1], Yoojin Chung[1], Brad Buran[2], Seth Koehler[1]

[1]*Regeneron Pharmaceuticals,* [2]*Oregon Health and Science University*

Category: Inner Ear: Anatomy and Physiology

Background: The auditory brainstem response (ABR) is an essential diagnostic indicator of overall cochlear health, used extensively both in animal model research and in human clinical studies. The key quantification of the ABR is threshold, the lowest sound level that elicits a response. Because the morphology of the ABR shifts with stimulus level and because the signal-to-noise ratio is low, estimation of threshold is not straightforward. Although several algorithmic approaches have been proposed, the current standard practice remains visual inspection of ABR waveforms as a function of stimulus level. This technique requires extensive training to ensure consistency and to minimize bias across raters. It is also time-intensive.

Methods: We developed an algorithm for determination of threshold based on the cross-correlation of two independent averages of responses to the same stimulus. The algorithm was tested on a database of ~2000 mouse ABRs, collected in response to 5 ms tone-pips of alternating polarity with 512 trials per level. The individual responses to each tone-pip were saved, enabling us to randomly split the trials into two groups, each containing 256 trials, and calculate the median waveform for each group, followed by the normalized cross correlation between these median waveforms. This process was repeated 500 times to obtain a bootstrapped cross-correlation distribution. The mean values of these distributions were computed for each level and fit with a sigmoid to find threshold. Results were compared with thresholds estimated by expert human raters.

Results: Algorithmic thresholds were highly similar to thresholds determined by expert human raters on a large pool of mouse data, with 94% of judgements within ±10 dB. This performance was better than that of several published algorithms on the same dataset. Across several gene therapy efficacy and tolerability studies, algorithmic thresholds yielded the same conclusion as that of expert human raters while decreasing variability across animals in the same treatment group.

Conclusions: The proposed algorithm is accurate, consistent, and objective. It has fully replaced estimation of mouse ABR thresholds by expert human raters at Decibel, saving time and increasing objectivity.

Encoding of Phasic and Tonic Signals by Non-Quantal and Quantal Transmission in the Vestibular Periphery

Dyllan Zhou[1], Wesley Schoo[1], Takashi Kodama[2], Sascha du Lac[1], Elisabeth Glowatzki[1], Soroush Sadeghi*[1]

[1]*Johns Hopkins University,* [2]*Johns Hopkins University, Tokyo Women's Medical University*

Category: Inner Ear: Anatomy and Physiology

Background: Phasic and tonic vestibular nerve afferents provide information about different components of head movements and the gravity vector. The most phasic afferents receive inputs from unique afferent terminals, the calyces that ensheath type I hair cells (HCs) in the central/striolar region of the cristae/maculae. The most tonic fibers receive inputs to their bouton terminals from type II HCs. The rest of the afferents with in-between properties receive inputs from both HCs. Type I HC – calyx synapse, besides quantal transmission, uses a unique non-quantal (NQ) transmission that is faster than the quantal (vesicular) transmission while type II HC – bouton synapse most likely uses quantal transmission. The functional contributions of quantal and non-quantal transmission to coding vestibular signals are not clear. Here, we investigated their contributions to vestibular nerve firing and behavioral responses to vestibular stimuli.

Methods: We used (1) vestibular sensory evoked potentials (VsEP) to evaluate in vivo responses of phasic otolith afferents to fast linear head movements (0.5 – 2 g/ms), (2) rotational vestibulo-ocular reflex (VOR, 0.2 – 6 Hz, 40 – 80 deg/s), and (3) righting reflex (contact and drop) tests to evaluate tonic otolith afferents that most likely encode changes in the gravity vector. For VsEP and VOR measurements we either used mice that lack quantal release of glutamate (vesicular glutamate transporter-3 knockout, vglut3 KO or we used intratympanic (IT) injection of NBQX (AMPA receptor antagonist) in WT mice.

Results: The vglut3 KO mice are known to be deaf, have no startle reflex, and show no auditory brainstem responses (ABR). Surprisingly, in both vglut3 KO mice (P30 – P60) and in WT mice post-NBQX injection (P30 – P60), VsEP and rotational VOR responses were normal, suggesting a role for NQ transmission between type I HC – calyx in generating these responses (5-10 mice in each group). We then used 'contact righting reflex' and 'drop test' in WT mice (before and after IT injection of NBQX, n = 2). Both tests depend on detection of gravity and are most likely encoded by tonic afferents that receive inputs mainly through quantal transmission. For the drop test, while control WT mice turned immediately after release and landed on all four legs, NBQX-injected mice did not turn so that they landed on their backs or sides. For contact righting reflex, control mice rolled and corrected their position relative to gravity in less than 5 s, but those injected with NBQX typically stayed in the upside-down position and moved around for 60 – 90 s before rolling to correct their position relative to gravity.
Conclusions: Together, the above results show that glutamate-independent NQ signals could effectively encode fast/phasic movements (normal VsEP and VOR), but quantal transmission is required for gravity dependent/tonic responses.

Podium Session 4 - Clinical Otology and Pathology: Human and Animal Studies

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

Platinum Salon 6

Mouse Models of DFNA78 Demonstrate Importance of Exon 21 of SLC12A2 for Hearing

Hideki Mutai*[1], Yukiko Kuroda[2], Shinobu Noji[2], Saki Ichikawa[3], Koichi Matsuo[2], Satoshi Tanaka[3], Naoyuki Kataoka[3], Tatsuo Matsunaga[1]

[1]*NHO Tokyo Medical Center,* [2]*Keio University School of Medicine,* [3]*The University of Tokyo*

Category: Genetics A: Genomics and Gene Regulation
Background: We have identified SLC12A2 as a gene responsible for autosomal dominant nonsyndromic hearing loss, DFNA78 (PubMed 32294086). DFNA78 patients exhibit congenital, severe to profound hearing loss. SLC12A2 encodes the Na+, K+, 2Cl- cotransporter (NKCC1). To date, all the reported pathogenic variants of SLC12A2 responsible for DFNA78 are amino acid-replacing missense variants on exon 21 or its splicing variant. SLC12A2 is predominantly expressed in the stria vascularis of mammalian cochlea and considered to be essential for the production and homeostasis of endolymph. In this study, we generated mouse models carrying heterologous exon 21-skipping variants of Slc12a2 and studied their phenotypes. We also investigated the effects of exon 21 splicing variants at the acceptor site.
Methods: Mice with genetic variants targeting splice acceptor site of the exon 21 of Slc12a2 were generated by Crispr/Cas9 system. Mice were subjected to ABR measurement. The cochleae were subjected to nano-scale computed tomography (nano3DX, Rigaku), histochemical studies, bulk RNA-seq analysis, and quantitative RT-PCR. The genomic region of the exon 21 in Slc12a2 and the variants were subjected to minigene splicing assay.
Results: We generated two mouse strains carrying heterologous Slc12a2 variants (Em1 (NM_009194.3:c.2912-2A greater than G) and Em2 (c.2912-4_2913del)). Slc12a2Em2/Em2 mice showed absence of ABR responses at all sound frequencies (8, 16, and 32 kHz) at 4 weeks of age (4w), while Slc12a2Em2/+ mice showed no ABR threshold shifts. Nano-CT analysis and hematoxylin-eosin staining confirmed shrinking stria vascularis and presence of endolymphatic space in the Slc12a2Em2/Em2 cochleae at 4w. While deafness was consistent with those observed in Slc12a2-ko mice, the morphological alteration was distinct from Slc12a2-ko mice, which showed severely reduced endolymphatic space (PubMed 10369265, 10480906, 10401008, 24009395). RNA-seq analysis followed by qRT-PCR demonstrated upregulation of set of genes related to cell-cell adhesion and downregulation of those related to nervous system in the cochleae of Slc12a2Em2/Em2 mice.
In contrast to Slc12a2Em2/Em2 mice, Slc12a2Em1/Em1 mice showed no ABR threshold shifts, even though Em1 was assumed to be equivalent to the splice variant detected in a patient (NM_001046.3:c.2930-2A greater than G, PubMed 32294086). RT-PCR demonstrated that Em1 variant led to generation of a cryptic acceptor site at 9 bp downstream of the original site for exon 21. The truncated transcript was predicted to retain the

molecular function of Slc12a2 sufficiently for normal hearing in Slc12a2Em1/Em1 mice. Minigene assays demonstrated that single nucleotide difference between human and mouse at the 5' end of exon 21 was critical for robustness of splicing of the exon 21.

Conclusions: Hearing loss in Slc12a2Em2/Em2 mice indicates that the exon 21, which encodes a part of cytoplasmic region of SLC12A2, is essential for normal hearing.

Risk Factors Determining Congenital Hearing Loss and Hearing Evolution in Children With Congenital Cytomegalovirus Infection

Elise De Cuyper*[1], Frederic Acke[1], Annelies Keymeulen[3], Els De Leenheer[1], Helen Van Hoecke[1], Elizaveta Padalko[3], An Boudewyns[4], Annick Gilles[4], Marie Muylle[5], Rudolf Kuhweide[5], Liesbeth Royackers[6], Christian Desloovere[6], Margriet Verstreken[7], Isabelle Schattemans[7], Ingeborg Dhooge[1]

[1]*Ghent University/Ghent University Hospital,* [3]*Ghent University Hospital,* [4]*Antwerp University Hospital,* [5]*Sint Jan Hospital, Bruges,* [6]*University Hospitals of Leuven,* [7]*GZA Hospitals Campus Sint Augustinus*

Category: Clinical Otolaryngology and Pathology

Background: Approximately 15% of newborns with a congenital cytomegalovirus (cCMV) infection has hearing loss at birth. Moreover, the hearing function of 10% of the children will progress during childhood. We aimed to assess risk factors for congenital hearing loss, hearing improvement, hearing deterioration, and late-onset hearing loss.

Methods: Long-term multicentric data have been collected for over 15 years in the Flemish CMV registry (Belgium). Patients were included after a confirmed diagnosis of cCMV infection and known hearing status at birth. To assess risk factors determining hearing evolution, only untreated children were selected with minimal 4-year audiological follow-up. The importance of gestational characteristics, clinical findings, timing of seroconversion, and viral load was investigated using effect sizes and/or logistic regression.

Results: A total of 1033 children were included, of whom 416 (40.3%) were diagnosed with symptomatic cCMV infection and 617 (59.7%) with asymptomatic cCMV infection. Congenital hearing loss was present in 159 newborns (15.4%). The regression model revealed three independent risk factors for congenital hearing loss: petechiae at birth (adjusted odds ratio [aOR], 6.7; 95%CI, 1.9-23.9), periventricular cysts on magnetic resonance imaging (MRI; aOR, 4.6; 95%CI, 1.5-14.1), and seroconversion in the first trimester (aOR, 3.1; 95%CI, 1.1-9.3). Lower viral loads were seen in patients with normal hearing compared with those with congenital hearing loss (median [IQR] viral load, 447.0 [39.3-2345.8] copies per milliliter of sample [copies/mL] vs 1349.5 [234.3-14 393.0] copies/mL; median difference, −397.0 [95%CI, −5058.0 to 174.0] copies/mL).

Amongst the 387 untreated children with long-term audiological follow-up, ninety percent (701/774) of the ears showed stable hearing (normal hearing or stable hearing loss since birth) over time. Late-onset hearing loss (normal hearing at birth followed by hearing loss) was present in 43 ears (43/683 [6.3%]). Among children with hearing loss present at birth, 70.6% (24/34) of the ears deteriorated and 6.6% (6/91) of the ears improved. Prematurity was associated with a higher chance of hearing improvement (OR, 12.80; 95% CI, 2.03-80.68). Late-onset hearing loss was more prevalent in a first trimester infection (OR, 10.10; 95% CI, 2.90-34.48). None of the 104 ears of children with a third trimester seroconversion developed late-onset hearing loss.

Conclusions: Newborns with cCMV infection and petechiae at birth, periventricular cysts on MRI, or a seroconversion in the first trimester have a higher risk of congenital hearing loss. Ongoing audiological follow-up for untreated children with congenital hearing loss is important as the majority will demonstrate hearing deterioration. The timing of seroconversion impacts the risk of developing late-onset hearing loss. These insights can aid in parental counselling, patient stratification, and follow-up. Future research should focus on the effect of treatment on hearing evolution and the study of eventual new risk factors in patients at high risk to develop hearing loss.

Molecular Specializations Underlying Phenotypic Differences in Hair Cells of Zebrafish and Mice

Kacey Yamane*[1], Kim Giffen[2], Huizhan Liu[3], Yi Li[4], Kenneth Kramer[1], David He[1]

[1]*Creighton University,* [2]*Medical College of Georgia,* [3]*Creighton University School of Medicine,* [4]*Beijing Tongren Hospital*

Category: Genetics A: Genomics and Gene Regulation

Background: Hair cells (HCs) are the sensory receptors of the auditory and vestibular systems in the ears of all vertebrates. HCs transduce mechanical stimuli into electrical activity. Although all HCs have the hallmark of the stereocilia bundle for mechanotransduction, HCs in nonmammals and mammals are different in many ways through specialization in the apical, basolateral and synaptic membranes. HCs of non-mammals, such as zebrafish, are electrically tuned to specific frequencies and possess an active process in the stereocilia bundle to amplify sound signals. Mammalian HCs, in contrast, are not electrically tuned and achieve amplification by somatic motility of outer HC. To understand the genetic mechanisms underlying their differences, we examined transcriptomes of adult zebrafish and mammalian HCs, with a focus on genes related to HC specializations.

Methods: A comparative analysis of zebrafish GRCz10 and mouse GRCm38.5 gene assemblies with Ensemble Biomart generated the comprehensive list of protein-coding gene orthologs used for this study. We analyzed the differential and shared expression of genes, quantified in our previous published RNA-seq datasets, in zebrafish inner ear sensory epithelia HCs and mouse inner and outer HCs. Validation of some expressed genes was confirmed by PCR and in situ hybridization. Finally, nonlinear capacitance of solitary zebrafish and mouse HCs were acquired using established electrophysiology techniques.

Results: A total of 10,995 of 17,498 zebrafish gene orthologs were expressed in zebrafish HCs, and 7,591 of these were commonly expressed in mouse inner and outer HCs. There were 174 genes expressed in both zebrafish HC and inner HCs, while 399 genes were expressed in zebrafish HCs and outer HCs. We showed that both zebrafish and mouse HCs express Tmc1, Lhfpl5, Tmie, and Cib2. Tmc2 was not detected in adult mouse HCs but tmc2a and b and cib3 were highly expressed in zebrafish HCs. Mouse HCs express Kcnj13, Kcnj16, and Kcnq4, which were not detected in zebrafish HCs. Both Chrna9 and Chrna10 were expressed in mouse HCs. In contrast, Chrna10 was not expressed in zebrafish HCs. Slc26a5, highly expressed in mammalian outer HCs, was weakly expressed in zebrafish HCs. Measures of nonlinear capacitance confirmed absence of electromotility and capacitance response in zebrafish HCs.

Conclusions: Our analyses unveil substantial differences in gene expression patterns that may explain different phenotypes of zebrafish and mouse HCs. Our datasets also establish a framework for future characterization of genes expressed in HCs in these two species as well as for the study of HC evolution from non-mammals to mammals.

SPI-1005 Reduces Tobramycin Ototoxicity in a Phase 2 Randomized Controlled Trial of Cystic Fibrosis Patients

Jonathan Kil*[1], Emily Harruff[1], Joel Mermis[2], Daniel Rosenbluth[3], Perry Brown[4], Douglas Conrad[5], Raksha Jain[6], Patrick Flume[7]

[1]*Sound Pharmaceuticals, Inc.,* [2]*KUMC,* [3]*Washington University,* [4]*St Luke's Cystic Fibrosis Center,* [5]*UCSD,* [6]*UTSW,* [7]*MUSC*

Category: Clinical Otolaryngology and Pathology

Background: Aminoglycosides are commonly used to treat infections in people-with-CF (pwCF) and are highly ototoxic. Our recent observational Ph1b study showed an ototoxicity rate of 89% to 93% (2-and 4-weeks) after receiving a standard course of IV-tobramycin. Our interventional Ph2b trial is testing SPI-1005, a novel anti-inflammatory that mimics and induces glutathione-peroxidase-1 activity, in similar IV-tobramycin receiving pwCF, to reduce ototoxicity. In other studies, SPI-1005 treatment has been shown to improve hearing and tinnitus after 21-28 days of oral treatment. This is the first statistical analysis comparing the ototoxicity rates between the two pwCF studies prior to unblinding.

Methods: pwCF were consented before standard treatment including ≥10-days of IV-tobramycin (10mg/kg/d) for acute pulmonary exacerbation. Adults received baseline pure tone audiometry (PTA) within 48hrs and were then randomized to oral SPI-1005 treatment (0, 200, 400 or 600mg BID) x21 days (1:1:1:1) within 72hrs. Follow-up audiometry occurred at 2-and 4-weeks post-IV tobramycin treatment and ASHA criteria for

ototoxic change were calculated: 1x20dB, 2x10dB or 3x5dB loss in the same ear at adjacent frequencies. Additional audiologic methods included words-in-noise (WIN) and distortion-product-otoacoustic-emissions (DPOAEs). Two patient-reported-outcomes (PRO) of tinnitus severity-Tinnitus Functional Index (TFI) and Tinnitus Loudness (TL) and vertigo severity-Vertigo Symptom Scale (VSS) were included. Fisher's Exact test was used to compare rates of ototoxicity.
Results: Ph2b adults (N=40) were 30 yo (19-56) and received 15.1 days (10-26) of IV-tobramycin on average. Ph1b adults (N=20) were 31 yo (18-59) and received 14.8 days (10-22) of IV-tobramycin on average. At 2-weeks post, the rate of ototoxicity was 73% in Ph2b vs 89% in Ph1b, an 18% relative reduction, p=0.2704. At 4-weeks post, the rate of ototoxicity was 63% in Ph2b vs 93% in Ph1b, a 32% relative reduction, p=0.0384. For WIN, the rate was 21% vs 17% and 31% vs 40%. For DPOAE, the rate was 68% vs 82% and 67% vs 80%. For TFI, the rate was 21% vs 12% and 10% vs 8%. For TL, the rate was 11% vs 12% and 0% vs 15%. For VSS, the rate was 4% vs 0% and 6% vs 8%. SPI-1005 was well tolerated and no DDIs were noted.
Conclusions: Overall Ph2b showed reductions in ototoxicity at 2-and 4-weeks after IV-tobramycin treatment when compared to Ph1b. This suggests a clinically relevant benefit to study drug since up to 75% of pwCF were randomized to active. No significant differences in age, duration of IV-tobramycin, concomitant medications, respiratory status, or baseline hearing loss were observed between the studies. SPI-1005 may prove to be the first drug to demonstrate safety and efficacy in preventing and treating aminoglycoside ototoxicity. An unblinded analysis will determine which active groups are contributing to the reduction in ototoxicity.

Comparison of Round Window Electrocochleography Across Pediatric Subjects Receiving Cochlear Implants With Auditory Neuropathy Spectrum Disorder (ANSD), Enlarged Vestibular Aqueduct (EVA), and Sensorineural Hearing Loss (SNHL).

Douglas Fitzpatrick*[1], William Dunn[1]

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

Category: Clinical Otolaryngology and Pathology
Background: There is a wide range of etiologies of hearing loss across children that ultimately receive a cochlear implant. It might be expected that these differing etiologies would have different effects on the cochlea and auditory nerve leading to hearing loss. These differences can be explored by electrocochleography (ECochG). Here, we applied ECochG to describe the range of hair cell and neural function between and within three groups with hearing losses clinically characterized as due to ANSD, EVA and SNHL.
Methods: The recordings were performed intraoperatively just prior to cochlea implantation. A stainless-steel recording electrode was placed in the round window niche, with reference on the opposite mastoid and ground on the high forehead. The stimuli were tones of 250-4000 Hz delivered at the high level of 90 dB nHL to each frequency and level series to some frequencies. Data analysis extracted amplitudes and waveshapes of the various ECochG components, including the cochlear microphonic (CM), compound action potential (CAP), auditory nerve neurophonic (ANN) and summating potential (SP).
Results: The largest responses overall were obtained to subjects with ANSD, followed by those of EVA and then SNHL, although these two were only slightly different from each other. The frequency range of the CM was much greater in ANSD subjects, but to high frequencies (greater than 1500 Hz) these responses were rarely associated with a CAP while to low frequencies there was a wide range of neural response in the form of the ANN. The frequency ranges for significant responses and 50% cut-off frequencies for the AC responses (CM alone for high frequencies, with ANN, if present, to low frequencies) were to lower frequencies overall in EVA and SNHL subjects with distributions similar to each other. The SP for ANSD subjects was predominantly negative to high frequencies but mixed polarity across cases to low frequencies. For EVA and SP subjects the magnitudes of the SPs were smaller and the proportion with negative and positive polarities were evenly distributed.
Conclusions: The periphery of ANSD subjects is characterized by functional hair cells to high frequencies with limited ability to drive auditory neurons synchronously to produce a CAP, while to low frequencies, neural activity can be pronounced in some cases. EVA responses are on average intermediate in size between EVA and SNHL, but these two groups show distributions of neural activity and of the SP that are similar to

each other. Distinctive features of EVA still being characterized include different patterns of SP morphology, which are less amenable to a simple numeric estimation.

AC102-201: A Phase 2 Clinical Trial Comparing the Efficacy of AC102, an Innovative Small Molecule, to Corticosteroids in Patients With Idiopathic Sudden Sensorineural Hearing Loss

Christin Galetzka[1], Ronald J.E. Pennings[2], Cris Lanting[2], Alena Meis[1], Maika Friedrich[1], Miroslawa Gachowska[1], Stefan Plontke[3], Reimar Schlingensiepen*[1]

[1]*AudioCure Pharma GmbH,* [2]*Radboud University Medical Center,* [3]*Martin Luther University Halle-Wittenberg*

Category: Clinical Otolaryngology and Pathology

Background: To date, there is no approved medication for the treatment of Sudden Sensorineural Hearing Loss (SSNHL). International guidelines recommend treatment with corticosteroids, but the therapeutic benefit remains unclear. AC102 is a new candidate for the treatment of SSNHL which significantly outperformed corticosteroids in preclinical models of hearing loss. In a Phase 1 clinical trial, a single intratympanic (i.t.) injection of AC102 was safe and well-tolerated. Treatment Emergent Adverse Events (TEAEs) were mild and associated with the injection procedure (pain) or the administered volume (short-term conductive hearing loss, suggesting that the formulation resides over days in the middle ear to release AC102). Healthy volunteers showed no other alterations in audiological or vestibular function. Therefore, a Phase 2 study to evaluate AC102 for the treatment of SSNHL was initiated.

Methods: AC102-201 is a randomized and blinded two-arm Phase 2 study in which AC102 is administered as a single intratympanic injection of up to 800µL and compared to oral corticosteroid treatment (prednisolone tablets, 60mg/day over 14 days followed by tapering down). Up to 210 patients with idiopathic SSNHL are being enrolled from up to 50 study sites across 8 European countries. The primary endpoint is the improvement of the hearing threshold (average of the three most affected consecutive frequencies) tested by pure tone audiometry (PTA) from baseline to Day 28. Secondary endpoints aim to further assess PTA and speech recognition up to three months as well as quality of life, tinnitus, and vertigo.

Results: The first Data Safety Monitoring Board (DSMB) Meeting took place after 20 patients had completed Day 28. The average age of the enrolled patients was 55 years, 54% were male while 46% were female. The average baseline PTA was 77 dB (3 most affected consecutive frequencies). Main TEAEs included ear discomfort after the injection procedure as well as hyperglycemia and palpitation. No other clinically significant abnormalities were observed in laboratory, vital signs, ECG or physical examination measures. Importantly, no clinically significant results were noted in otoscopy or tympanometry measures. The DSMB reported no safety concerns and recommended the continuation of the AC102-201 trial.

Conclusions: Preclinical results show great potential for AC102 to treat SSNHL as it outperformed corticosteroids, the current standard of care. AC102 was shown to be safe and well tolerated in a First-In-Human Phase 1 study in healthy volunteers. This positive safety profile in healthy volunteers was confirmed in the first treated patients of an ongoing Phase 2 study. After a thorough review of all available data by the DSMB, there are no safety concerns to continue recruitment of patients with idiopathic SSNHL in the AC102-201 clinical trial.

Machine Learning-Based Prediction Model for Longitudinal Outcomes in GJB2-Related Sensorineural Hearing Impairment

Pey-Yu Chen*[1], Ta-Wei Yang[2], Yi-Shan Tseng[2], Cheng-Yu Tsai[3], Chiung-Szu Yeh[2], Tien-Chen Liu[4], Yen-Hui Lee[5], Pei-Hsuan Lin[4], Ting-Chun Lin[2], Yu-Jen Wu[2], Ting-Hua Yang[4], Yu-Ting Chiang[6], Jacob Shu-Jui Hsu[2], Chuan-Jen Hsu[7], Pei-Lung Chen[4], Chen-Fu Chou[2], Chen-Chi Wu[5]

[1]*MacKay Memorial Hospital,* [2]*National Taiwan University,* [3]*National Taiwan University Hospital; National Taiwan University College of Medicine,* [4]*National Taiwan University Hospital,* [5]*National Taiwan University*

Hospital Hsin-Chu Branch; National Taiwan University Biomedical Park Hospital; National Taiwan University Hospital, [6]National Taiwan University College of Medicine, [7]Taichung Tzu-Chi Hospital,

Category: Clinical Otolaryngology and Pathology

Background: Pathogenic variants in GJB2 are the most common genetic cause of sensorineural hearing impairment (SNHI). There is increasing evidence that hearing in patients with GJB2 variants is progressive. However, the rate of progression is difficult to estimate based on traditional linear regression, which is inadequate to reflect the non-linear hearing change in the real world. The aim of our study is to build an accurate predictive model for this population using big data through machine learning, enabling personalized medical planning.

Methods: We retrospectively collected hearing impaired patients with bi-allelic GJB2 pathogenic variants from 2005 to 2022 at a tertiary referral center. We used a combination of different data preprocessing protocols and computational algorithms to build prediction models. The data set was divided into a training set and a validation set at a ratio of 5:1. The performance of the models was evaluated using the mean absolute error (MAE), which refers to the discrepancy between the predicted and actual hearing levels.

Results: A total of 449 patients (including 247 males and 202 females) with 3,148 audiograms were available for deep learning analysis after data augmentation. The mean age at first visit was 7.8 ± 8.2 years (range 0.1-38.0 years), and the mean follow-up was 3.0 ± 2.7 years (range 0.1-25.8 years). Of the patients in our cohort, 81% were homozygous for p.V37I, 13% had compound heterozygosity for p.V37I, and the remaining 6% had other compound heterozygous variants.

All of the machine learning models we evaluated demonstrated progression of SNHI, independent of age, sex, and GJB2 genotypes. Performance improved as the amount of prior audiologic data increased. The long short-term memory model provided the best prediction of hearing level with an average MAE of 3.44 dB HL with five audiological inputs. The models using at least three audiological inputs could achieve optimal accuracy (MAE less than 5 dB) for a prediction period of two years and acceptable accuracy (MAE less than 10 dB) for up to four years.

Conclusions: We have developed a machine learning prognostic model that approximates the realistic hearing outcome of GJB2-related SNHI. This model shows promise for guiding hearing monitoring strategies and designing individualized treatment plans. More longitudinal audiological records are needed to extend the prediction period.

Longitudinal Variability in Vestibular Evoked Myogenic Potentials and Endolymphatic Hydrops for Patients With Hearing Instability

Julia Telischi*[1], Jennifer Chisholm[2], Christopher Zalewski[2], Julie Christensen[2], Noelle Allemang[2], Anhelina Bilokon[2], Hui Cheng[3], John Butman[4], Carmen Brewer[2], Michael Hoa[5]

[1]*University of Miami Miller School of Medicine,* [2]*Audiology Unit, National Institute on Deafness and Other Communication Disorders, National Institutes of Health,* [3]*Bioinformatics and Biostatistics Collaboration Core, National Institute on Deafness and Other Communication Disorders, National Institutes of Health,* [4]*National Institutes of Health,* [5]*Auditory Development and Restoration Program, National Institute on Deafness and Other Communication Disorders, National Institutes of Health*

Category: Clinical Otolaryngology and Pathology

Background: Hearing Instability (HI) disorders such as Meniere's disease, autoimmune inner ear disease, and sudden sensorineural hearing loss are characterized by endolymphatic hydrops (EH). This increase in endolymphatic fluid volume within the inner ear space can affect both cochlear and vestibular structures. The purpose of this work is to evaluate variability in cervical and ocular Vestibular Evoked Myogenic Potentials (cVEMP and oVEMP) testing over time in patients with HI to determine if variability in vestibular test results correlates to clinical presentation and disease status.

Methods: A cohort of 14 patients with HI was evaluated at 3-6 month intervals with MRI and audiovestibular testing. Presence of EH was identified at each visit by review of contrast-enhanced delayed FLAIR MRI images by an experienced neuroradiologist (JB). Threshold changes between sequential audiograms were used to determine hearing stability: hearing was labeled as unstable if a threshold changed by 20 dB for at least one frequency or by 15 dB for at least two consecutive frequencies. Vestibular testing included cVEMP and

oVEMP recordings from 500Hz and 1000Hz tone burst stimuli and at 750Hz when the VEMP response was present at only 500 or only 1000Hz. Amplitude and latency of VEMP response were recorded and frequency/amplitude ratios (FAR) were determined using 500 and 1000 Hz. Changes in VEMP responses were examined for all ears in HI patients in comparison to hearing stability and EH status over time.
Results: Preliminary analysis suggests that patients with HI exhibit less FAR variability in cVEMP responses when hearing is unstable with FAR variance of 0.15 in stable ears and 0.0049 in unstable ears, although this difference was not statistically significant (p=0.055). Less difference in cVEMP response variability was seen when hydropic ears were compared to non-hydropic ears (0.12 vs 0.15). OVEMP responses had more similar variance across stable and unstable ears (0.49 vs 0.55) and hydropic and non-hydropic ears (0.42 vs 0.47). Absolute latency and amplitude values were also compared, with significant differences seen only in oVEMP N1 and P1 latency at 500Hz in hydropic vs non-hydropic ears (N1: p=0.001, P1: p=0.00058) and in stable vs unstable ears (N1: p=0.012, P1: p=0.011).
Conclusions: Patients with HI disorders show decreased variability in VEMP during episodes of EH and hearing instability. However, the differences in variance between stable and unstable ears were not statistically significant in the context of fewer data in the unstable groups. Even so, these findings suggest that vestibular testing may offer additional objective information for clinicians when monitoring patient status and response to therapy and investigations should continue in this area to further clarify the relationship between VEMP responses and hearing stability.

Young Investigator Symposium 3 - Multisensory Signals Along the Auditory Processing Pathway

10:15 a.m. - 12:15 p.m.

Grand Ballroom Salon E

Multisensory Signals Along the Auditory Processing Pathway

Chair: Rebecca Norris*, UCL*
Co-Chair: Nathan Vogler*, University of Pennsylvania*

Cortical Mechanisms for Integration of Auditory and Olfactory Information

Nathan Vogler

University of Pennsylvania

Individual Abstract: In complex environments, the brain must integrate information from multiple sensory modalities, including the auditory and olfactory systems. However, little is known about how the brain integrates auditory and olfactory stimuli. Here, we investigated the mechanisms underlying auditory-olfactory integration using anatomy, electrophysiology, and behavior. We first used viral tracing strategies to investigate the circuits underlying auditory-olfactory integration. Our results demonstrate direct inputs to the auditory cortex (ACx) from the piriform cortex (PCx), mainly from the posterior PCx, suggesting an anatomical substrate for olfactory integration in ACx. We next developed an experimental system for delivering combinations of auditory and olfactory stimuli during in vivo electrophysiology and tested the effect of odor stimuli on auditory cortical responses to sound in awake mice. Odor stimuli modulate the responses of ACx neurons in a stimulus- and sound level-dependent manner, suggesting a neural substrate for olfactory integration in ACx. Finally, we trained mice on a Go/No-Go task, to assess how odor stimuli affect auditory perception and behavior. Odors modulate sound detection thresholds. Together, our findings suggest novel circuits for auditory-olfactory integration in the ACx.

Behavioral Origin of Sound-Evoked Activity in Mouse Visual Cortex

Celian Bimbard

University College London

Individual Abstract: Sensory cortices can be affected by stimuli of multiple modalities and are thus increasingly thought to be multisensory. For instance, primary visual cortex (V1) is influenced not only by images but also by sounds. Here we show that the activity evoked by sounds in V1, measured with Neuropixels probes, is stereotyped across neurons and even across mice. It is independent of projections from auditory cortex and resembles activity evoked in the hippocampal formation, which receives little direct auditory input. Its low-dimensional nature starkly contrasts the high-dimensional code that V1 uses to represent images. Furthermore, this sound-evoked activity can be precisely predicted by small body movements that are elicited by each sound and are stereotyped across trials and mice. Thus, neural activity that is apparently multisensory may simply arise from low-dimensional signals associated with internal state and behavior.

Temporal Integration of Audio-Visual Stimuli in the Mouse Superior Colliculus

Gaia Bianchini

The Francis Crick Institute

Individual Abstract: The relative timing of different sensory input signals is an important factor in multisensory integration and perception. When multiple forms of sensory stimuli arise from a single source, the degree of synchrony in the arrival of these stimuli can provide distance cues. The difference between the velocities of light and sound introduces a distance dependent lag for auditory signals with respect to visual information. Audio-visual delays therefore carry information about the distance to the stimulus source. Here we investigated the representation of audio-visual delays in the mouse superior colliculus (SC), a midbrain area that represents the location of visual and auditory targets topographically. Neuronal activity was recorded with Neuropixels probes in awake animals presented with visual and auditory stimuli with staggered onset times (ranging from 0 to 100ms). We found that 30% of the recorded neurons are modulated by both visual and auditory stimuli. These neurons exhibit a broad range of audio-visual delay preferences and exhibit nonlinear multisensory interactions. We used a random forest classifier to decode audio visual delays from population responses. We found differences in decoding performance across the anterior-posterior and the medio-lateral axes of the SC, with the highest accuracy in the posterior-medial region. This result suggests that there is a functional specialization of multisensory integration across anatomical regions of the SC. While separability of audio-visual delay representations is enhanced in the upper visual field, perceptual binding is favoured in the lower visual field.

Functional Specificity of Auditory Inputs to the Visual Cortex

Alexander Egea Weiss

The Francis Crick Institute

Individual Abstract: The brain continuously receives a wealth of sensory information and uses this information to guide behaviour. However, the process by which different sensory streams are integrated to form a unified percept remains poorly understood. Cortical regions dedicated to a specific sensory modality nonetheless receive input related to other modalities. The mouse auditory cortex sends numerous projections to the primary visual cortex and surrounding higher visual areas. These inputs may be crucial to the integration and binding of auditory and visual signals. Yet the exact nature of the auditory information carried by these inputs, and how they are organised within the visual cortices remains undetermined. We characterised the functional response properties of auditory cortex axonal projections to the mouse visual cortex using two-photon calcium imaging. We found that while all visual cortical areas studied receive axons tuned to a wide range of sound frequencies and sound source locations, the encoding of these features is not uniformly distributed across target regions. In particular, sound frequency varies along the anterior-posterior axes of the visual cortex. Furthermore, while information about lateral sound locations is conveyed widely across the visual cortex, information about sounds originating from the centre of the visual field is excluded from the primary visual cortex and most prominently conveyed to the rostrolateral visual cortex. Together, these results suggest that segregated auditory input to the visual cortex might lead to distinct multisensory representations across cortical areas.

Origin of Visual Information in Deep Layers of Mouse Auditory Cortex
Timothy Olsen
UCSF

Individual Abstract: Emerging evidence shows that neocortex is inherently multisensory, even in sensory areas previously thought to be unisensory. In primary auditory cortex (AC) of mice, many cells in the deep layers respond to visual stimulation as well as to auditory stimulation. The brain regions driving these visual responses must be established in order to manipulate these inputs and dissect the potential functions of visual information in the AC. Here we combine viral circuit tracing, facial movement analyses, and optogenetic silencing techniques to determine which visual areas send visual information to the AC.

Visual Signals in Ferret Auditory Cortex
Rebecca Norris
UCL

Individual Abstract: Multisensory integration is a fundamental property of mammalian sensory systems, allowing the brain to combine information across sensory modalities to facilitate perception and action in complex environments. Audio-visual integration is of particular interest to understanding auditory processing, as visual information influences auditory scene analysis, multi-modal object formation and speech processing. We now know that cross-modal integration happens at many stages of the processing hierarchy, including primary sensory cortices once thought to be unimodal. In this talk, I will present electrophysiological findings from our laboratory regarding visual signals in ferret auditory cortex: what they look like, where they come from, and how they can impact the representation of sounds across different behavioural states and contexts. I will additionally describe recent work to elucidate which features of visual stimuli are most important for driving audio-visual integration at the neural level.

Podium Session 5 - Aging

10:15 a.m. - 12:15 p.m.

Platinum Salon 5

The Proteomic Changes of Mouse Cochlear Nucleus of Mice During Aging

Weijun Zhou[1], Huihui Liu[1], Ruijie Cai[1], Ting Zhao[1], Ling Tong[1], Sidi Liu[1], Yunge Gao[1], Zhaoyan Wang[1], Hao Wu[1], Meijian Wang*[1]

[1]*Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine*

Category: Aging
Background: Age related hearing loss (ARHL), also known as presbycusis, is one of the most prevalent health conditions that affect the elders. However, there is limited knowledge regarding the alterations occurring in the cochlear nucleus (CN), which is the first central auditory nucleus, during ARHL. The previous evidences have showed the changes in CN associated with aging in perspectives of neurology, electrophysiology, immunology, et al. One or several protein markers were usually involved in them correspondently, albeit from different perspectives. To facilitate further research into the diverse mechanisms underlying ARHL in the CN, it would be beneficial to develop a comprehensive atlas of aging proteomic biomarkers. This resource would provide informative and systematic information for studying ARHL in the CN.
Methods: In this study, four age groups of C57BL/6J mice, namely 1, 3, 10, and 16 months of age, were utilized. The CN of the mice were dissected and the proteins were extracted. Subsequently, trypsin was employed to enzymatically digest the proteins into peptides, which were subsequently subjected to mass spectrometry (MS) scanning. The resulting MS data were then searched against the Uniprot database to identify and quantify the proteins.

Results: Groups of proteins changing during aging were revealed by comparisons of the proteomic profiles among the four age groups. The potential influences of these changes were addressed categorically based on the notations of gene ontology biological process (GOBP), Kyoto encyclopedia of genes and genomes (KEGG) pathway, as well as the proteins we were concerned with. The results indicated that the proteomic changes might affected multiple functions and components in the CN throughout the aging process.
Conclusions: The current study provided an aging atlas of proteomic biomarkers, which would be informative references to understand the ARHL mechanisms in CN in regards of signal pathways, electrophysiology functions, inflammatory processes, and et al. The pathology changes are gradually accumulating and eventually account for ARHL after some age. This indicates that compensating the proteomic changes might slow down the progress of ARHL.

Inhibitor of Mitosis Reduces Supporting Cell Regeneration in the Neonatal Mouse Cochlea

Julia Abitbol*[1], Sungwon Choi[1], Alan G. Cheng[1]

[1]*Stanford University*

Category: Regeneration
Background: While the adult mammalian cochlea does not proliferate or regenerate, the non-mammalian cochlea can regenerate following damage by conversion of supporting cells (SCs) to hair cells through mitotic and non-mitotic mechanisms. Selective SC subtypes (inner phalangeal cells (IPhCs) and inner border cells) from the neonatal mouse cochlea can regenerate after damage, however, the mechanisms by which this occurs remain unknown. We utilized a previously described mouse model (Lgr5DTR/+) that allows for specific ablation and subsequent regeneration of SCs to assess the mechanisms of SC regeneration.
Methods: DT (4 ng/g) was administered on postnatal day 1 (P1) to selectively ablate cochlear Lgr5+ SCs. Cochleae were harvested at P4 and P7. Lgr5DTR/+; GLASTCre/+; R26RTdTomato/+ mice were used to fate-map GLAST+ cells in the greater epithelial ridge (GER). Mice were injected with DT and tamoxifen (0.2 mg/g) at P1, and EdU at P3, P4, and P5. Lgr5DTR/+; Ki67Cre/+; R26RTdTomato/+ mice (DT at P1 and 0.25 mg/g tamoxifen at P3) were used to fate-map proliferating cells. Additionally, Lgr5DTR/+ and wildtype mice were administered DT at P1, dissected at P2, and their cochleae cultured as explants with EdU for 48hr and 72hr in vitro. Aphidicolin, an inhibitor of mitosis, was added to culture media. IPhCs were quantified by counting Fabp7 positive cells.
Results: In Lgr5DTR/+ mice, the human diphtheria toxin (DT) receptor is expressed specifically in inner pillar cells, inner phalangeal cells (IPhCs) and the third row of Deiters' cells. After DT injection at P1, Lgr5DTR/+ cochleae had a significant loss of IPhCs in the apical (80.6%±3.9%), the middle (79.2%±3.7%), and the basal (70.9%±4.1%) turns at P4 in vivo compared to controls. At P7, IPhCs significantly increased and regenerated to control levels in all cochlear turns (81.9%±1.89% in apical, 91.3%±2.1% in middle, and 86.4%±3.6% in base). Fate-mapping revealed that many regenerated IPhCs arose from GLAST+ cells in the GER region. In both Lgr5DTR/+; GLASTCre/+; R26RTdTomato/+ and Lgr5DTR/+; Ki67Cre/+; R26RTdTomato/+ mice, ~50% of regenerated IPhCs had undergone proliferation (Edu+, Fabp7+, or tdTomato+, Fabp7+ cells, respectively) in both apical and middle turns. In the base, only ~20% of regenerated IPhCs underwent proliferation. Aphidicolin treatment in vitro decreased the number of CyclinD1+ and EdU+ GER cells. Lastly, aphidicolin treatment in cultures significantly reduced IPhC regeneration in the apical and middle cochlear turns, but not in the base.
Conclusions: After selective SC damage in the neonatal cochlea, cells in the GER regenerate IPhCs through mitotic and non-mitotic mechanisms, with the former significantly contributing to and required for regeneration in the apical and middle cochlear turns. Ongoing experiments using live cell imaging and are under way to investigate the dynamics of GER cells regenerating SCs.

Age-Related Auditory Temporal Processing Deficits in Behavioral and Electrophysiological Measurements of Forward Masking

Chengjie Huang*[1], Taylor M. Evans-Beinke[1], Roksana Soleimanpour[1], Milena Costantino[1], Samira Anderson[1], Matthew J. Goupell[1]

[1]*University of Maryland - College Park*

Category: Aging

Background: Aging has profound effects on sensory systems where older adults can report problems in hearing despite having normal audiometric thresholds. The literature suggests that there are age-related reductions in inhibitory neural transmission that may lead to age-related temporal processing deficits. However, there is limited understanding of how these deficits interact with sound level effects, particularly with regards to the central auditory system. Therefore, we wanted to integrate these aspects to investigate whether aging has consequential effects on central processing resulting from forward masking that vary with masker level. We hypothesized that auditory brainstem responses (ABR) Wave V amplitudes will decrease across conditions as a function of increasing age. Furthermore, we hypothesized that participants will demonstrate later masking release at higher masker-to-target intervals (MTIs) for both behavior and electrophysiology as a function of age.

Methods: Adult participants with ages across the lifespan performed a behavioral perception task (3-interval 2-alternative forced choice) to detect a single-pulse target following a masker interval stimulus presented monaurally through the right ear as well as recorded EEG responses to the same stimulus. The masker level was fixed at 65- or 80-dB SPL. The target stimulus level was adapted in the behavioral task and was fixed at 90 dB peSPL for EEG. Additionally, we presented the target stimulus alone during EEG recording to establish baseline responses. To test for temporal processing deficits and the effect of forward masking, we presented the masker stimuli at different MTIs at 5, 50, 100, and 200-ms.

Results: The EEG data demonstrated that ABR Wave V amplitudes decreased as a function of age. We found a significant decrease in amplitude and increase in latency (i.e., increased forward masking) at the 5-ms conditions in comparison to the target-only condition and this effect decreased with increasing age. Using linear mixed-effect modelling, we also found a significant interaction between MTI and age. We determined that masking release had occurred for conditions at which latencies and amplitudes were equivalent to those obtained to the target alone. The behavioral data demonstrated that the masking release occurs at a later MTI as a function of age, which correlated with our electrophysiology results.

Conclusions: Our results help elucidate the fundamental effects of aging on temporal processing in the central auditory system. Despite having near-normal audiometric thresholds, older listeners can experience hearing difficulties in real-world situations outside of the clinical environment. Thus, our study gains insight into further understanding this “hidden hearing loss” and future experiments should focus on extending stimuli to more complex time-varying signals such as speech. Delineating these effects of aging will pave the way for better clinical diagnosis of hearing disorders and personalized treatments for different forms of age-related hearing loss.

Probing the Role of Epigenetics Mechanisms in Presbycusis Using a Genome-Wide Methylation Analysis

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Category: Aging

Background: Presbycusis or age-related hearing loss (ARHL) is the most frequent type of sensory disorder among the elderly population, resulting in irreversible sensorineural hearing loss. Presbycusis is characterized by bilateral hearing loss, pronounced in high frequency, that leads to psychophysical issues and low quality of life. It is well-defined that aging is associated with functional and morphological changes in the sensory organs, including the auditory system. Accumulating evidence shows epigenetic factors, in addition to genetics as determinants of this complex trait. Here we present a study examining whether DNA methylation; an epigenetic modifier is involved as a biomarker in Presbycusis.

Methods: Hospital-based cohort study of adults with presbycusis, comprising 55 ARHL subjects and 79 controls. The hearing measurements were employed to determine 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 Methylation-specific Polymerase Chain Reaction assay was used to confirm methylation levels at the identified genetic loci in ARHL patients.
Results: Our data demonstrate a strong correlation between patients' audiometric thresholds and CpG sites methylation in forty hearing-related genes. Interestingly, CpG sites located in ESPN and TNFRSF25 show an increase in methylation at each hearing frequency as the patient's hearing declines. Additionally, by Sanger sequencing, we identified a donor splice-site variant (c.745+ 1G greater than A) and a missense sequence change (E189Q) in the TNFRSF25 gene (NM003790) that were significantly increased in ARHL patients compared to controls. Preliminary characterization of the hearing on C57BL/6J-Tnfrsf25 (Cyagen) knockout mice generated by CRISPR /Cas-mediated genome engineering revealed that they have hearing loss by the age of 4 months. Clues to the Tnfrsf25 gene function are being examined.
Conclusions: We present an investigation of the involvement of DNA methylation in presbycusis by probing the methylation status across the genome of ARHL patients and age-matched controls. Our study can help in establishing the correlation between patients hearing thresholds and methylation status of hearing genes. We have sequenced presbycusis patients and controls, and data analysis have been undergone. We have generated and are characterizing the hearing and morphological changes in a Tnfrsf25 mouse model.

Relevance of Supporting Cell Pairing for Hair Cell Resilience to Age and Noise

Ignacio Garcia-Gomez*[1], Jemma L. Webber[1], Yingjie Zhou[1], Berta Soria Izquierdo[1], Trevor D.M. Harriman[1], Chloe G. Brink[1], Xiaodong Tan[1], Claus-Peter Richter[1], Mary Ann Cheatham[1], Jaime Garcia-Anoveros[1]

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Category: Aging
Background: The mammalian cochlear sensory epithelium, the organ of Corti, has two types of mechanosensors, inner (IHCs) and outer hair cells (OHCs), each surrounded by uniquely specialized supporting cells: IPhCs and IBCs support IHCs; OPCs and DCs support OHCs. Hair cells are particularly vulnerable to acoustic trauma through metabolic and mechanical damage, with the rate of OHC loss much higher than that of IHCs. Here we assess the contribution of the specialized support for each hair cell type to its survival with age and after noise exposure.
Methods: We examined mutant mice (Atoh1-Cre; Insm1(F/F), in which many OHCs convert into IHCs embryonically such that IHCs are found amidst OHCs (oc-IHCs) and are surrounded by outer compartment supporting cells (OPCs and DCs). Conversely, we also examined mutants in which OHCs convert into IHCs (ic-OHCs) either embryonically (Atoh1-Cre; Tbx2(F/F)) or postnatally (Fgf8-CreER; Tbx2(F/F) exposed to tamoxifen). These ic-OHCs are surrounded by inner compartment supporting cells (IPhCs and IBCs). We assess the resilience of out-of-place IHCs and OHCs in the outer or inner compartments, respectively, to either aging or exposure to loud noise.
Results: We find that oc-IHCs are more vulnerable than their OHC counterparts when found in the outer compartment and accompanied by OPCs and DCs, and that ic-OHCs in the position of IHCs and surrounded by IBCs and IPhCs) are more vulnerable than either OHCs in the outer compartment or IHCs in control animals. We also observe that OHCs are rendered less resilient to age and loud noise when the outer compartment supporting cells are disrupted (with a conversion of OPCs to DCs, which only occurs in the embryonic Tbx2 cKOs).
Conclusions: Our results imply that the cellular context of each hair cell type (surrounded by its appropriate supporting cell) is essential for their resilience and longevity. In other words, IHCs must be supported by IBCs and IPhCs while OHCs need the support of OPCs and DCs. This highlights the importance of focusing on specialized supporting cells in research towards preventing hair cell loss, as well as for the generation of appropriately paired (inner or outer) hair and supporting cells for regenerative therapies.

Longitudinal Assessment of Auditory Nerve and Brainstem Function Older Adults

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Category: Aging

Background: Estimates of age-related changes in auditory nerve (AN) function have been based exclusively on cross-sectional studies of older adults. As a result, little is known about how AN function changes over time within individuals and how differences in rates of change at the AN relate to changes in auditory function at the brainstem. Although AN response amplitudes are typically smaller for older than younger adults, neural response amplitudes at the brainstem are often similar, which has been attributed to hyperexcitability (central gain). In this study, we assessed changes in AN and brainstem function in older adults across two time points. We predicted that due to hyperexcitability, smaller changes in brainstem auditory function over time would be observed than changes in AN function.
Methods: Neural response amplitudes from the AN (Wave I or CAP) and brainstem (Wave V) were measured in older adults. Responses were elicited by a high level click. We examined the rate of change by measuring differences in response amplitudes between two time points (baseline and T2). We examined the extent to which age at baseline (45 to 88 years), time between tests (1.8 to 23.6 years), and hearing thresholds and change in hearing thresholds (average thresholds from 0.5-4.0 kHz) contributed to rates of change in AN and brainstem response amplitudes.
Results: We used linear mixed (LMER) modeling and model testing to assess response amplitudes at each time point. Age (baseline and T2) was a significant predictor of AN and Wave V amplitudes (baseline and T2), indicating that both AN and brainstem responses decreased with increasing age from baseline to T2. The rate of change was highly variable across subjects. Therefore, we tested several additional LMER models, where visit number was categorically entered into the model, with additional predictors of age at baseline, time between tests, and hearing thresholds. Only visit number was a significant predictor of AN or brainstem response amplitudes. To examine changes in the AN relative to the brainstem we used an LMER model that included AN and brainstem response amplitudes. There was a significant interaction of visit by neural response (AN versus brainstem). That is, AN amplitudes declined more precipitously than Wave V amplitudes, consistent with hyperexcitability, or preserved brainstem responses, as reported previously.
Conclusions: Consistent with our prediction, AN and brainstem function decreased over time in older adults. Moreover, rates of change were highly variable and not predicted by age at baseline, time between tests, or differences in hearing thresholds. Findings of significantly greater decreases over time in response amplitudes at the AN than at the brainstem is consistent with results of cross-sectional studies that suggest that decreases in AN activity contribute to hyperexcitability in the central auditory system.

QuickSIN as a Predictor of Subsequent Clinical Hearing Loss: Insights From the Baltimore Longitudinal Study of Aging

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Category: Aging
Background: Even though hearing difficulties in noise with a normal audiogram have been suggested as a potential precursor of later clinical hearing loss, the evidence for this is mixed in humans. This study aimed to determine whether speech perception difficulties in noise, as measured by QuickSIN (Etymotic Research, 2001), could be used to predict subsequent clinical hearing loss.
Methods: We conducted an analysis using data from the Baltimore Longitudinal Study of Aging (BLSA) (Shock 1984), which includes 1122 participants who had their hearing tested at least once. Of those, we focused on individuals who were initially normal hearing or had mild hearing loss and had undergone at least two hearing tests. During the hearing tests, audiometric threshold (0.5-8kHz), speech discrimination score (SDS), and QuickSIN were collected.
Results: Using a linear mixed model, the Age, Number of years between a visit and the first visit, Gender, the previous visit's SDS and QuickSIN scores were included as independent variables, while the change in average audiometric threshold (across ears and frequencies) between the current and first visit was treated as the dependent variable. The results revealed an interaction between the previous visit's QuickSIN score and the number of years between the current visit and the first visit in predicting the overall change in the audiometric threshold from the first visit. Specifically, the longer the duration from the first visit and the higher the QuickSIN score (poorer speech perception in noise) at the previous visit, the greater the increase in

audiometric threshold in the current visit. Importantly, a similar interaction did not exist for SDS (single word perception in quiet).
Conclusions: This suggests that a poorer QuickSIN score might be a precursor of clinical hearing loss, especially with advanced age. These findings offer some insights into early interventions (such as wearing ear protection during high noise exposure) and monitoring strategies for individuals at risk of hearing impairment, which could ultimately improve the management of age-related hearing difficulties.

Podium Session 6 - Vestibular, Hearing and Brain Dysfunction: Diseases, Drugs and Genes

10:15 a.m. - 12:15 p.m.

Platinum Salon 6

Evaluating the Vestibulotoxic Potential of Cisplatin in a Clinically Relevant Mouse Model of Cisplatin Ototoxicity

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Category: Vestibular: Basic Research and Clinical
Background: Despite its effectiveness in treating cancer, cisplatin can induce cochleotoxicity in up to 50-60% of patients. However, the vestibulotoxic potential of cisplatin remains unclear. Reported incidences are highly variable; vestibular symptoms are often underappreciated, and objective measures of vestibulotoxicity are frequently limited to assessment of horizontal semicircular canal function. As a result, many patients experiencing vestibulotoxicity likely go unevaluated.
Our lab previously developed and optimized a multi-cycle model of cisplatin administration in mice that results in hearing loss similar to that observed clinically with virtually no mortality.

The purpose of this study was to comprehensively characterize the functional and morphological consequences of cisplatin on the entire vestibular periphery in this mouse model.

Methods: Adult CBA/CaJ mice were randomly assigned to control and cisplatin-treated groups. Cisplatin-treated mice received three consecutive cycles of once-daily intraperitoneal injections of 4.0 mg/kg cisplatin for four days followed by a 10-day recovery period, for a cumulative cisplatin dose of 48 mg/kg.

All mice underwent VsEP testing prior to cisplatin administration, after the final cycle of cisplatin, and 6 months after treatment cessation. Thresholds were designated as the stimulus level halfway between the lowest level at which a repeatable response was obtained and level at which no response was obtained. Mice also underwent vestibulo-ocular reflex (VOR) testing and vestibular afferent single-unit recordings 6 months after treatment cessation. For VOR testing, eye velocity signals in response to sinusoidal head rotations and translations were recorded using infrared eye tracking. Rotational and translational VOR gains and phases were calculated from these signals. For single unit recordings, afferents' spontaneous activity and responses to head rotations and translations were recorded. Afferent regularity was determined by calculating normalized coefficient of variation of interspike intervals (i.e., CV*). Gains and phases relative to head velocity were calculated at 1Hz.

Following functional testing, temporal bones were extracted, fixed, and decalcified. Utricles, saccules, and ampullae were micro-dissected and immunostained with hair cell and afferent neuron markers.

Results: In cisplatin-treated mice, VsEP thresholds were significantly elevated and utricular/saccular hair cell densities were reduced. Differences in rotational VOR gain and vestibular afferent regularity were also observed in cisplatin-treated mice compared to saline-treated controls.
Conclusions: Our results demonstrate cisplatin's potential to adversely and differentially affect the otolith organs (i.e., utricle, saccule) and semicircular canals. Clinically, objective vestibular testing is often limited

to assessment of horizontal semicircular canal function, leaving the status of 4/5 vestibular end organs largely unknown. Our mouse model of cisplatin vestibulotoxicity suggests the vestibulotoxic potential of cisplatin may be underestimated and emphasizes the need for more comprehensive vestibular testing in patients treated with cisplatin.

Superior Semicircular Canal Dehiscence and Subsequent Closure Through Osteoneogenesis Induces Reversible Impaired Decision-Making.

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[1]*Rutgers Robert Wood Johnson Medical School,* [2]*Rutgers University*

Category: Vestibular: Basic Research and Clinical

Background: Vestibular loss and dysfunction has been associated with cognitive deficits, decreased spatial navigation, spatial memory, visuospatial ability, attention, executive function, and processing speed among others. Superior semicircular canal dehiscence (SSCD) is a vestibular-cochlear disorder in humans in which a pathological third mobile window of the otic capsule creates changes to the flow of sound pressure energy through the perilymph/endolymph. The primary symptoms include sound-induced dizziness/vertigo, inner ear conductive hearing loss, autophony, headaches, and visual problems; however, individuals also experience measurable deficits in basic decision-making, short-term memory, concentration, spatial cognition, and depression. These suggest central mechanisms of impairment are associated with vestibular disorders; therefore, we directly tested this hypothesis using both an auditory and visual decision-making task of varying difficulty levels in our model of SSCD.

Methods: Adult Mongolian gerbils (n = 69) were used to test the effect of SSCD on auditory brain stem response (ABR) thresholds and cervical positive vestibular-evoked myogenic potential (c+VEMP) amplitudes or to behavioral decision-making on a Go-NoGo task (N=33). Thirty-six animals received surgical fenestrations of varying size of the left superior semicircular canal. ABR and c+VEMP testing were carried out prior to surgery and over acute (10 days) or prolonged recovery (28 days).Micro-CT analysis was completed with representative samples of control, day 3 and 10 post-SSCD animals. Thirty-three were trained on one of four versions of a Go-NoGo stimulus presentation rate discrimination task that included standard ("easy") or more difficult ("hard") auditory and visual stimuli. After 10 days of training, preoperative ABR and c+VEMP testing was followed by a surgical fenestration of the left superior semicircular canal. Animals with persistent circling or head tilt were excluded to minimize effects from acute vestibular injury. Testing was resumed at postoperative day 5 and continued through postoperative day 15 at which point final ABR and c+VEMP testing was carried out.

Results: The SSCD created a significant worsening of ABR hearing thresholds and increased c+VEMP amplitude by postoperative day 7. However, progressive osteoneogenesis resulted in resurfacing of the SSCD and a return towards baseline physiology, which was apparent by the second week. Behavioral performance (d-prime) was measured during the peak of SSCD induced ABR and c+VEMP impairment (post 7) and during the return of physiology towards baseline (post 14). There were significant decreases in behavioral performance that were highly correlated with persistent deficits in c+VEMPs at the end of training (postoperative day 15). The controls demonstrated additional learning post procedure that was absent in the SSCD group.

Conclusions: These results suggest that aberrant asymmetric vestibular output is associated with decision-making impairments in these discrimination tasks. This holds implications for cognitive impairments associated with other vestibular disorders.

Glucocorticosteroid Treatment in Acute Unilateral Vestibulopathy, a Multicentric, Randomized, Double-Blind, Placebo-Controlled Study

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Category: Vestibular: Basic Research and Clinical

Background: Acute unilateral vestibulopathy is a common cause of peripheral vestibular vertigo. It is a benign affection of still unknown etiology. The aim of this study is to assess the effect of a 10-day course of oral corticosteroids on the recovery of the vestibular function and on the symptoms.

Methods: A multicentric, randomized, double-blind, placebo-controlled study was performed. Patients with acute unilateral vestibulopathy were recruited at University Hospitals of Geneva (HUG) and at the Lausanne University Hospital (CHUV), Switzerland. They were randomly assigned in the prednisone or the placebo group. They received a 10-day oral treatment. Caloric asymmetry (classical Jongkees formula), the primary outcome, and the impact of symptoms assessed with the Dizziness Handicap Inventory (DHI), the secondary outcome, were measured at inclusion (T0) and at 1 year (T1). Data were analyzed using a mixed between-within ANOVA test.

Results: A total of 94 patients were included and randomized. 44 patients were assigned in group A (mean age 59 +/- 23 yr, 14F and 30M) and 50 patients in group B (mean age 54 +/- 13 yr, 20F and 30M). At one year, 39 patients of group A and 36 patients of group B had available data for the primary outcome (caloric asymmetry), respectively, 35 patients of group A and 36 patients of group B for the secondary outcome (DHI). For the primary and secondary outcomes statistical analysis showed a significant effect of time in both groups (T0 vs T1, p less than 0.001) and no group effect (A vs B, p=0.590, respectively p=0.896).

Conclusions: At one year, we found a significant recovery of the vestibular function and a significant decrease of symptoms, with, however, no significant differences between the prednisone and placebo groups.

Contribution of Mirror-Image Hair Cell Orientation to Mouse Otolith Organ and Zebrafish Neuromast Function

Amandine Jarysta*[1], Kazuya Ono[2], Natasha Hughes[3], Alma Jukic[4], Vanessa Chang[3], Michael Deans[5], Ruth Anne Eatock[6], Kathleen E. Cullen[3], Katie Kindt[4], Basile Tarchini[1]

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Category: Vestibular: Basic Research and Clinical

Background: Otolith organs in the inner ear and neuromasts in the fish lateral line harbor two hair cell populations of opposing orientation. In both species the transcription factor EMX2 is regionally expressed in just one hair cell population and enables polarized localization of the GPR156 receptor to reverse hair cell orientation compared to the rest of the organ. Emx2 also impacts HC mechanosensory properties in zebrafish and afferent organization in both species. While mirror-image hair cell organization confers neuromasts with bi-directional sensitivity to fluid flow stimuli it remains unclear how the ability to detect stimuli of opposing directions serves vestibular function in mammals. In addition, it is unclear whether GPR156 has other roles besides orienting hair cells.

Methods: To address these questions, we leveraged mouse and zebrafish models lacking GPR156 and adopted a broad multidisciplinary approach. We used immunolabeling and confocal microscopy to assess macular and neuromast anatomy, electrophysiology in the mouse utricle to assess hair cell and afferent function, calcium imaging in neuromasts to assess hair cell mechanotransduction properties, and a battery of behavioral tests including off--vertical-axis rotation (OVAR) to probe mouse vestibular function.

Results: We find that zonal and type I-II hair cell organization in otolith organs is unaffected in Gpr156 mouse mutants. Electrophysiological recordings suggest normal mechano-electrical transduction properties in Gpr156 mouse mutants, including in non-reversed Emx2-positive hair cells. In gpr156 zebrafish mutants, however, calcium imaging shows that Emx2-positive hair cells lose their distinctively smaller mechanically-evoked signals, resulting in more uniform transduction across the neuromast. In contrast, loss of GPR156 does not affect direction-selectivity of afferent contacts in the mouse utricle or zebrafish neuromast, with normal afferent firing patterns in mouse mutants. This suggests that GPR156 relays both orientation and transduction information downstream of EMX2, but not selectivity for direction-specific afferents. Based on these results, the Gpr156 null mouse is a useful model to tease apart how mirror-image organization serves vestibular function. We find that although Gpr156 mutant mice do not show overt vestibular dysfunction (spinning or head tilt), performance on two tests that engage otolith organs was significantly altered (swimming and off-vertical-axis rotation, OVAR).

Conclusions: These results help show how mechanisms that confer bi-directionality to sensory organs contribute to function, from single hair cell physiology to animal behavior.

The Transcription Factor Six2 Regulates Morphogenesis of Vestibular Epithelia and Maturation of Vestibular Hair Cells

Sumana Ghosh*[1], Punam Thapa[1], Vineel Vanga[1], Beth Baker[1], Steven . Gressett[1], Bradley Walters[1]

[1]*University of Mississippi Medical Center*

Category: Vestibular: Basic Research and Clinical

Background: Six2 belongs to the Sine Oculis Homeobox Homolog (Six) homeobox family of transcription factors. It has been shown to be critical for development and patterning of multiple tissues including kidney, and heart. Recent transcriptomic data suggest that expression Six2 is enriched in adult cochlear outer hair cells. However, the distribution and function of Six2 during inner ear development remains unexplored.

Methods: To investigate the expression of Six2 during otic development, CD-1 embryos and pups were collected at different stages (E10.5, E12.5, E14.5 and E17.5, P0, and P21). Six2 expression was investigated by RNAscope in-situ hybridization. We used germline knockout (Six2-/-) mice to study the role of Six2 in morphogenesis of vestibular epithelia. Whole-mounted utricles, and cristae were immunolabelled for beta-spectrin, espin, SOX2, MYO7A and SPP1, as well as oncomodulin (OCM) to examine the effects of Six2 deletion on the striola. To investigate the effect of Six2 deletion in planar cell polarity (PCP) the samples were also immunolabelled for PCP proteins VANGL2 and Gαi3. The hair cell orientation with respect to the refence frame was measured using ImageJ and the data were analyzed and plotted as rose diagrams in Oriana software.

Results: Our data suggest that Six2 is expressed in the otic vesicle as early as E10.5, prior to differentiation of pro-sensory cells to hair cells. Six2 is expressed both in the dorsal and ventral sides of the otic vesicle, most prominently in the superior half. It's expressed in both sensory and non-sensory cells throughout development. In late embryonic (E17.5) and early postnatal stages, Six2 is expressed in both the type I and type II hair cells and the supporting cells of the vestibular epithelia. Six2 deletion results in expansion of the overall area of the utricle and anterior crista, but not lateral crista. In congruence with overall increases in area, there is an increase in the lateral extrastriola area of Six2-/- utricles compared to the controls. Furthermore, there is also an increase in the OCM+ striola area in Six2-/- utricles, although total number of OCM+ cells is decreased. The density of immature hair cells significantly increases in the in the Six2-/- utricles suggesting that germline deletion of Six2 may delay hair cell maturation. In accordance with that, preliminary data suggest that Six2 deletion increases the density of Sox2+ve Myo7a+ve immature hair cells and reduces total number of SPP1+ve cells. Additionally, there is hair cell misorientation in the Six2-/- utricles compared to wild type, suggesting Six2 may play a role in planar cell polarity of utricular hair cells.

Conclusions: Taken together our data suggest that Six2 regulates development of the vestibular epithelia. Future studies will further investigate the molecular mechanisms of Six2- mediated regulation of hair cell maturation and morphogenesis of vestibular epithelia.

Dissecting the Divergent Impact of Cochleovestibular Tumors on Auditory and Vestibular Dysfunction in Neurofibromatosis Type 2

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Category: Vestibular: Basic Research and Clinical

Background: Neurofibromatosis type 2 (NF2) is an autosomal dominant condition resulting from mutation of merlin, a tumor suppressor gene, and is characterized by bilateral cochleo-vestibular schwannomas (CVS) and other nervous system tumors. The pathognomonic presentation of NF2 includes tinnitus and hearing loss due to cochlear dysfunction. However, the origins and mechanisms of disequilibrium and vestibular dysfunction remain unknown.

Methods: Participants in a prospective natural history study (NCT NCT00598351) of NF2 with specific audiovestibular phenotype were included in this study. The study comprised 72 treatment naïve participants

(age 28.9 ± 18.01 years; 32 males) from 06/2008 To 04/2023. Comprehensive vestibular test battery included videonystagmography, computerized dynamic posturography, cervical vestibular evoked myogenic potentials (VEMP), sinusoidal harmonic acceleration, and velocity step testing. Additional deep phenotypic profiles also included self-reported functional measures, lifetime interventions (surgical, radiation and drug treatments), and imaging (tumor number, type, and volume). Analysis was performed using a linear mixed-effects model (LMEM), which accounted for random effects of individual patients to investigate relationships between gross left- and right-sided CVS tumor volumes and 21 vestibular parameters of interest (R software). Matched mixed-effects models were also used to compare vestibular ocular reflex (VOR) responses via rotational testing and caloric irrigations for those patients with multiple visits.

Results: Upon preliminary analyses of gross VOR function (rotational and caloric assessments) we found significant declines in VOR gain for most angular frequencies from 0.02Hz to 0.64Hz during the study period. We also found significant declines in peak eye velocity in response to high velocity 240-degree step accelerations with time in each participant. We did not find significant declines in caloric VOR response or low-frequency rotational VOR gain (0.01Hz), suggesting a primary mid-to-high frequency impact on vestibular function. LMEM analysis extended these findings by identifying robust significant relationships between gross VS tumor volume and lateralized high velocity step acceleration VOR gain and mid-to-high frequency angular VOR gain. We did not find a significant decline of low-frequency caloric responses to gross CVS tumor volume increase. We did not identify any significant relationship between audiometric pure-tone average and gross CVS tumor volume for both the right and left ears, supporting previous reports that fail to show any robust relationship between hearing sensitivity and CVS tumor volume.

Conclusions: Our findings indicate a measurable impact of cochleovestibular schwannoma growth on vestibular function, specifically on the mid-to-high frequency vestibular response, in individuals with NF2. While the hallmark symptoms of NF2 largely revolve around cochlear disturbances leading to tinnitus and hearing loss, our study underscores the significant relationship between CVS tumor volume and compromised vestibular function. Further, the lack of correlation between CVS tumor volume and hearing sensitivity reaffirms that cochlear and vestibular disturbances in NF2, although interconnected, may operate via different mechanistic pathways.

Data-Driven Analysis From Audiometric Profiles of 1045 Patients Consulting for Dizziness and Vertigo

Cécile Puel[1], Jérôme Bourien[1], Jean-Luc Puel[1], Jean-Charles Ceccato*[2]

[1]*INM, Inserm; University of Montpellier, Montpellier Hospital,* [2]*Audiocampus, University of Montpellier*

Category: Vestibular: Basic Research and Clinical

Background: Dizziness and vertigo are the most frequent complaints in adults with prevalence rates ranging from 20% to 30%. In routine practice, the E.N.T. doctor performs clinical investigations to determine the localization of central and/or vestibular deficits. Since auditory and vestibular system are ontogenetically and phylogenetically related, we explore the relationship between hearing loss and vestibular deficits using machine learning approach.

Methods: To do so, we performed data-driven analysis from audiometric profiles of 1045 patients consulting for dizziness and vertigo. The selected patients were examined by the same clinician (Cecile Nicolas-Puel). The data were anonymized, centralized by the Inserm, and stored on secure data server. Patients provided written informed consent. The retrospective study was conducted in accordance with the ethical standards of the Helsinki Declaration of 1975, as revised in 1983.

Results: Principal component analysis (PCA) was used to reduce the dimensionality of the tonal audiogram (14 dimensions, with 7 frequencies from 250 Hz to 8 kHz for both ears) to 2 components. The first two components explaining respectively 93 % and 3% of the variation, the audiograms could be projected in a two-dimensional space. Interestingly, the first component of PCA correlated with the degree of hearing loss while second component was strongly related to the asymmetry between the two ears. Based on the two-dimensional distance (Mahalanobis) between patients' audiometry, we choose to segregate the population in 7 clusters (~150 patients per cluster) to explore sub-population characteristics. Quantitative analysis of the audiograms shows that one third of the population consulting for dizziness and vertigo displays normal hearing with the mean age of 39-year-old (cluster 1). Resembling to classical presbycusis, high frequency hearing loss progressively increased with age from 52- to 74-year-old (clusters 2,3,4). Flat asymmetrical hearing loss also

increase with age, but the patients were ~10 years younger, and predominantly women (clusters 5 ,6,7). This segmentation of the audiometric profiles was compared with vestibular tests. Caloric irrigation and rotatory chair tests were used to assess lateral semi-circular canal function. Interestingly, a clear correlation was seen between the asymmetrical audiograms and the vestibular side deficits. The video head impulse test was used to evaluate the functional state of all the canal. Worthy of note is that the gain of the lateral semicircular canal is better than those of the anterior and the posterior canal. For all canals, the gain decrease correlated with age, at least up to the 74-year-old, but not with the hearing loss. All these data-driven results were finally confronted to the diagnostics provided by the ENT.
Conclusions: Altogether, these results support the need to explore hearing to refine the diagnosis in patients suffering from balance disorders.

Reduced Saccular Function is Associated With Cortical Surface Growth in Broca's Area

Dominic Padova*[1], J. Tilak Ratnanather[2], Andreia Faria[1], Yuri Agrawal[1]

[1]*Johns Hopkins School of Medicine,* [2]*Johns Hopkins University*

Category: Vestibular: Basic Research and Clinical
Background: Emerging evidence highlights an association between vestibular function and higher cognitive abilities, including attention, executive function, and motor planning and execution. However, the specific neuroanatomical pathways underlying this association remain elusive. Clinical neuroimaging of vestibular patients reveals widespread structural abnormalities, notably in the prefrontal cortex. Functional neuroimaging during vestibular stimulation unveils quick, robust activations in the prefrontal cortex, suggesting a potential vestibular-thalamus-prefrontal cortex trisynaptic pathway. However, the neural circuits affected by the aging peripheral vestibular system, which deteriorates structurally and functionally over time, are still unidentified. This study aims to elucidate the relationship between age-related saccular function and surface atrophy in eight prefrontal cortex subfields, accounting for age, intracranial volume, and sex.
Methods: Data from 58 participants from the Baltimore Longitudinal Study of Aging, who underwent concurrent cVEMP tests and T1-weighted MRI scans, were analyzed. MRICloud segmented the MRI scans automatically, and triangulated surface meshes were generated through Delaunay refinement. Independent population templates were created for each hemisphere of every region of interest and mapped to individual surfaces to measure surface atrophy. To streamline statistical testing, the 800 surface vertices were clustered into k patches based on surface geometry, reducing the number of comparisons tenfold. Continuous variables were standardized before linear regression, and quality control was enacted at each stage. Hypotheses were tested using 10,000 permutations of residuals, with a rejection threshold set at the 0.05 level based on the maximum test statistic across the surface.
Results: Permutation testing revealed a significant relationship between saccular function and surface shape in the pars opercularis of the left inferior frontal gyrus (a component of Broca's area) ($p\_perm \approx 0.023$). The model indicated that a one standard deviation increase in saccular function correlated with approximately 0.1 standard deviation compression tangent to the cortical surface in the inferior medial region of the left pars opercularis ($p \approx 0.027$), signifying surface atrophy. This atrophy may be tentatively attributed to a reduced number of cortical columns within the significant patch.
Conclusions: Our findings demonstrate a correlation between reduced saccular function and cortical surface growth in the pars opercularis of the inferior frontal gyrus. This region, situated above the insular cortex and activated during vestibular stimulation, plays a crucial role in various functions, including semantic tasks, music perception, motor aspects of speech, and, intriguingly, theory of mind abilities. Furthermore, the pars opercularis is also implicated in vestibular-modulated persistent developmental stuttering. Future research will further explore the relationships between age-related vestibular end-organ function and the structure and function of the sensorimotor cortex. Subsequent analyses will investigate the combined effects of age-related vestibular function and surface atrophy on executive ability and motor planning and execution, considering variables such as age, education, and sex.

Poster Session 2

1:15 p.m. - 3:15 p.m.

Marquis Ballroom

SU1. Recovery From Auditory Neuropathy Upon Induction of Schwann Cell Loss in Mature Adult DTA Mice

Shreeya Sawant*[1], Nicole Zoghby[1], Elizabeth Markuson[1], Jessica Georgopulos[1], Hongji Zhang[1], Maria Traka[1], Michael Ebeid[1]

[1]*Midwestern University*

Category: Auditory Nerve

Background: Myelination is essential for rapid propagation of action potentials along neuronal axons. In the peripheral nervous system (PNS), including the auditory system, Schwann cells (SCs) are responsible for myelination. Demyelinating diseases of the PNS affect an increasing number of people, and can be either inherited, such as Charcot-Marie-Tooth, or acquired from an autoimmunological insult, trauma or nerve injury. In these diseases there is substantial damage to SCs leading to loss of the myelin sheath and subsequent impaired peripheral nerve conduction. As a result, affected individuals suffer from sensorimotor peripheral neuropathy and auditory neuropathy. Enhancing the endogenous remyelination potential of the PNS is a promising therapeutic approach for promoting functional recovery and axonal survival in these demyelinating diseases. PLP-CreERT;ROSA26-eGFP-DTA (DTA) mouse is an established model of cell-specific ablation of Plp1-expressing glial cells in the nervous system upon induction with tamoxifen administration. DTA mice developed a demyelinating disease characterized by significant motor and physiological defects at 5 weeks post-induction, followed by a full recovery of symptoms a few weeks later. Previous work has shown that robust SC regeneration and axonal remyelination occur in the auditory nerve a few weeks after SC ablation in young DTA mice. The process of glial cell regeneration and ultimately remyelination in mature-adult auditory system has not been investigated.

Methods: Two age groups (4-month-old and 8-9 month-old) DTA mice are treated with tamoxifen for four continuous days to induce recombination and expression of the diphtheria toxin A subunit and subsequent death in glial cells. Recovering mice are collected at different time points post-induction (1, 3 and 5 weeks) to analyze glial cell regeneration within the auditory neurons. Inner ears are fixed, then spiral ganglion and auditory fibers are dissected and immunostained as whole mount or sectioned then immunostained to label different types of glial cells, as well as myelin and node of Ranvier markers. Proliferation in glial cells is analyzed using 5-ethynyl-2′-deoxyuridine (EdU) proliferation marker injected 24 hours before collecting mice. Auditory brain stem responses (ABR) are used to assess hearing thresholds at 4 different frequencies: 4, 8, 16 and 32kHz. Transmission electron microscopy is used to identify myelin and neuronal axon ultrastructure.

Results: In the 4-month-old group, Schwann cell density was 56%, 76% and 81% compared to controls at 1-, 3-, and 5-week post-induction respectively, indicating a gradual recovery of SCs. SC proliferation showed 3.8-fold increase compared to controls at 1-week post-induction, indicating activated cell proliferation after glial cell ablation. The ratio of satellite cells to spiral ganglion neurons was 58% and 86 % compared to controls at 3- and 5-week postinduction respectively, indicating recovery of the satellite cells. Functional recovery data is pending.

Conclusions: Robust Schwann cell and satellite cell regeneration occurs after ablation in mature adult auditory system.

SU2. Representations of Relative Frequency in Human Auditory Cortex

Emily Allen*[1], Juraj Mesik[1], Anahita Mehta[2], Kendrick Kay[1], Andrew Oxenham[1]

[1]*University of Minnesota,* [2]*University of Michigan*

Category: Auditory Cortex and Thalamus: Human Studies

Background: Representations of absolute frequency have a well-established tonotopic representation throughout the auditory system, from the cochlea up to auditory cortex. In contrast, representations of relative frequency, which are critical for speech and music perception, have remained less explored. Previous work,

using a combination of natural speech sounds and direct neural recordings in humans undergoing brain surgery for epilepsy, has identified a population of neurons in auditory cortex that primarily encode absolute pitch, as well as neurons more anterior and lateral in auditory cortex that encode relative pitch. However, additional studies in neurologically healthy participants are needed in order to support these results, as well as to determine whether there exists any systematic organization of relative pitch, perhaps akin to tonotopy.

Methods: Using fMRI, we measured BOLD responses in 12 normal-hearing participants as they actively listened to sequential pairs of pure tones, separated into blocks of lower frequencies (100-800 Hz) and higher frequencies (800-6400 Hz). Tone pairs varied in both direction and magnitude of change (i.e., relative change in short-term contexts). Including 800 Hz in both ranges allowed us to assess whether longer-term context (i.e., a low frequency block vs a high frequency block) would affect the cortical representation of a given stimulus.

Results: Encoding and decoding models were used to investigate the contributions of absolute and relative frequency in auditory cortex. Comparing baseline models based on absolute frequency alone to full models that incorporate relative change tuning revealed that absolute frequency explained the majority of BOLD response variance, with relative tuning only marginally improving model fits. Moreover, while absolute frequency showed clear tonotopic organization, we did not observe systematic organization of relative direction or magnitude tuning.

In contrast, analyses focused on longer-term context effects on the representations of the 800-Hz trials revealed profound context-dependent shifts in the distribution of evoked BOLD. Specifically, at the macroscopic level of the cortical tonotopic map, we found that 800-Hz stimuli within low-frequency blocks consistently evoked peak responses in voxels with nominally higher tuning than 800-Hz stimuli within the high-frequency blocks. This is consistent with context-dependent tuning shifts, likely driven by neural adaptation within these populations.

Conclusions: Overall, our results show that although relative frequency explained substantially less variance than absolute frequency in a short-term context (i.e., within tone pairs), and there was no clear evidence of its systematic mapping, there was strong evidence of longer-term context effects. Further studies will be needed to elucidate the nature of short-term relative frequency encoding, as well as to explore the perceptual counterparts of tuning shifts associated with longer-term spectral context observed in the present work.

Supported by NIH grant R01 DC005216

SU3. Can Speech Induced Mismatch Negativity Used as a Biomarker for the Hidden Hearing Loss in Human Adults?

Lichun Zhang*[1], Florian Schmidt[1], David Mißler[1], Karsten Ehrt[1], Wilma Großmann[1], Robert Mlynski[1]

[1]*Rostock University Medical Center*

Category: Auditory Cortex and Thalamus: Human Studies

Background: In the early stage of presbycusis, patients primarily complain about reduced speech perception in noisy environments even though their routine audiometry does not show an elevation in hearing thresholds. This condition is often referred to as ‘hidden hearing loss’ (Schaette and McAlpine 2011). Current diagnostic metrics, such as a reduced amplitude of the ABR Wave I, elevated hearing threshold in the extended high-frequency range (EHT), a decreased amplitude of middle ear muscle reflex etc., mainly focus on finding the suitable biomarkers for pathological changes in the peripheral auditory system (Kujawa and Liberman 2009, 2015; Liberman et al., 2016; Bramhall et al., 2019). However, the results are inconsistent.

Mismatch negativity (MMN) is a long-latency cortical auditory evoked potential and is generated when an individual automatically detects a change in sound. This change detection relies on the brain's memory traces of the regularity of sound stimuli. The aim of this study was to prove if MMN could also be used as a diagnostic metric for hidden hearing loss in human adults.

Methods: 73 subjects with normal hearing were included in this study. All participants underwent an extended pure-tone audiogram examination ranging from 0.125 to 16 kHz, a middle ear muscle reflex assessment and a subsequent MMN assessment with 2 different stimuli: two verbal (da/ba) and two non-verbal stimuli (1/2 kHz). The MMN’S amplitude and latency were calculated and analyzed.

Results: Pearson correlation analyses revealed a statistically significant negative correlation between age and the amplitude of MMN elicited by verbal stimuli (R= -0.18, p=0.029). Regarding the correlation between the amplitude of MMN induced by verbal stimuli and the EHT, no statistically significant correlation was found. However, when we divided all participants into two group based on their EHT, the MMN amplitude in the group with EHT less than 0 dB was significantly higher than that in the group with EHT greater than 0 dB (p = 0.05).
Conclusions: Thus, speech-induced MMN may serve as a potential biomarker for hidden hearing loss in human adults.

SU4. Compact and Explainable Decoding of Auditory Selective Attention

Jusung Ham*[1], Jinhee Kim[2], Kyogu Lee[2], Inyong Choi[1]

[1]*University of Iowa,* [2]*Seoul National University*

Category: Auditory Cortex and Thalamus: Human Studies
Background: More than 10% of people with normal hearing thresholds suffer from difficulty hearing in noisy and complex acoustic environments. A prospective solution to the problem is perceptual training that strengthens the attentional modulation of cortical sensory encoding, enhancing the target sound's neural representation while suppressing the surrounding noise. We previously showed that an audiovisual neurofeedback training paradigm improved attentional modulation and speech perception in the noise of normal-hearing listeners.
Since the neurofeedback is determined by the attention decoded from single-trial EEG signals, accurate auditory attention decoding (AAD) is key to the success of the training. An AAD algorithm should be compact enough to train on a small dataset and operate in real-time while remaining reliable to properly direct and motivate participants. It is also required to be explainable to interpret the neural basis of the training. To satisfy the above requirements, we built a feature engineering pipeline that can integrate spatiotemporal information of EEG signals within a small number of features to minimize performance loss and maximize explainability.
Methods: Twenty-eight normal-hearing participants were recruited. For each trial, they were indicated to focus on one speech stream out of two concurrent streams: a female saying “up” five times, and a male saying “down” four times. 64-channel EEG was recorded during the trials. We used the digitized amplitudes of single-trial EEG waveforms as the features for the classification. To integrate spatial information, we compared simple concatenation of channels, averaging across channels, and global field power. Additionally, we utilized source-localized EEG time courses. The features were put into three different binary classifiers: logistic regression, random forest, and support vector machine (SVM). Classification performance was evaluated using cross-validation of leave-one-trial-out for the within subject, and leave-one-subject-out for the across subjects. For each classifier, we estimated the contribution of each feature to the classification.
Results: We achieved significant improvements in AAD performance as we integrated more channels and used SVM. The “importance map” of EEG channel locations or source time courses demonstrated that the auditory cortex makes the most contribution to the AAD performance. The amplitude around 100-200 ms latency was most important for the classification.
Conclusions: In our study, we developed a compact, reliable, and explainable AAD algorithm that can be utilized for neurofeedback training of auditory selective attention. More reliable decoding will improve participants’ engagement with the training and can lead to better training effects. An interpretable and straightforward process will verify that the training targets the neural circuit of auditory attention.

SU5. The Cortical Frequency-Following Response to Continuous Speech in Musicians and Non-Musicians

Jasmin Riegel*[1], Alina Schüller[1], Achim Schilling[2], Patrick Krauss[3], Stefan Rampp[4], Tobias Reichenbach[1]

[1]*Friedrich-Alexander-University Erlangen,* [2]*Neuroscience Lab, University Hospital Erlangen,* [3]*Neuroscience Lab, University Hospital Erlangen; Pattern Recognition Lab, Friedrich-Alexander-University Erlangen-Nürnberg,* [4]*University Hospital Erlangen; University Hospital Halle (Saale)*

Category: Auditory Cortex and Thalamus: Structure and Function
Background: Previous research indicated that neural responses to acoustic signals can differ in musicians and non-musicians. For instance, subcortical responses to speech sounds were found to be stronger in musicians and to decline less with increasing age [1]. Furthermore, subcortical responses as well as cortical evoked potentials to pitch, timing, and timbre of music were larger in musicians [2,3]. However, these studies did involve many repetitions of short acoustic signals and not more ecological non-repetitive stimuli such as continuous speech. Neural responses to the latter emerge, for instance, at the fundamental frequency (speech-FFR) and include a cortical contribution. The cortical contribution is modulated by attention [4], however, its potential dependence on musical experience is not yet known.
Methods: 35 healthy and young participants with different levels of musical experience were assessed. They were simultaneously presented with two audiobooks from different speakers and were asked to attend one while ignoring the other. Meanwhile, MEG recordings of their brain activity were conducted. The data was analyzed using temporal response functions (TRFs) for the source-reconstructed activity in the auditory cortex, for two acoustic features of the speaker that were related to the fundamental frequency and thus described the speech-FFR.
Results: Our data showed a significant attentional difference for both groups and both acoustic features, comparable to our previous findings [4]. Furthermore, the non-musicians had significantly higher cortical responses than the musicians. Additionally, the variance in the neural response was higher in the non-musicians. No significant difference between musicians and non-musicians emerged regarding the attentional modulation.
Conclusions: In summary, our data showed a stronger cortical contribution to the speech-FFR in non-musicians than in musicians. As previous behavioral work showed that musicians are better at recognizing speech in noise [1], one possible explanation for our results could be our task was less demanding for musicians and therefore resulted in lower neural responses. However, more work is needed to understand the interplay with the subcortical speech-FFR and the possible impact of selective attention.
[1] Anderson, S., Hittner, E., and Kraus, N. (2012) Musical experience strengthens the neural representation of sounds important for communication in middle-aged adults. Frontiers in Aging Neuroscience 4: 35814.

[2] Tzounopoulos T, Kraus N. (2009) Learning to encode timing: mechanisms of plasticity in the auditory brainstem. Neuron 62:463.

[3] Pantev C, Roberts LE, Schulz M, Engelien A, Ross B. (2001) Timbre-specific enhancement of auditory cortical representations in musicians. Neuroreport 12:169.

[4] Schueller A., Schilling A., Krauss P., Rampp S., Reichenbach T. (2023) Attentional modulation of the cortical contribution to the frequency-following response evoked by continuous speech. bioRxiv 2023.07.03.547608.

SU6. Vasoactive Intestinal Peptide Signaling Within Auditory Cortex

Christine Junhui Liu*[1], Cathryn MacGregor[2], Carolyn Sweeney[1], Lucas Vattino[1], Kasey Smith[2], Anne Takesian[1]

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Category: Auditory Cortex and Thalamus: Structure and Function
Background: Auditory learning can induce structural and functional changes in the auditory cortex; however, the neural mechanisms underlying cortical plasticity require further investigation. Previous studies have demonstrated that a population of cortical GABAergic neurons expressing vasoactive intestinal peptide (VIP) is important for auditory plasticity. Although many studies have leveraged VIP expression to genetically target this specific interneuron population, few have evaluated the function of the signaling molecule VIP in auditory plasticity and learning.
Methods: To understand when VIP is released in mouse auditory cortex (ACtx), we performed in vivo imaging of a G-protein Coupled Receptor (GPCR)-Activation-Based (GRAB) peptide sensor using fiber photometry in mice while they listen to passive sound presentations or undergo associative auditory learning. To characterize the VIP receptor expression in ACtx, we used in situ hybridization to quantify the expression

levels of mRNA encoding the VIP receptor 1, Vipr1. Ongoing studies are using in vitro electrophysiology to determine the functional postsynaptic effects of VIP receptor activation.
Results: Our results show that sound stimuli elicited VIP sensor responses in ACtx from a subset of mice. Furthermore, as mice learned to associate specific sounds with water rewards, VIP release is modulated by the behavioral relevance of the sound stimuli. Consistent with previous studies in other sensory cortices, our anatomical studies reveal that Vipr1 in ACtx is expressed within 77% of excitatory pyramidal cells, marked by expression of vesicular glutamate transporter 1 (Slc17a7) and 18% of GABAergic neurons, marked by expression of the GABA synthesizing enzymes Gad1 and Gad2.
Conclusions: These studies reveal that VIP release in ACtx changes across auditory associative learning and VIP receptors are widely distributed across both excitatory and inhibitory cell types in ACtx. Together, these results may elucidate the effects of VIP within auditory cortical circuits, laying the necessary foundation for future loss- and gain-of-function experiments to evaluate the function of VIP release in auditory perception and learning.

SU7. Cortical Synaptic Zinc Signaling Contributes to Increased Gain and Perceptual Recovery After Noise Trauma

Manoj Kumar*[1], Thanos Tzounoupoulos[2]

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

Category: Auditory Cortex and Thalamus: Structure and Function
Background: Noise-induced hearing loss (NIHL) 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 increased gain of A1 PNs and recovery of perceptual hearing remain unknown. Our previous work has established that synaptic zinc signaling contributes to cortical gain modulation and sound frequency discrimination.
Methods: Here, we employed in vivo two-photon Ca2+ imaging in awake mice and operant behavior to determine the contribution of synaptic zinc signaling to the increased gain of A1 PNs and perceptual recovery after NIHL.
Results: We found that genetic or pharmacological elimination of synaptic zinc in specific A1 neurons blocked the increased cortical gain and the recovery of perceptual sound-detection thresholds after NIHL.
Conclusions: Synaptic zinc signaling contributes to A1 compensatory plasticity after NIHL.

SU8. Dynamic Membrane Potential Responses to Complex Sound Features in Mouse Auditory Cortical Fields

Chung-Wei Chiang*[1], Matthew McGinley[1]

[1]*Baylor College of Medicine*

Category: Auditory Cortex and Thalamus: Structure and Function
Background: Discriminating higher-order features in natural sounds is critical to animals. Many studies show that neurons in the auditory cortex have complex tuning characteristics, and the nonlinear spectrotemporal integration is one key determinant of such ability, and has been extensively studied at the cortical level. One critical, and understudied, component of this complex response is the subthreshold nonlinear synaptic integration by the membrane potential, which bridges the input and output of individual neurons. Here we compare, at single neuron level, the difference of subthreshold responses between simple and complex sounds, and among multiple auditory cortical fields.
Methods: We first identified different mouse auditory cortical regions by their tonotopic organization with imaging of GCaMP6 in Thy1 mice, and subsequently used the resulting map coordinates to systematically target the different cortical fields. Next, we performed in vivo whole-cell recordings from awake animals that

were passively receiving pure tone pips as well as log-frequency spaced, frequency-modulated (lsFM) stimuli. We structured the complex lsFM stimuli with 3 distinct and parametrically varied features: carrier frequency, bandwidth, and modulation rate.
Results: We found that neurons from different cortical regions had a uniform depolarization at sound offset but with distinct activities at onset and sustain period for both tone pip and complex lsFM stimuli. We are currently looking into the response characteristics corresponding to each lsFM property and quantifying differences between cortical fields.
Conclusions: By modeling the membrane potential integration, and dissecting how neurons dynamically respond to higher-order sound features, we should have a better understanding of the nonlinearity of spectrotemporal integration that takes place in the auditory cortex.

SU9. Spiral Ganglion Neurons Activation by pH Changes

Enrique Soto*[1], Katya Rosas[1], Esteban Urrieta[1], Rosario Vega[1]

[1]*Universidad Autonoma de Puebla*

Category: Auditory Nerve
Background: Since their discovery, Acid Sensing Ionic Channels (ASIC) were involved in chemo-nociceptive sensitivity. The ASIC participate in various sensory processes including nociceptive transduction, touch and pressure, somatosensory sensibility, smell, taste, and sight. Their role in hearing, and in the vestibular sensory processes is still not clear. ASICs are abundantly expressed in the afferent neurons of the cochlea and vestibular system. Where they mediate a significant excitatory input to these neurons.
Methods: In this work we have studied the functional responses of spiral ganglion neurons to fast pH changes (which leads to ASIC activation) and the subsequent depolarization and activation of voltage dependent calcium currents in isolated spiral ganglion neurons (SGN). The SGN were isolated from C2 Long Evans (P7-10) rats. Intracellular calcium changes were recorded by preloading the cells with Fluo-4 AM and fluorescence changes were followed using a Leica oil immersion 40x and a video intensified system. Fluorescence intensity and its changes were recorded using J image.
Results: As a positive control we studied the fluorescence changes induced by KCL (10 to 30 mM) perfusion of the SGN. KCl perfusion produced a concentration dependent change of the fluorescence of the SGN indicating that calcium fluorescence is a valid proxy of the SGN depolarization. The pH changes from pH 6.1, 5.5 and 4.5, produced a significant increase in the basal fluorescence of the SGN indicating the functional expression of ASIC in these neurons. In some neurons it was possible to detect calcium waves, although most of the cells showed a nearly stable basal fluorescence.
Conclusions: Our results demonstrate that ASIC activation produced a significant depolarization whose magnitude activates voltage gated calcium channels and allows the detection of ASIC channel activity by means of measuring the fluorescence change produced by an intracellular calcium channel fluorophore. It is known that processes related to the accumulation of extracellular H+ are capable of activating ASICs expressed at the synaptic and extra-synaptic levels. This mechanism has been considered potentially relevant in synaptic transmission and in metabolic induced responses of neuron networks.

SU10. Human Pluripotent Derived Neurons Survive in the Guinea Pig Cochlea

Lisa Beyer[1], Diane Prieskorn[1], Aleksandara Poole[2], Rami Skaliter[2], Dana Hayoun Neeman[2], Ofer Wiser[2], Olga Mizrahi[2], Yehoash Raphael*[1]

[1]*University of Michigan,* [2]*Lineage Cell Therapeutics, Inc*

Category: Auditory Nerve
Background: Loss of auditory nerve cells can lead to auditory neuropathy, even when the hair cells and the cochlear nucleus remain intact. Cell-based therapy for replacing lost or dysfunctional auditory neurons may restore hearing in these cases. In more severe cases, where both hair cells and many neurons are lost, the degree of success of a cochlear implant procedure may be enhanced by repopulating the cochlea with transplanted, functional auditory neurons. Here we tested a guinea pig model for eliminating neurons and assessing the fate of LCTANP1 derived human auditory neurons when transplanted into the cochlea.

LCTANP1 cells are human auditory neurons derived from proprietary pluripotent cells with direct differentiation to otic neuronal progenitors.
Methods: Initially, three young Hartley albino guinea pigs were given 5 µl of 10 mM ouabain via scala tympani and ouabain-soaked gelfoam on the round window. One month later, the cochleae were processed and assessed as plastic sections to confirm an extensive loss of spiral ganglion neurons in Rosenthal's canal. Then, a group of eight guinea pigs received ouabain in a similar way, and one month later their cochleae were infused with 5 µl of red fluorescent protein labeled LCTANP1 cells via a cochleostomy into scala tympani or via a needle inserted into the modiolus, both procedures at the base of the cochlea. Seven days later animals were euthanized, and ears were harvested for histology. Ears were fixed in paraformaldehyde followed by dissection using a fluorescence stereoscope to visualize and image the LCTANP1 labeled cells within the cochlea.
Results: In all animals, large areas of the cochleae contained red-labeled cells. The cells appeared mostly around the site of injection, but many cells migrated to adjacent flanking areas. Qualitative observations confirmed that both injection routes, into perilymph and into the modiolus, resulted in a large number of LCTANP1 cells a week after the injection.
Conclusions: Ouabain can be used to eliminate auditory neurons in the guinea pig. Human derived auditory neurons, LCTANP1 cells, can survive in the ouabain-traumatized cochlea for at least 7 days and spread to areas flanking the injection site.

Supported by Lineage Cell Therapeutics, Inc.

SU11. Enhanced Gain at the Ribbon Synapse May Constitute a Mechanism to Compensate Auditory Nerve Fiber Loss After Excitotoxicity

Artem Diuba[1], Jerome Bourien*[1], Sharon Kujawa[2], Jean-Luc Puel[1]

[1]*Institute for Neurosciences Montpellier, University of Montpellier, Institut National de la Santé et de la Recherche Médicale,* [2]*Eaton-Peabody Laboratories, Massachusetts Eye and Ear; Harvard Medical School, Harvard University*

Category: Auditory Nerve
Background: Noise overexposure can induce a loss of synapses between the inner hair cells (IHCs) and auditory nerve fibers (ANFs) without destroying the hair cells. Recent studies suggest that synapses regenerate spontaneously during the months following noise exposure (Hickman et al, Front Cell Neurosci, 2021 Aug 9;15:684706). Here, we investigate whether synapses can recover after round window infusion of kainate in gerbils.
Methods: Artificial perilymph (AP) containing 25 mM kainic acid (KA) was infused into the round window niche for 1 hour in young adult Mongolian gerbils. AP alone was infused in control gerbils. Cochlear function was assessed measuring distortion product otoacoustic emissions (DPOAEs) and compound action potentials (CAP) of the auditory nerve. Synapses were quantified using CtBP2 and GluA2 immunolabeling and confocal imaging. Morphological and functional assessments were performed at different cochlear locations from 2 to 16 kHz and different stages after drug application from 3 hours to 4 months. Spontaneous and sound-driven activity of single auditory nerve fibers were measured at 4 months post-KA.
Results: Although completely abolished 3 hours after the KA application, the CAP thresholds and amplitudes progressively recovered starting from the 1st day to 4 months, especially at 8 and 16 kHz where the CAP amplitudes reached their control values. Whatever the time point recorded, the amplitude of the DPOAEs remained mostly unaffected. Ultrastructural examination of the cochlea 3 hours after KA infusion showed swelling of the afferent terminals and disruption of the ribbon synapses. One day after KA infusion, less than 25% of cochlear ribbon synapses (re: control) were observed all along the tonotopic axis. Two weeks after, the partially recovered synapse count was still under 50% at all cochlear locations. In contrast to CAP amplitudes and thresholds, synapse counts did not fully recover 4 months post-KA, reaching 50% in the regions of 2, 4 and 8 kHz and 70% in the 16 kHz region. Single-fiber recordings at 4 months post-KA reveal that the regenerated ANFs exhibit higher spontaneous discharge rates leading to a better synchronization to sound onset as attested by the recordings of the peri-stimulus time histograms.

Conclusions: Our results show for the first time that the compound action potentials of the gerbil auditory nerve can fully recover from KA-induced excitotoxicity, despite persistent synapse loss. This phenomenon occurs because the regenerated fibers change their phenotype by responding more synchronously to sounds. Enhanced gain at the ribbon synapse may constitute a mechanism to compensate for ANF loss after excitotoxicity.

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SU12. Neural Response Recovery Despite Persistent Synapse Loss in the Noise-Exposed Gerbil: Characterization in Ears With and Without Hair Cell Loss

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Category: Auditory Nerve

Background: Synaptopathic noise exposure may preferentially target low-spontaneous rate auditory neurons. Previously, we reported on a model of noise-induced synaptopathy in gerbil in which hair cells remain intact. Here, we include higher-level exposures that produce outer hair cell (OHC) damage/loss in the cochlear base, where synaptopathy also is greatest. For all models, with and without OHC damage, we consider a battery of functional measures that may help predict the extent and frequency location of synapse loss, as well as the nature of the fibers/synapses affected and their patterns of recovery.

Methods: Mongolian gerbils (M,F; 14wk) were noise-exposed then tested, with age-matched controls, at one of five post-exposure time points, from 24hr to 36wk. We recorded sound-evoked distortion product otoacoustic emissions, compound action potentials, and peri-stimulus time responses, across a broad range of frequencies, as well as unstimulated spontaneous neural activity ('noise'). Hair cells and synapses were quantified in immunolabeled cochlear tissue.

Results: A single 2-hour octave-band noise exposure at 100 or 103 dB SPL yielded threshold shifts and response amplitude reductions (DPOAE, CAP) that recovered by 2wk post exposure, without hair cell loss. CAP and PSTR peak responses and spontaneous neural noise all recovered, substantially exceeding control values at some post-exposure times. PSTR plateaus also recovered but never exceeded controls. In the same ears, synapse loss was persistent, even 36wk post exposure. With increased exposure level (112 or 115 dB SPL), permanent synaptopathy was accompanied by permanent threshold shifts and persistent OHC injury or frank loss. For 112 dB exposure, permanent threshold shifts (to ~18 dB) and mild OHC loss (~18%) were restricted to the highest frequencies/cochlear regions evaluated. CAP amplitudes recovered incompletely, but more fully than DPOAE amplitudes. For 115 dB exposure, large threshold shifts and suprathreshold amplitude declines remained evident at the longest (36wk) post exposure time. OHC loss was largest in the extreme base. For both higher-level exposures, spontaneous neural noise declined significantly, with incomplete post-noise recovery.

Conclusions: For a range of noise exposure levels, we observed persistent synaptopathy with recovery of neural response thresholds and amplitudes and augmented spontaneous activity. Through multiple functional assays and offline analyses, we focused on characterizing this neural response recovery. Post-noise recovery, even overshoot of control values for high-SR dominated onset responses, was surprising given the synapse loss evident even in our longest held animals, perhaps signifying a compensatory mechanism. Our data suggest that there may be a range of noise doses that activates such a dynamic recovery process, whereas other exposures may be too low to activate it or too damaging to benefit from it.

Supported by the Office of Naval Research and the Department of Defense and by the Sheldon and Dorothea Buckler Research Fund

SU13. Open Board

SU14. Understanding Adaptation to Single-Sided Hearing Loss

Kehinde Adeaga*[1], Katherine Wood[1], Maria Geffen[1]
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Background: Learning is a fundamental function of the brain: sensory representations must be flexible to adjust to environmental changes, thus allowing us to adapt to the world around us. Understanding the neuronal mechanisms of learning are important not only for normal function of the brain but also in disease, for example, after hearing loss.
Methods: To study the role of feedback during learning, sound localization tasks provide a precise measure of spatial hearing performance. Unilateral hearing loss severely disrupts spatial hearing. Re-learning to localize sounds after unilateral loss requires corticofugal feedback projections from the auditory cortex (AC) to the inferior colliculus; ablating the feedback neurons prevented restoration of spatial hearing following prolonged unilateral ear plugging in ferrets (Bajo et al. 2009).
Results: Our goal was to test the time scale over which the feedback was required for spatial hearing restoration. We first designed a novel spatial sound localization task and tested whether optogenetically modulating the activity of cortico-collicular feedback affected the mouse performance on this task. Mice learned to respond to the stimulus location by licking or withholding the lick depending on whether the louder sound was presented on a specific side. 1 47th Annual ARO MidWinter Meeting Activation of cortico-collicular feedback led to a decrease in the accuracy of the performance of the mice during test trials (N = 2). Reaction time during Go and No-Go trials was not significantly affected by activation.
Conclusions: So far, our results suggest that mice can be trained reliably on the spatial localization task, and that the cortico-collicular feedback plays a role in their performance. We are presently repeating the experiment with a greater number of mice and beginning to measure the effect of ear-plugging on performance in the localization task. This experiment will ultimately reveal the time scale over which feedback from the cortex to peripheral structures acts to restore sensory function following sensory deprivation.

SU15. Myogenic Artifacts Masquerade as Neuroplasticity in the Auditory Frequency-Following Response (FFR)

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Category: Brainstem: Structure and Function
Background: The frequency-following response (FFR) is an auditory neurophonic that provides a "neural fingerprint" of complex sound encoding in the brain. FFRs have been widely used to characterize speech and music processing, experience-dependent neuroplasticity (e.g., auditory learning, musicianship, and biomarkers for hearing and language-based disorders that distort receptive communication abilities. It is widely assumed FFRs stem from a mixture of phase-locked neurogenic activity from brainstem and cortical structures along the hearing neuraxis. Here, we challenge this prevailing view by demonstrating upwards of ~50% of the FFR can originate from a non-neural source: contamination from the postauricular muscle (PAM) vestigial startle reflex.
Methods: Using EEG, we first establish PAM artifact is present in all ears, varies with electrode proximity to the muscle, and can be experimentally manipulated by directing listeners' eye gaze toward the ear of sound stimulation.
Results: We show this muscular noise easily confounds auditory FFRs, spuriously amplifying responses by 3-4x fold with tandem PAM contraction and even explaining putative FFR enhancements observed in highly skilled musicians.

Conclusions: Our findings expose a new and unrecognized myogenic source to the FFR that drives its large inter-subject variability and cast doubt on whether changes in the response typically attributed to neuroplasticity or pathology are solely of brain origin.

SU16. Examining Efferent Effects on Neural Processing of Concurrent Vowel Processing

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Category: Brainstem: Structure and Function

Background: Concurrent vowel mixtures have been used extensively to study how competing speech is represented in auditory nerve fibers of animal models. However, the use of such stimuli to study neural processing of competing speech in human listeners is sparse. The primary aims of this ongoing study are to: 1) parametrically examine concurrent vowel representation in the human auditory brainstem and 2) assess if attending to a target vowel in a concurrent mixture enhances its subcortical neural representation via efferent modulation.

Methods: Normal hearing listeners continue to be recruited for this study with a planned enrollment of 30 participants. Neural responses are evoked by concurrent vowel mixtures (/i/, /ɛ/, /u/, and /ɔ/) of one male (f0 = 130 Hz) and one female (f0 = 210 Hz) speaker. Each trial begins with a click-train cue presented at the rate of the target speaker's f0 (130 or 210 Hz) followed by 20 repetitions of a concurrent vowel mixture (token duration = 250 ms; ISI = 10 ms) for a total trial length of 5.2 seconds. 50 trials of each concurrent vowel mixture are collected for each cue type (male or female). Responses are collected in passive and active listening conditions with equal probability of male and female cues. In the passive condition, stimuli are presented while the listener watches a silent TV show or movie on a monitor with subtitles. During the active condition, listeners are asked to identify the vowel in the pair with the same pitch (130 or 210 Hz) as the preceding click-train cue. Neural data are filtered to extract brainstem (70-2000 Hz) and cortical (1-30 Hz) evoked potentials to click-train cues and concurrent vowel mixtures. Spectral amplitudes of brainstem responses at speaker f0 and formant frequencies are measured as a function of cue type (male or female) and listening condition (passive or active) to quantify brainstem attention effects. Similarly, cortical response amplitudes to click-train cues and concurrent vowel mixtures are used to quantify cortical attention effects.

Results: Preliminary results indicate robust attention effects on cortical responses to the click-train cue and concurrent vowel mixtures, consistent with similar previous literature. Brainstem spectral representations of speaker f0 and/or formants are not observed for male and female vowels in every concurrent mixture, likely due to energetic masking effects. There is not a systematic effect of attention on brainstem representation of target speaker vowels in conditions where f0 and formant neural components are clearly seen.

Conclusions: This project presents an efficient method for measuring brainstem neural responses to concurrent vowel mixtures, which may be useful for studying sensory processing of competing speech. A lack of attention effects on brainstem processing is consistent with some previous reports.

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SU17. Three-Dimensional Mapping of Isofrequency Bands in the Mouse Cochlear Nucleus With c-fos Expression Upon Hierarchical Acoustic Stimulation

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Category: Brainstem: Structure and Function

Background: Cochlear nucleus (CN), the first central processors in the auditory pathway, receives electrical signals from spiral ganglion neurons as well as transmitting complex auditory information. The neuron distribution in CN follow the frequency tonotopic map, with neurons on the ventral side receiving input from low-frequency fibers and neurons on the dorsal side receiving input from high-frequency fibers. This study aims to describe the frequency tonotopic map of the CN at the single-neuron level, objectively demonstrating

the iso-frequency band structures under different stimulus frequencies, and constructing a three-dimensional frequency tonotopic map of the cochlear nucleus.
Methods: After three quiet hours, we exposed Thy1-YFP16 mice to 8k, 17k, 26k, 35k and 44k at 60dB pure tone stimuli for one hour, respectively. Subsequently, we combined TESOS tissue clearing method with whole-tissue immunofluorescence staining to describe the expression of c-fos, an inducible neuronal activation marker, in the cochlear nucleus at the single-cell level. The images were acquired using a Leica SP8 confocal microscope, stitched using Fiji, and reconstructed in 3D using Imaris.
Results: Thy1-YFP16 mice is an ideal model for visualizing morphology of neurons in the subdivision of CN. There were scarcely any c-fos+ neurons observed under the quiet state, with the exception of a few signals in the granule cells layer. The continuous pure tone stimuli of 8, 17, 26, 35 and 44 kHz at 60dB of 1h generated clearly defined iso-frequency bands, with the arrangement of ventral to dorsal that represents low to high frequencies. Moreover, we observed the distance between iso-frequency bands and the edge of the cochlear nucleus is not linearly related to the corresponding frequency in spatial analysis, with bushy cells receive lower frequency input occupying a wider band, in agreement with the distribution of hair cells in the cochlea. Intriguingly, sound induced c-fos+ neuron responses were inhibited under anesthesia, even with increasing sound intensity. Three-dimensional reconstruction revealed over a thousand c-fos+ cells in both the AVCN and DCN, whereas only around 300 positively activated neurons appeared in PVCN. There is no difference in number of cells activated for each frequency band. Based on the characteristic genes identified by scRNA-sequencing (personal communication), we found that major types of neurons in the cochlear nucleus express c-fos, but the octopus cells and the unipolar brush cells located in the deep layer of DCN are two exceptions.
Conclusions: Whole-tissue immunofluorescence c-fos staining revealed the iso-frequency bands formed by activated neurons in the cochlear nucleus. Most types of neurons in different subdivisions of the cochlear nucleus are activated, although the number may vary to some extent.

SU18. Enhancement of Phase-Locking in Bushy Cells Benefits Binaural Coding in the Auditory Brainstem

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Category: Brainstem: Structure and Function
Background: Bushy cells in the mammalian anteroventral cochlear nucleus convey acoustic information from auditory nerves (ANs) to the superior olivary complex, where binaural neurons encode relevant cues for sound localization. Bushy cells are anatomically and physiologically subcategorized into two groups. Globular bushy cells (GBCs) that are innervated by ~20 AN fibers present so-called primary-like-with-notch peristimulus time histograms (PSTHs) in response to high-frequency tonal stimulation. Spherical bushy cells (SBCs) receive a small number of large endbulb synapses as well as a larger number of small bouton synapses, and mostly inherit the spiking patterns of AN fibers showing primary-like PSTHs. Previous physiological recordings reported, in several mammalian species, that both types of bushy cells show more prominent phase-locking than AN fibers for frequencies below 1 kHz. This enhancement of spike synchrony has long been hypothesized, yet remained unconfirmed, to be beneficial for temporal coding in binaural neurons.
Methods: In the present study, we investigate this question in a computational modeling framework of binaural circuity, in which the spiking pattens of bushy cells can easily be modified. We adopt the GBC model from our previous study and adjust it to the input configuration of SBCs. By varying the relative strengths of the modeled endbulb and bouton synapses, the degree of phase-locking can be systematically changed, while the overall spike rate is kept constant. The spiking output of the SBC model is then fed into a binaural neuron model of the lateral superior olive (LSO).
Results: In response to high-frequency tonal inputs for which no phase-locking occurs, the LSO model is sensitive only to the binaural intensity differences, regardless of the synaptic configuration of the SBC model. For amplitude-modulated tones with low-frequency envelopes, however, the LSO model varies its spike rate according to the envelope phase difference of the bilateral inputs. And the resulting modulation depth of LSO (i.e., the difference between the maximum and minimum spike rates) roughly linearly changes with the degree of phase-locking of the SBC model. We also test the hypothetical circuit configuration in which AN output is directly fed into the modeled LSO (in other words, bushy cells are considered as a simple relay with no

additional functions). Removing the active function of bushy cells leads to a reduced peak and trough of binaural phase-tuning curve in LSO.
Conclusions: These results provide computational evidence for the benefit of enhanced phase-locking in bushy cells for binaural coding and possibly for sound localization.

SU19. Unipolar Brush Cells Directly Target Fusiform and Cartwheel Cells and Can Drive Action Potential Firing

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Category: Brainstem: Structure and Function
Background: One of the key challenges of studying the auditory system is unlocking how sound is processed as it ascends through the brain. The dorsal cochlear nucleus (DCN) is crucial for this processing. It receives acoustic signals from the auditory nerve, as well as non-auditory signals from various sources, to perform complex functions such as canceling self-generated sounds and localizing sounds relative to the head and body. Understanding how this cerebellum-like circuit processes information requires knowledge about how the different cell types in DCN are connected and how their synapses transform signals. Granule cells receive multimodal sensory signals and project parallel fiber axons to fusiform cells, the principal output neurons of the DCN, as well as inhibitory cartwheel cells. Unipolar brush cells (UBCs) also receive multimodal input and are presumed to project to other UBCs and granule cells. However, their location in the deep layer of DCN, relatively distant from the granule cell domains, suggests the possibility that they target additional cell types.
Methods: Whole-cell patch-clamp recordings of fusiform cells, cartwheel cells, and vertical cells were made in acute brain slices from P15-24 mice, and patched cells were filled with biocytin. Transgenic mice were used to express channelrhodopsin-2 (ChR2) specifically in a subtype of UBCs to investigate their connectivity and synaptic effects. Slices were fixed and fluorescently labeled for confocal analysis of anatomical connections between ChR2-expressing UBCs and biocytin filled cells.
Results: Optogenetic stimulation of UBCs evoked EPSCs in fusiform and cartwheel cells with latencies averaging less than 4 ms, in addition to the longer latency polysynaptic EPSCs that were expected due to the UBCs' activation of granule cells averaging greater than 9 ms. The presumed monosynaptic latencies were similar to those of EPSCs recorded in granule cells evoked by the same optogenetic stimulation. In current clamp, UBC stimulation drove a population of fusiform and cartwheel cells to fire action potentials, suggesting that they have a significant influence on the activity of these cells. Confocal imaging of biocytin-filled cells revealed putative anatomically defined synaptic contacts between UBC axons and fusiform cells.
Conclusions: Our data support the conclusion that UBCs target fusiform and cartwheel cells directly, in addition to granule cells, and can drive them to fire independently. These findings, combined with previous knowledge that UBCs amplify signals, suggest that this pathway may have a more direct influence on the output of DCN than previously appreciated.

SU20. Glial Glutamate Transporters are Essential for Auditory Coding in the Ventral Cochlear Nucleus

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Category: Brainstem: Structure and Function
Background: Auditory signaling in the cochlear nuclei (CN) is mediated by the transmitter glutamate acting on fast-gating postsynaptic AMPA receptors. Following generation of the EPSP, glutamate rapidly diffuses away and is taken up by glial glutamate transporters (e.g., EAATs1-3). At many synapses, this uptake process is slow compared to the timescale of fast transmission. We explored how this removal process impacts signaling by T-stellate neurons of the ventral CN. These neurons ably encode signal intensity by effectively integrating auditory nerve inputs that fire as sound level is elevated. We recorded from T-stellate cells in brain slices from mouse, stimulated auditory nerve fibers electrically, and used the selective transporter blocker TBOA to examine how transporters regulate the signaling at auditory nerve synapses.

Methods: Slices were made from mice aged P18-27. Auditory nerve fibers were stimulated with glass or metal electrodes, and the intensity or duration of the stimulus was used to vary the number of auditory nerve inputs. T-stellate cells in PVCN were identified by their apparent morphology, soma size, and tonic firing properties, and were recorded in either voltage or current clamp modes using a patch-clamp amplifier.
Results: In control solutions, each strong EPSP generated a single spike regardless of the stimulus rate, and thus spiking quickly terminated after the period of activity. As more fibers were recruited in a stimulus, more spikes were obtained up to a saturating level. By contrast, at low stimulation rates, transporter blockade caused doubling of spikes upon each stimulus, while at high frequencies the neurons continued to fire for hundreds of milliseconds after the end of the stimulus train. A linear relation between stimulus number and spike output was lost upon transporter blockade. Delayed firing after the stimuli was caused by a large buildup of glutamate, evidenced by an exceedingly slow decay of synaptic current that was blocked by AMPAR blockers. Increasing the number of active auditory nerve synapses by varying stimulus duration produced more profound effects of the transport blocker, and maximal firing was reached by activating fewer fibers.
Conclusions: Transporters rapidly clear glutamate at T-stellate cell synapses, effectively isolating transmitters from nearby synapses to prevent pooling and spillover. As a result, these neurons can respond to increases in the rate presynaptic spikes and number of active synapses with parallel increases in postsynaptic spike output. Thus, the activity of glial cells is essential to fast coding of signals in CN.

SU21. Structure-Function Effects in the Auditory System After Impact Acceleration Traumatic Brain Injury in CBA/CaJ Mice

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Category: Brainstem: Structure and Function
Background: Hearing deficits are associated with traumatic brain injuries (TBIs) acquired from car accidents, falls, or a hit to the head. Diffuse axonal injury (DAI) caused by TBI involves shearing of axons in the brain which leads to functional consequences for the auditory system. Impact acceleration-TBI (IA-TBI) models the physical force of DAI allowing the causal study of its hearing implications using a mouse model. By measuring auditory function before and after IA-TBI and examining histopathology in the auditory brainstem we can link structural damage to functional deficits. Here we aimed to provide a characterization the short-term effects of auditory system structure and function after IA-TBI by assessing: 1) auditory brainstem responses (ABRs), 2) evaluating waveform morphology, and 3) assessing brainstem neuropathology in auditory regions before and 3 days after injury.
Methods: Subjects were 2 month old CBA/CaJ mice (N = 7, 3 male 4 female, 4 IA-TBI 3 Sham). ABRs were recorded to clicks and tone bursts (8, 12, 16, 24, and 32kHz; 10-90 dB SPL) at baseline and 3 days after IA-TBI. ABR waveforms were analyzed using custom software (https://github.com/mattbke63/Auditory-Brainstem-Response-Waveform-Analysis) for group comparisons. Subjects were perfused transcardially, and tissue was postfixed for 7 days. Brains were embedded in gelatin, coronally sectioned, and stained using a Gallyas silver kit for signs of neurodegeneration. Key structures of the auditory brainstem and midbrain were compared between IA-TBI and sham subjects including the cochlear nucleus, superior olivary complex, lateral lemniscus, and inferior colliculus; the cerebellum served as a positive control.
Results: We found a minimal non-significant ABR threshold increase in IA-TBI subjects compared to shams. Analysis of waves 1-5 of the ABR revealed a slight decrease in the amplitude of waves 2 and 4. Silver was assessed semi-quantitatively by region, with the amount of silver ranging from absent (0), to partial (.5) to high (1). There was statistically significantly more silver stain in IA-TBI subjects than sham subjects in the cochlear nucleus, lateral lemniscus, inferior colliculus, and the cerebellum, consistent with neurodegeneration in auditory brain regions from IA-TBI. There was a positive trend between higher amounts of silver and reduced ABR wave amplitudes, in particular with the amplitude of wave 2 correlating with a greater presence of silver in the cochlear nucleus.
Conclusions: Mice with IA-TBIs have worse auditory function and neurodegeneration in auditory regions of the brain. These findings are consistent with the idea that TBIs acutely lead to hearing deficits, and that analysis of the ABR wave morphology may help to reflect underlying brain trauma not reflected in auditory thresholds.

SU22. Effects of C1qa Deletion on Auditory Brainstem Development

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Category: Brainstem: Structure and Function

Background: Neural circuits in the auditory brainstem compute interaural time and intensity differences used to determine the locations of sound sources. These circuits display features that are specialized for these functions. The projection from the ventral cochlear nucleus (VCN) to the medial nucleus of the trapezoid (MNTB) body travels along highly myelinated fibers and terminates in the calyx of Held. This monoinnervating synapse emerges during development as multiple inputs are eliminated. We previously demonstrated that elimination of microglia with a colony stimulating factor-1 inhibitor results in impaired synaptic pruning so that multiple calyceal terminals reside on principal cells of MNTB. This inhibitor also resulted in impaired auditory brainstem responses (ABRs), with elevated thresholds and increased peak latencies. The mechanisms underlying these effects are not known. Here we investigated the role of the classical complement pathway initiator, C1q, in auditory brainstem maturation.

Methods: We investigated the expression of C1q in the developing auditory brainstem using immunolabeling and fluorescent microscopy. We used super-resolution imaging to assess co-localization with calyces and synaptic proteins. We used anatomical tracing methods with confocal microscopy and 3D reconstruction to determine whether pruning of calyceal terminals was impaired in C1qa-/- mice. We measured ABRs in P28 mutant and wild type control mice.

Results: We found C1q expression throughout MNTB during postnatal development, with variation across the topographic axis. Expression in medial regions decreased after hearing onset while expression in lateral regions increased after hearing onset. C1q expression was associated with microglia and strong expression was seen in close apposition with both RDA labeled and VGlut1/2 labeled calyces of Held. We showed that the source of C1q in the developing brainstem is likely microglia, as expression was absent after microglia depletion and returned with microglial repopulation. These results are consistent with the view that microglia deposit C1qa protein onto developing calyces of Held. While calyceal pruning appeared normal in C1qa-/- mice, ABR measures showed decreased peak latencies compared to wild type mice.

Conclusions: Our findings show that C1q is produced by microglia in the developing auditory brainstem and suggest that C1q is apposed to or deposited near calyces of Held. C1q has been shown to have a role in synaptic pruning in some, but not all, regions of the nervous system. Our data suggest that this role does not extend to pruning of the excitatory projections from VCN to MNTB. However, deletion of C1q results in alterations of auditory brainstem function. Future studies will explore the mechanisms through which C1q acts on auditory circuits.

SU23. A Comparison of Auditory Brainstem Response for Chirp Stimuli Across Three Different Manufacturers

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Category: Brainstem: Structure and Function

Background: The use of broadband (BB) and narrow-band (NB) chirp stimuli in auditory brainstem response (ABR) and Auditory steady-state responses (ASSR) testing continues to grow, owing to larger amplitude responses compared to their counterparts (Sininger, 2019). ABR equipment manufacturers have produced their own chirp stimuli differing in timing, magnitude, and bandwidth, impacting ABR amplitude and latency. While most studies compare differences in BB chirps versus clicks and NB chirps versus tone-burst stimuli, studies have yet to compare commercially available chirp stimuli among manufacturers. This study compares the ABR obtained from normal-hearing adults using Interacoustics Level Specific (LS) CE-Chirp, Vivosonic VF Chirp, and Intelligent Hearing Systems iChirp in terms of ABR wave V latency and amplitude across multiple frequencies and intensities.

Methods: The study enrolled sixteen participants with normal hearing, defined as thresholds at 20 dB or lower across frequencies ranging from 250 to 8000 Hz. ABR was measured for BB chirp, 500Hz NB chirp, and 4000Hz NB chirp using equipment from three different manufacturers. A 2-channel recording montage was

utilized, employing M1 and M2 as inverting electrodes, the high forehead as the non-inverting electrode, and the mid-forehead as the ground, following standard ABR recording procedures.
Results: The study's results indicate significant differences in wave V latency and amplitude across manufacturers for all three chirp stimuli considered. For BB chirp, iChirp had longer latencies than CE-chirp and VF Chirp; for 500Hz, iChirp and VF chirp had longer latencies than CE-chirp. No latency differences were observed between chirp stimuli for 4000Hz. CE chirp and iChirp consistently had larger amplitudes than VF chirp for wave V amplitude.
Conclusions: Altogether, significant differences exist in ABR latency and amplitude generated by the three manufacturers' chirp stimuli. Clinicians must be aware of the differences in responses for educated use of the different equipment. This information will also inform clinicians about the potential benefits of one piece of equipment over another for specific diagnostic tasks.

SU24. Impact of Scn2a Haploinsufficiency on Auditory Processing Abnormalities in an ASD Mouse Model

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Category: Brainstem: Structure and Function
Background: Auditory processing abnormalities, including auditory hypersensitivity and diminished auditory spatial attention, are notable and frequent characteristics of autism spectrum disorder (ASD). The Scn2a gene, which encodes the sodium channel NaV1.2, is recognized as a significant genetic determinant for ASD.
Methods: In this study, we investigate the influence of Scn2a haploinsufficiency on the cellular, synaptic, and behavioral functions within the auditory brainstem circuitry, using conventional Scn2a heterozygous mice (Scn2a+/−) that have a loss-of-function mutation in Scn2a.
Results: Through in vivo auditory function tests, Scn2a+/− mice demonstrated normal responses in distortion product otoacoustic emissions (DPOAE) and auditory brainstem responses (ABRs). However, a noteworthy observation was the pronounced startle response that Scn2a+/− mice displayed to sudden, loud auditory stimuli in the acoustic startle response (ASR) tests. This response is indicative of heightened sensory perception. Furthermore, whole-cell recordings of medial nucleus of the trapezoid body (MNTB) principal neurons did not show significant differences in the intrinsic properties of MNTB neurons between the Scn2a+/− mice and wild-type mice during auditory brainstem development.
Conclusions: These data suggest that while Scn2a haploinsufficiency does not appear to affect auditory transmission (as evidenced by normal ABRs), it does impair sensory gating, resulting in increased ASR. The findings offer valuable insights into the role of Scn2a in auditory processing abnormalities associated with ASD.

SU25. Inferior Colliculus Neurons are Sensitive to Sub-Millisecond Variations in Sound Onset Duration

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Category: Brainstem: Structure and Function
Background: Rapid acoustic transients, including onsets, are prominent in natural sounds. Examples include speech, such as after gaps, and adventitious sounds, such as rustling noises. Octopus cells (OCs) in the cochlear nucleus are highly responsive to these rapid onsets and drive precisely timed inhibition in the inferior colliculus (IC) via the ventral nucleus of the lateral lemniscus. However, the function of OC-driven onset inhibition in the IC remains unknown. There are several divergent models for the role of this onset inhibition in the circuit function of the IC, including suppressing spectral splatter or increasing sensitivity to acoustic transients.
Methods: To begin to test these models, we examined the impact of onset gate duration in Neuropixel recordings of neural responses to single tones in IC of mice and computational models of cochlear response. We characterized the theoretical limit, and simulated cochlear extent, of spectral splatter in natural sounds and in tones with varying onset gate durations and find that inherent spectral splatter due to sub-millisecond

differences in onset gate duration is closely recapitulated by simple cochlear models. During presentation of single tones with a range of onset gate durations, we record neural response properties in the IC of head-fixed, awake mice.
Results: We find that sub-millisecond gate duration affects tone-evoked firing by up to 2-fold (N=3 mice; n=172 neurons) particularly at carrier frequencies away from the neuron's best frequency, indicating that population responses in IC are strongly impacted by spectral splatter.
Conclusions: In ongoing work, we are investigating the interaction of the spectral content of rapid acoustic events, via presentation of a narrow, gaussian gated, bandwidth-limited carrier (which we call a 'clicklet'), with ongoing sound. Ongoing analyses are determining the implications of our results for models of the function of rapid onset inhibition in IC.

SU26. Dynamic Changes in Pre- and Post-Synaptic Markers of Neurotransmission Across the Inferior Colliculus in a Model of Noise Induced Temporary Threshold Shift

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Category: Brainstem: Structure and Function
Background: Our previous imaging studies using manganese (Mn2+) as a surrogate for calcium, show changes in Mn2+ uptake after a single noise exposure (NE). This demonstrates a relationship between noise-induced permanent or temporary threshold shift (PTS or TTS) and increased neuronal activity in the inferior colliculus (IC). 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 post-synaptic CaVs with changes in pre-synaptic markers of neurotransmission in central auditory nuclei may provide important evidence for identifying mechanisms contributing to the imbalance between excitation and inhibition that occurs after NE. Therefore, in the current study, we examined the distribution of CaV1.3 and vGluT1 (excitatory pre-synaptic) in auditory nuclei following noise-induced TTS and CaV blockade.
Methods: Sprague-Dawley rats were divided into three groups: saline injection only (Saline), saline injection before noise exposure (Noise), and verapamil injection before noise exposure (Verapamil). The groups were administered either verapamil or saline intraperitoneally. After treatment, the noise groups were exposed to a 16 kHz, 106 dB SPL tone for one hour, while Saline group was maintained in ambient noise conditions for one hour. Five days after treatment, following transcardial perfusion, rat brains were collected, post-fixed, and cryoprotected. Serial cryostat sections (40 μm) were collected for immunolabeling. The density and intensity of Immunolabeled CaV1.3 and vGluT1 were compared across groups in subdivisions of the CN and IC.
Results: In the Saline 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. The intensity of CaV1.3 immunolabeling in the Noise and Verapamil groups was increased compared to the Saline group. The intensity of vGluT1 immunolabeling was decreased in the Noise group compared to the Saline group. However, the intensity of vGluT1 labeling was highest in the Verapamil group. Noise reduced both CaV1.3 and vGluT1 immunolabeling in the dorsal cortex of the CN. The intensity was further reduced in the Verapamil group.
Conclusions: The differential distribution of CaV1.3 across IC subdivisions correlates with the tonotopic organization of the IC. Following NE, our CaV1.3 result suggests an upregulation of CaVs, which may underlie the greater activity following NE observed in previous studies of the IC. Our results in the CN correlate with the literature and may be an indicator of CaV1.3 downregulation. These findings suggest sustained changes in synaptic transmission with NE that is not prevented by CaV blockade. Future studies should further delineate changes in distribution and levels of CaV1.3 over time.

SU27. Biochemical Diversity Amongst Calyx-Only Afferents in the Vestibular Nuclear Complex

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Category: Brainstem: Structure and Function

Background: The peripheral vestibular end-organs are innervated by regular and irregular afferent fibers which slowly and rapidly adapt to stimuli, respectively. The range of rapid vestibular stimulation to which irregular fibers can adapt is relatively broad with some afferents demonstrating a wider range than others. This observation leads to the suggestion that this functional heterogeneity among irregular afferents may result from biochemical heterogeneity, but this interpretation has not been proven. To begin to address this gap in knowledge, calyx-only afferents were assessed in the vestibular nuclear complex (VNC). Calcium buffering proteins (CBPs) have been used to distinguish amongst vestibular ganglion neurons. 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. Calyx-only neurons are excitatory. Vesicular glutamate transporters 1 and 2 (vGluT1 and vGluT2) have been previously used to define subsets of excitatory neurons. Therefore, colocalization of calretinin with vGluT1 and/or vGluT2 in the VNC may prove useful in discriminating subsets of calyx-only afferents.

Methods: Following transcardial perfusion (4% paraformaldehyde), Sprague-Dawley rat brains were collected, post-fixed, cryoprotected, and serially sectioned (40 μm) using a freezing sliding microtome. Immunohistochemistry was performed for calretinin and vGluT1, or calretinin and vGluT2 in free-floating sections containing either rostral or caudal subdivisions of the VNC.

Results: Puncta that were immunolabeled for vGluT1-2, 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 and vGluT2 was localized to terminals and had a distribution that correlated well with previous reports in the literature. In the spinal vestibular nucleus, puncta immunolabeled for both calretinin and vGluT1 showed less than 95% overlap. However, immunolabeling for calretinin and vGluT2 resulted in 75% of colocalized puncta.

Conclusions: Our data indicate that subpopulations of irregular afferent fibers exist and project to the VNC. These subpopulations may have different functional properties, such as firing rate, which may be attributed to the subtype of vGluT being utilized. Given that the rate of adaptation to stimuli can be end-organ specific (e.g., canal vs otolith), one possibility is that fibers using vGluT1 or vGluT2 vs vGluT1 and vGluT2 may have different end-organs of origin. Also, these irregular fiber subpopulations may have differential effects on descending vestibular pathways, affecting movement and posture. Future studies will use this biochemical signature to evaluate the contributions of these subpopulations to vestibular function centrally. In addition, the effects of mechanically- and noise-induced over stimulation will be evaluated in calyx-only subsets within the VNC.

SU28. A Systems-Neurophysiologic Toolkit for Studying Dorsal Cochlear Nucleus' Role in State-Dependent Spatial Saliency

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Category: Brainstem: Structure and Function

Background: Early auditory spatial processing by the dorsal cochlear nucleus (DCN) integrates acoustic information with multimodal inputs from the head, neck, and ears. This integration has been proposed to be critical to ethological hearing functions, including localizing sounds in the vertical plane or suppressing responses to self-generated sounds (Oertel and Young, 2004). It has been difficult to test these models, due to the technical challenges of recording from specific cell types in the DCN, as well as a complete inability to selectively manipulate them. Here we have developed a head-fixed behavioral approach in mice to study spatial sound processing and molecular-genetic transgenic mouse lines to record from and manipulate DCN cell types during behavior.

Methods: In the auditory spatial oddball paradigm, sounds are played rarely from an unexpected spatial location. We track pupil size, as a measure of arousal state and sound saliency, as well as treadmill wheel movements, and also perform videography of the ear position and movement. Our results show that the pupil dilates more to sounds from an unexpected spatial location.

Results: To explore the underlying neural processes, we are developing and validating mouse lines to use the cre-lox system to target fusiform (necab2-cre, nts-cre), granule (gabra6-cre), cartwheel (Stac-cre, foxp2-cre, lepr-cre), and vertical/tuberculoventral (penk-cre) cells within the DCN, based on our recent atlas of gene expression in the cochlear nucleus (Junzhan et al., 2023).
Conclusions: In ongoing work, we are developing 3D video tracking for volumetric video rendering of ear position in order to track ear state, with the goal of performing causal manipulations of the DCN cell-types. We expect that these approaches will shepherd the next generation of deeper understanding DCN circuit function, including the role of multimodal inputs.

SU29. Behavioral Task Modulates Neural Activity in the Dorsal Inferior Colliculus Neurons of Mice

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Category: Midbrain: Structure and Function
Background: Sensory perception can vary depending on different behavioral contexts. The activity of neurons throughout the mammalian brain has been shown to be modified during task engagement in many sensory modalities, and the auditory system is no exception. Many previous studies have shown that task engagement modulates neural activity at higher levels in the auditory pathway, including the thalamus and cortex. A recent study also found that task-engagement-related modulation is present as early as in the inferior colliculus (IC). Understanding the role of task engagement-related modulation in IC is important to understanding the process of how perception changes under different listening conditions. Such knowledge will prove to be highly valuable as more sophisticated hearing aid devices are developed, whose properties can change with listening context. Currently, it is not clear if modulation occurred throughout the IC or if it is specific to particular subregions. The spatial pattern of the modulation is also not clear. In this study, we used 2-photon microscopy to study the neural activity in the dorsal IC under task and passive conditions to look for answers to the above questions.
Methods: The F1 progeny of Tg(Thy1-jRGECO1a)GP8.20Dkim/J (RCaMP) strain mice crossed with CBA/CaJ strain were used in the experiment. The mice were trained to perform a 2-alternative-forced-choice (2AFC) behavioral paradigm where they lick one of the two tubes when they heard a pure tone linked to that tube to get the reward. After the mice reached a high performance level, a cranial window was opened over the IC with a glass well covering it. Then the mice's dorsal IC neurons were imaged using 2-photon microscopy while they were performing the same 2AFC task or they were passively listening to the same pure tone stimuli.
Results: The neurons in dorsal IC showed different response types to the pure tone stimuli which were either excitatory or inhibitory. Comparing the neural activity to pure tone stimuli in the 2AFC task and passive listening condition revealed that the task modulated the neural activity mostly in an excitatory manner. By training a support vector machine algorithm to classify the neural population activity into task or passive conditions with a subset of the data, it could predict the class of the held-out data with high accuracy which showed that the neural population activity carried distinct information between active and passive conditions.
Conclusions: Our study shows that dorsal IC neural activity is modulated by task-engagement. It raises further questions as to what the mechanism of this modulation is and whether cortico-collicular projections are involved in this modulation which require further investigation.

SU30. Predicting Auditory Midbrain Responses to Natural Sounds With Interpretable Gabor Integrate and Fire Receptive Field Models

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Category: Midbrain: Structure and Function
Background: Spectrotemporal receptive fields (STRFs) are widely used in auditory neuroscience to model the time-frequency sensitivity of auditory neurons. Frequently, STRFs are derived using unbiased synthetic stimulus ensembles, such as dynamic ripples, which can easily be estimated using spike-triggered averaging.

When using natural sounds, decorrelation and regularization techniques are needed to remove residual stimulus correlations that can distort the estimated STRFs. Furthermore, nonlinearities and non-stationarities (such as adaptation) make it difficult to predict neural responses to natural sounds.
Methods: We obtained neural recordings from the inferior colliculus of unanesthetized rabbits in response to both a sequence of natural sounds and dynamic moving ripples (DMR). We developed a model-based approach for deriving auditory STRFs and predicting single trial spike trains to either the DMR or natural sounds. The model consists of a nine parameter Gabor STRF (gSTRF; Qiu et al. 2003), which accounts for the neuron's spectro-temporal integration of the stimulus and a four parameter nonlinear integrate-and-fire compartment which incorporates intrinsic noise, cell membrane integration, and nonlinear thresholding in order to generate simulated output spikes. We used Bayesian optimization to fit neural data and derive optimal model parameters by maximizing the model's log-likelihood.
Results: To validate our spiking gSTRF model, we compared the optimal gSTRFs with more common approaches including regularized regression and generalized linear model (GLM) and subsequently compared the spike train predictions obtained from each model. We also carried out these comparisons with simulated data where the "ground truth" STRF and spiking activity was known a priori. Although regression approaches produced elongated STRFs and did not match the original model, we demonstrate that the gSTRF converges to the original simulation parameters and replicates the spiking activity from the original simulations down to millisecond precision. Similar observations were observed for real neural data. Regression based STRFs were elongated and spectrally smeared, whereas gSTRFs produced compact receptive fields in time and frequency. The optimal gSTRF parameters, including the neuron's best frequency, delay, and best temporal and spectral modulation frequency are interpretable and provide insight into the neuron's integration. Furthermore, when we used the estimated gSTRFs to re-simulate neural activity with the spiking model to natural sounds, the measured regression STRFs were once again spectro-temporally smeared, indicating residual stimulus correlations were not effectively removed. By comparison, for this same simulated spike train, the optimized gSTRF produced compact receptive fields that matched the original, suggesting the optimization approach more effectively removes natural stimulus correlations that bias the estimated receptive field structure.
Conclusions: This new approach allows one to derive auditory STRFs and predict neural spiking activity to natural sounds using functionally interpretable basis functions. The small number of functionally interpretable parameters make exploration of nonlinear and nonstationary effects due to natural sound statistics more feasible.

SU31. Effect of Spectro-Temporal Characteristics of Sound Sequences and Anesthesia on Sound Processing in the Inferior Colliculus

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Category: Midbrain: Structure and Function
Background: The auditory system has evolved to extract behaviorally relevant information from the continuous flux of sound stimuli. During communication many species use rhythmic calls with typically short time intervals. The common practice in the field, however, is to use relatively long inter-stimulus intervals (ISIs) when studying response properties of auditory neurons. Further, many labs perform these experiments under anesthesia. However, it is not clear whether these results reflect auditory neurons performance during animals' communication. The main objective of our research was to investigate how neurons in the auditory midbrain process sound sequences with species-specific rhythmic structure in unanesthetized mice.
Methods: Extracellular recordings were conducted in the inferior colliculus (IC) of 3–6-month-old CBA/CaJ mice with glass microelectrodes. Once a single neuron was isolated, its spiking responses to a sequence of pure tones 60 ms duration were recorded. Each sequence contained 2,993 combinations of different sound frequencies (4-40 kHz, 500 Hz step) and sound levels (0-80 dB SPL in 2 dB step) presented pseudo-randomly. Every IC neuron was tested with four identical sequences presented at different ISIs (500ms, 300ms, 100ms, and 70ms) in unanesthetized mice. These data were used to reconstruct frequency response areas (FRAs) for every IC neuron. Several of these experiments were performed under ketamine/xylazine anesthesia which is typically used for mice anesthesia.
Results: In this study, we found that all IC neurons demonstrated similar FRAs at four different ISIs. About half of these neurons showed an enhancement in their responses when stimulated with shorter ISIs, which are

behaviorally relevant. However, when we conducted experiments under ketamine/xylazine anesthesia, we observed significant alterations in the FRAs of IC neurons. Almost all neurons in the anesthetized group exhibited considerably higher response thresholds, and there was a noticeable reduction in the diversity of FRA shapes when compared to the unanesthetized group.
In fact, the majority of neurons in anesthetized mice exhibited a similar, classic V-shaped FRA pattern.

Conclusions: Overall, short, or behaviorally relevant ISIs have little effect on sound processing in unanesthetized mice. Response enhancement in a selected group of IC neurons under short ISIs further advocates for the use of these ISIs during assessment of the auditory system performance in sound processing. The effect of ketamine/xylazine anesthesia should be considered during interpretation of experimental results obtained at least under this type of anesthesia.

SU32. Lineage-Tracing Reveals an Expanded Population of NPY Neurons in the Inferior Colliculus

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Category: Midbrain: Structure and Function
Background: Growing evidence suggests that neuropeptide signaling shapes auditory computations. We previously showed that neuropeptide Y (NPY) is expressed in the inferior colliculus (IC) by a population of GABAergic stellate neurons and that application of NPY strongly regulates local circuits. NPY neurons were initially characterized using the NPY-hrGFP reporter mouse in which hrGFP expression reports current expression of NPY, i.e., an expression-tracing approach. Since NPY expression can vary dramatically, the hrGFP mouse might miss NPY neurons that are not expressing NPY proximal to the experiment date. We hypothesized that NPY is expressed by a larger population of IC GABAergic neurons than previously reported. We used a lineage-tracing approach to irreversibly tag neurons that expressed NPY at any point prior to the experiment. We compared the physiological and anatomical features of neurons labeled with the lineage-tracing approach to our prior data collected with an expression-tracing approach.
Methods: To selectively target NPY neurons, we crossed NPY-IRES2-FlpO mice with Ai65F reporter mice (lineage-tracing) or used the NPY-hrGFP reporter mouse (expression-tracing), allowing us to visualize NPY neurons by expression of tdTomato or hrGFP. To validate the NPY-FlpO x Ai65F mouse we performed in situ hybridization with probes targeted to Vgat, Npy and tdTomato. We next compared the distribution and soma size of tdTomato+ and hrGFP+ neurons across IC subdivisions. We then performed whole-cell current clamp experiments to evaluate the intrinsic physiology of tdTomato+ and hrGFP+ neurons.
Results: We found that 92.9% of tdTomato+ neurons co-label with Vgat and 75.6% co-label with Npy. While the GABAergic phenotype is similar among mouse lines (92.9% vs 98.5%), a larger proportion of neurons co-label with NPY in the hrGFP reporter line (75.6% vs 94.7%). tdTomato labels a larger population of neurons than in the hrGFP reporter line, resulting in a different distribution across IC subdivisions and more variability in soma size. Despite these anatomical differences, tdTomato+ neurons exhibited sustained firing patterns and stellate morphology, consistent with the properties in the NPY-hrGFP mouse. Using principal component analysis, we found that the physiological properties of tdTomato+ and hrGFP+ neurons almost completely overlap and were not separable into distinct clusters.
Conclusions: Our data suggest that a lineage-tracing approach reveals a larger population of NPY neurons than found with an expression-tracing approach. Despite some differences in anatomical features, NPY neurons from both labeling approaches had similar physiological and morphological properties, suggesting that they are part of the same neuron class. This suggests that NPY expression may be transient in some neurons during development and/or is strongly regulated by intrinsic or extrinsic factors, such that some NPY neurons do not express NPY under our standard experimental conditions.

SU33. Cortical Responses Time-Locked to Continuous Speech in the High-Gamma Band Depend on Selective Attention

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Category: Primary Auditory Cortex

Background: Auditory cortical responses to speech are one mechanism by which the auditory system preserves temporal information about sounds. Such responses measured by magnetoencephalography (MEG) show robust speech tracking in the high-gamma band (70-200 Hz) that is time-locked to the fundamental frequency (F0) of speech. However, little is currently known about whether these responses depend at all on the focus of selective attention.

Methods: In this study we investigate differences in high-gamma cortical responses to male and female speech. We address whether these responses, thought to originate from primary auditory cortex, depend on selective attention. Twenty-two human subjects listened to concurrent speech from male and female speakers and selectively attended to one speaker at a time while their neural responses were recorded with MEG. The male speaker's pitch range coincided with the lower range of the high-gamma band. In contrast, the female speaker's pitch range was higher, and only overlapped the upper end of the high-gamma band. Neural responses were analyzed using the temporal response function (TRF) framework.

Results: As expected, the responses demonstrate robust speech tracking in the high gamma band, but only to the male's speech. Responses present with a peak latency of approximately 40 ms indicating an origin of primary auditory cortex. The response magnitude also depends on selective attention: the response to the male speaker is significantly greater when male speech is attended than when it is not attended.

Conclusions: This is a clear demonstration that even very early cortical auditory responses are influenced by top-down, cognitive, neural processing mechanisms.

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SU34. Pioneering Cortical Assays of Gap Detection to Explore Temporal Processing in Chinchilla Using a Multi-Channel Mini-EEG Cap

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Category: Primary Auditory Cortex

Background: Precise encoding of temporal envelope – slow amplitude modulations of acoustic stimuli – is essential for perception of speech and species-specific communication. The ability to utilize temporal processing cues varies amongst individuals and can deteriorate with age, peripheral pathology, or central auditory system changes. Animal models of hearing provide a controlled way to explore the consequences of aging and hearing loss on temporal processing. Here, we measured a cortical correlate of gap detection, a well-established tool for evaluating the encoding of envelope fluctuations, by recording EEG responses in chinchillas. While subcortical components of auditory evoked potentials in small-animal models using subdermal electrode placements are well-defined, the realm of animal studies concerning auditory-evoked cortical responses that are more spatially complex remains largely unexplored. To address this, we adapted a 32-channel mini-EEG cap (Cortech Solutions' ActiveRat) to non-invasively record cortical potentials from chinchillas to gain insight into processing of real-world stimuli along the auditory pathway.

Methods: In this ongoing study, we are recording cortical responses in chinchillas (Young Normal Hearing; Middle-Aged Normal Hearing; TTS-exposed) to varying gap durations (16, 32, and 64 ms) within a 4000 Hz tone embedded in a 4000 Hz octave-band noise. A light sedation protocol (ketamine/acepromazine/atropine) was iteratively developed and utilized to minimize movement artifacts while preserving cortical responses. Data is simultaneously recorded from the mini-EEG cap and three subdermal electrodes (mastoid, vertex, ground) using the BioSemi ActiveTwo system. EEG data is analyzed using the same framework (Python, MNE) used to process these measures in humans (to support cross-species translation).

Results: Preliminary data suggest the presence of cortical responses to the three gap durations - 16, 32, and 64 ms. A trend of increasing amplitude of the gap response with increasing gap duration is also observed. The waveform morphology recorded by the EEG cap and subdermal electrodes appears similar, though it is observed that the SNR of the responses from individual channels in the mini-cap is marginally lower than the

subdermal electrodes. This can be mitigated by optimizing a subset of channels to aggregate data and increasing the number of trials in the data acquisition process.
Conclusions: Eliciting reliable cortical responses to fundamental gap stimuli in an animal model is the gateway to exploring cortical auditory processing to complex stimuli. This development enables research on the subcortical and cortical consequences of specific pathologies of the auditory system, like loss of outer/inner hair cells, loss of synapses, by using controlled-hearing-loss animal models. Furthermore, the EEG mini-cap not only facilitates animal research but also enhances its translational potential, fostering a more comprehensive understanding of auditory processes across species.

SU35. Dissecting the Functional Connectome of the Lateral-Line System in Zebrafish

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Category: Hair Cells: Anatomy and Physiology
Background: Fish and amphibia depend on their lateral-line system to detect water movements around their body, which facilitates a number of important behaviors. Water flow information is detected by hair cells organized in clusters called neuromasts. Each neuromast transmits this information to 4-6 afferent neurons for further deciphering in the brain. Our previous work demonstrated that the majority of hair cells in each neuromast are synaptically silent. We have also shown that silent synapses are a mechanism to protect against cellular stress. Importantly, silent hair cells act as a reserve, and can be recruited when the active cells are damaged. Currently it is unclear how the activity of silent and newly recruited hair cells are reflected in the downstream circuitry.
Methods: For our analyses we have used GCaMP-based calcium imaging to examine activity in lateral-line neuromasts and afferent neurons. We used a custom-built light-sheet microscope to perform functional in vivo imaging in all afferent neurons while stimulating a single neuromast. We also performed similar imaging in all afferent neurons where we stimulated all anatomically connected neuromasts. This approach enabled us to create a functional connectivity map between the afferent neurons and the hair cells in every neuromast.
Results: Our map revealed that only half of the afferent neurons that are anatomically connected with hair cells response to hair cell stimulation. Further, we have performed hair cell ablation experiments that indicate that a single hair cell is sufficient to activate an afferent neuron. These results suggest that the functional connectome and morphological connectome are not equivalent at the level of hair cells or primary afferents. In addition, very few hair cells and afferent neurons are needed for the sensory system to function. We next seek to investigate how the afferent-hair cell circuit responds when silent hair cells are recruited after hair cell damage.
Conclusions: This research helps us understand how a mechanosensory system functions in vivo. We found that the lateral line system functions with many silent connections. Understanding the role of these silent connections may provide valuable insight on how sensory information is encoded in mammals.

SU36. Kif1aa-Based Transport and Microtubules Maintains Synaptic Vesicle Populations in Hair Cells

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Category: Hair Cells: Anatomy and 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. In neurons, the motor protein Kif1a has been shown to transport synaptic vesicles along microtubules to the presynapse. How synaptic vesicles reach the hair cell presynapses is not known.
Methods: To study the role of microtubules and Kif1a in hair cells we study the zebrafish lateral-line system. For our analyses we have created CRISPR-Cas9 kif1aa zebrafish mutants to examine the role of Kif1aa in hair cells. We have also used pharmacology (nocodazole) to destabilize microtubules. To visualize synaptic

vesicles, we used immunohistochemistry against Rab3 and Vglut3, markers of synaptic vesicle populations, along with the vital dye Lysotracker. In addition, we used transmission electron microscopy to quantify the number of vesicles at the synapse in kif1aa mutants. We have also assayed the behavioral output of hair cell systems in kif1aa mutants by monitoring the acoustic startle response and through a rheotaxis assay using constant water flow. Lastly, to assess synaptic function we have used electrophysiology and calcium imaging.
Results: Our immunohistochemistry using Vglut3 and Rab3 and our Lysotracker stain show an enrichment of synaptic vesicles at the base of wild-type hair cells, near the presynapses. This enrichment is absent in kif1aa mutants and after microtubule destabilization. Using TEM, we found significantly fewer synaptic vesicles tethered at the ribbon and within 200nm from the ribbon in kif1aa mutants compared to wild-type siblings. Our behavioral results indicate that despite a depleted vesicle population, there is no significant difference in the acoustic startle response between kif1aa mutants and their wild-type siblings at three levels of stimulus intensity or in a habituation assay using a five second inter-stimulus interval, though mutants appear to recover slightly slower after habituation. In our rheotaxis assay, kif1aa mutants show fewer rheotaxis events and the trend becomes more pronounced in the later part of the stimulus. Finally, our functional results reveal a lower rate of spontaneous vesicle release and reduced evoked calcium responses in afferent terminals in kif1aa mutants compared to wild-type siblings.
Conclusions: Our results indicate that Kif1aa and microtubules are required to enrich synaptic vesicles at the base of sensory hair cells. Synaptic vesicle enrichment is important to maintain synaptic activity. Importantly, only behaviors required sustained function, such as rheotaxis are perturbed by synaptic vesicle depletion in kif1aa mutants. Overall, this work reveals how synaptic vesicles are transported to and maintained at the hair cell synapses and will shed light on the significance of synaptic vesicle pools for proper hearing and balance.

SU37. Characterizing the Functional and Molecular Features of Smpx in Cochlear Hair Cells

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Category: Hair Cells: Anatomy and Physiology
Background: Smpx (small muscle protein, X-linked) is an 9kDa molecule expressed abundantly in skeletal and cardiac muscle cells. Since its initial discovery, it has also been characterized as potent deafness gene, causing non-syndromic progressive hearing loss in both humans and mice, particularly in males. Preliminary studies from our lab and published work from others provided evidence that Smpx is crucial for maintaining long term resiliency of the F-actin-based cuticular plate and stereocilia of outer hair cells in the cochlea. Evidence suggests Smpx responds to mechanical stress by stimulating actin nucleation, locally bolstering focal adhesion sites and stabilizing actin filaments. Yet, the Smpx peptide sequence contains no well characterized functional domains and its molecular dynamics remain unknown.
The goal of the present study was to investigate the role of Smpx in hair cell maintenance. In the muscle, Smpx was reported to protect myocytes against mechanical stress. Smpx in the muscle colocalizes with f-actin and co-precipitates with the focal adhesion protein vinculin. We will therefore test the hypothesis that Smpx, analogous to its role in myocytes, protects F-actin based structures in hair cells against mechanical stress.

Methods: We utilized confocal microscopy and immunohistochemical techniques to examine the spatial distribution of Smpx the cuticular plate and stereocilia. To test whether Smpx responds to noise-induced mechanical stress, we will determine the expression and localization of Smpx both in the presence and absence of acoustic trauma. Auditory brainstem response assays were employed to test hearing capabilities in Smpx KO. Future work will incorporate live imaging studies using a newly developed GFP-Smpx KI mouse line.
Results: Screening of multiple commercially available antibodies identified a Smpx-specific antibody suitable for immunofluorescence imaging. Smpx is abundantly localized in the cuticular plate but also pervades the entire cellular structure, including the stereocilia. Mice deficient in Smpx develop perforations in the cuticular plate and hair bundle degeneration and early onset progressive decline in auditory function. This phenotype mirrors the morphological and functional degeneration we observed after traumatic noise exposure in mice. We are therefore testing whether Smpx KO mice are more susceptible to noise-induced hearing loss. We are also investigating the interactome of Smpx in hair cells using CoIP-MS. Finally, we are studying the dynamic behavior of GFP-tagged Smpx in live hair cell stereocilia and the cuticular plate.

Conclusions: Our data further supports a critical role for Smpx in safeguarding the structural integrity of F-actin-rich hair cell structures such as the cuticular plate and the hair bundle. We propose that Smpx is a crucial component of the repair and maintenance machinery of the hair cell.

SU38. Kv1.8 Channels Enhance Vestibular Hair Cell Signaling and Performance on Challenging Vestibulomotor Tasks

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Category: Hair Cells: Anatomy and Physiology

Background: Distinct basolateral voltage-gated potassium (Kv) conductances distinguish vestibular type I and II hair cells (HCs). Type I HCs, notable for their giant calyx synapse, express a low-voltage-activated Kv conductance, g-K,L, that is thought to augment non-quantal transmission. In contrast, type II HCs, contacted by more typical bouton synapses, express Kv conductances that activate positive to rest, both fast-inactivating (g-A) and slowly or non-inactivating (delayed rectifier). We have found that Kv channel subunit Kv1.8 is essential for 3 distinct conductances: g-K,L, g-A, and part of the type II HC delayed rectifier. Here, we present results on how absence of Kv1.8 affects different ends of the vestibulomotor pathway: HC receptor potentials and behavior. Any effects likely reflect Kv1.8 absence from the inner ear, given the low expression of Kv1.8 in other tissues (Lee et al. Hear Res 300:1, 2013).

Methods: We recorded from semi-intact utricles excised from Kv1.8-null, wildtype, and heterozygous littermates (postnatal days 10-60). We deflected hair bundles with step or sinusoidal waveforms delivered by stiff probes, and recorded whole-cell HC currents and receptor potentials. In KV1.8-null and heterozygous littermates (2-6 months), we tested activities that involve vestibular input: free swimming, open field activity, balance beam traversal, gait, and rotarod performance.

Results: For step stimuli, the absence of Kv1.8 increased the gain and rise time of the voltage response. Sinusoidal bundle displacement revealed how these changes altered receptor potential tuning. Lowpass corner frequencies of receptor potentials fell from greater than 100 Hz to ~20 Hz in type I HCs and from ~70 Hz to ~25 Hz in type II HCs. For stimuli greater than 10 Hz, absence of Kv1.8 significantly increased phase (timing) lag of the receptor potential in both hair cell types. These effects degrade temporal fidelity of receptor potentials in both hair cell types, and are likely to contribute to the degradation of vestibular evoked potentials in response to jerk stimuli (Lee et al. 2013).

Kv1.8-null mice had normal posture in their cages, and no differences relative to heterozygous mice were evident in rotarod performance (in light), gait, or muscular strength. Other tests, however, revealed motor and balance abnormalities. Kv1.8-null mice did not maintain horizontal swimming posture, were less likely to rear and jump, and, on a narrow balance beam, more frequently lost balance and wrapped their tails around the beam to anchor themselves.

Conclusions: Absence of Kv1.8-dependent conductances from the vestibular inner ear reduced performance on challenging vestibular tasks. These deficiencies may reflect in part the decreased temporal fidelity of receptor potentials in both type I and type II HCs at frequencies of natural head motions (Carriot et al. 2017 J Physiol 595:2751).

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SU39. The 3D Morphology of the Outer-Hair-Cell Hair Bundle Increases Its Dynamic Range

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Category: Hair Cells: Anatomy and Physiology

Background: Outer-hair-cell hair bundles (OHBs) convert sound-induced forces into receptor currents, which drive cochlear amplification. The dynamic range of hearing is set in large part by the dynamic range of the

OHB receptor current, but we lack understanding of how the dynamic range of the OHB receptor current is regulated.
An OHB comprises actin-packed stereocilia inserted in the outer-hair-cell apex. Stereocilia are arranged in columns and rows. In a column, stereocilia of increasing height are linked by gating springs that gate mechanoelectrical transduction (MET) channels, through which the receptor current flows. Rows are defined by stereocilia of similar height. Sound-induced stimulus forces on the tallest row of stereocilia (row 1) deflect the OHB stereocilia, modulating the gating-spring forces that open and close the MET channels in the shorter rows (rows 2 and 3). This varies the MET channel currents, which sum to produce the oscillating receptor current. The 3D morphology of an OHB is defined by the orientations of its columns and the shapes of its rows.

Methods: We use published data on OHB morphology and our measurements of the stereocilium insertion points to build a 3D mathematical model of the OHB. The mathematical model allows us to calculate orientations of the columns and shapes of the rows from the insertion point positions in the hair-cell apex. We find that the OHB columns are far from parallel. Physiological measurements from the literature constrain the physiological properties of the 3D OHB model, including the stiffness and damping of the stereocilia and the properties of the MET channels. The dynamic range of the 3D OHB model matches the experimentally measured dynamic range. To determine how the 3D morphology of the OHB affects the receptor current, we also calculate the receptor current for an OHB with identical columns (i.e., with parallel columns and lacking a V shape) for comparison.
Results: Owing to the OHB's far-from-parallel columns and V shapes of the rows, stereocilium deflections differ in direction from the stimulus force and differ substantially within columns and within rows. These differences cause large differences in the currents passing through the MET channels. Consequently, the dynamic range of the receptor current in the 3D OHB is more than double that of the identical-columns OHB.
Conclusions: We find that the dynamic range of the receptor current is greatly affected by changing the 3D morphology of the OHB. Based on these results, we propose that the dynamic range of the OHB can be changed tonotopically, during development, and in mutants by changing the stereocilium insertion point positions. We conclude the stereocilium insertion point positions in OHBs regulate their receptor-current dynamic range.

SU40. Exploring the Function of KLHDC7b, a Novel Gene Associated With Hearing Loss

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Regeneron Pharmaceuticals, Inc.

Category: Hair Cells: Anatomy and Physiology
Background: The Regeneron Genetics Center recently conducted a genome wide association analysis to identify alleles associated with hearing loss in adults [1]. Mutations in KLHDC7B, a gene of unknown function, increased the risk of hearing loss in adults. Regeneron generated a knockout mouse that completely removes KLHDC7b.

1. Praveen, K., et al., Population-scale analysis of common and rare genetic variation associated with hearing loss in adults. Commun Biol, 2022. 5(1): p. 540.

Methods: We performed qPCR, RNA scope, SEM and immunofluorescence.
Results: The mice have profound hearing loss at the onset of hearing and near complete hearing loss by 8 weeks. Hair cells develop normally, showing functional mechanotransduction complexes in culture, but begin to die around postnatal day 11-12 as indicated by histology and scanning electron microscopy. RNA scope results show that KLHDC7b is expressed specifically in hair cells. A custom antibody generated against KLHDC7B confirms hair cell specificity. We surveyed cell lines by qPCR for expression of KLHDC7B and confirm the presence of KLHDC7B protein localization in these model systems. Additionally, we've explored functional assays to assess function and disfunction resulting from loss of function.
Conclusions: Our results suggest a role for KLHDC7B in the maintenance of cochlear hair cells.

SU41. Application of Synchrotron-Based Imaging in Visualization of the Spiral Ganglion and Outer Hair Cells in Mice

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Category: Inner Ear: Anatomy and Physiology

Background: Spiral ganglion neurons and cochlear hair cells play a key role in the transduction of sound. However, these structures are difficult to quantify using conventional imaging modalities due to a lack of contrast and resolution. Recently, synchrotron-radiation phase-contrast imaging (SR-PCI) has been proven an effective alternative to histology for comprehensive three-dimensional (3D) visualization of cochlear soft tissues, such as the basilar and Reissner's membranes. The aim of this study was to use SR-PCI to visualize and measure the spiral ganglion and cochlear hair cells in mouse.

Methods: Five mouse cochleae were stained with osmium tetroxide and imaged using SR-PCI at the Biomedical Imaging and Therapy Facility at The Canadian Light Source Inc. (Saskatoon, SK, Canada). The images were collected using a setup with a 0.7 μm voxel size. The reconstructed images were then used to visualize the spiral ganglion and outer hair cells. Feasibility of quantitative measurements of spiral ganglion cells, from base to apex, were investigated.

Results: The reconstructed images allowed for excellent visualization of individual spiral ganglion cells, outer hair cells, cochlear membranes (basilar, Reissner's, and tectorial), and dendrites. The contrast was sufficient to isolate individual cells in order to quantify the size of the cells at different locations of the cochlea.

Conclusions: SR-PCI was effective for visualizing and quantifying fine cochlear structures, such as the spiral ganglion and outer hair cells, in mouse samples. This technique could be used to study the effects of a variety of pathologies on cochlear anatomy, including Meniere's disease or genetic mutations. Future studies are required to investigate similar outcomes in cadaveric human specimens.

SU42. The Cochlea is Built to Last a Lifetime

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Category: Inner Ear: Anatomy and Physiology

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 as a result 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 fibrillin 2, 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.

SU43. Group 2 Innate Lymphoid Cells in the Mouse Cochlea

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Category: Inner Ear: Anatomy and Physiology
Background: The immune cell population of the cochlea has been studied with increasing interest in the last several years with focus on the most plentiful immune cell type, cochlear macrophages. Macrophages are myeloid cells of the innate immune system that are important for spiral ganglion neuron survival after noise exposure and hair cell death (Kaur et al, 2019; Kaur et al., 2015). While both myeloid and lymphoid cells make up the immune response after noise exposure (Rai, Wood et al., 2020), very little is known about inner ear lymphocytes. As lymphoid cells can affect macrophage activity by releasing cytokines and through direct cell interaction, these cells must be studied in the cochlea. This study targets innate lymphoid cells (ILCs) that may reside in the cochlea and respond immediately to insults.
Methods: Flow cytometry was performed on 6-week-old C57BL6/J mice. The soft tissue of both cochleas from a mouse were combined to form one sample. The tissue was dissected into RPMI containing Liberase TL and DNaseI before being processed into a single-cell suspension. Cells were stimulated with PMA and ionomycin to amplify transcription factor expression before staining. Cells were stained for surface markers and intranuclearly stained for cell type-specific transcription factors (GATA3, T-bet, RORgammat, FoxP3). The surface proteins labeled for flow cytometry analysis included: CD45, CD3, CD4, CD8, NK1.1, and gamma delta TCR along with a lineage (lin) dump which included CD19, Gr1, CD11b, CD11c, CD64, and FCepsilonRI. To verify the location of lymphoid cells, cochlear whole mount tissue was immunostained for GATA3, KLRG1 and either CD3 or CD45. CD3 negative, GATA3+, KLRG1+ cells were determined to be ILC2 cells.
Results: ILC2 cells were found in both wildtype and Rag1-/- mice. Lin- CD3- Nk 1.1- cells were examined for expression of the transcription factors that are indicative of ILC1 (T-bet), ILC2 (GATA3), and ILC3 (RORgammat) cells. Using flow cytometry, we found that of these three lineages, ILC2s were the largest population of ILCs expressed in both male and female wildtype mice. Consistently, cochlear whole mounts of wildtype mice showed a population of KLRG1+ GATA3+ ILC2 cells in the spiral limbus and osseous spiral lamina. As GATA3 also marks a subset of T cells, we confirmed our data using Rag1-/- which lack B and T cells (CD3+). Similar to wildtype animals, we also found KLRG1+ GATA3+ ILC2 cells in similar locations in cochlear whole mounts of Rag1-/- mice.
Conclusions: Using a combination of flow cytometry and immunohistochemistry ILC2 were identified in the mouse cochlea. These cells could provide important modulation of cochlear function, including the regulation of macrophage activity and, therefore, should be explored further to understand their function in the cochlea.

SU44. BK Channels are Required for Synchronized Response of Vestibular Nerve Afferents During Fast Head Movements in Vivo

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Category: Inner Ear: Anatomy and Physiology
Background: Vestibular nerve afferents that have the most phasic response properties receive inputs from central regions of the neuroepithelia in the inner ear, mainly through calyx nerve terminals that cover the basolateral walls of type I hair cells. These afferents respond to fast head movements and show highly reproducible spike patterns in response to complex head movements. We have previously shown that in vitro inhibition of potassium channels affects membrane properties of calyces and their response properties as shown by a decrease in spike adaptation, first spike latency, and response threshold as well as a change in their frequency tuning. Here, we investigated large conductance calcium activated potassium (BK) channels that have fast kinetics and are activated at more depolarized membrane potentials, play a role in the reliability of vestibular nerve responses that could provide synchronous activity between fibers in response to fast head movements.
Methods: We investigated the role of BK channels on the synchronous response of phasic afferents in mice using vestibular sensory evoked potentials (VsEP). BK null mice were produced by breeding mice lacking a single allele (+/−) of the Kcnma1 (slo1) gene and results were compared to heterozygote and WT littermates.
Results: We found that the amplitude of VsEP responses in BK knockout mice (n = 15 homozygotes and heterozygotes) were decreased by about 35% compared to their WT littermates (n = 13). In this sample of recordings, responses of KO and heterozygote mice seemed to be grouped separately, with heterozygotes somewhere in between KO and WT littermates.
Conclusions: The synchronous response of phasic afferents during the 2 ms stimulation for VsEP, requires high precision of spiking. It is known that the fast kinetics of BK channels play an important role in the temporal precision of neuronal firing. We propose that lack of BK activity in the vestibular periphery results in increased variability and decreased precision of spike times, similar to that shown in the auditory nerve. This lack of precision affects the synchrony of afferents and the observed decrease in VsEP amplitudes. These findings suggest an important role for BK channels in coding of fast head movements by phasic vestibular afferents.

SU45. Exploring HOMER1 as a Postsynaptic Marker for Ribbon Synapses in the Peripheral Vestibular Epithelia

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Category: Inner Ear: Anatomy and Physiology
Background: Synaptic ribbons are integral elements of the visual, auditory, lateral line, and vestibular sensory epithelia. Immunohistochemical methods using antibodies targeting the ribbon (CtBP2) allow for their visualization, though colabeling with a postsynaptic marker enhances confidence that CtBP2-positive puncta are associated with viable synapses. Labeling afferent dendrites (e.g. β3-tubulin, TUBB3) provides an alternative postsynaptic marker, but does not specifically identify a postsynaptic density complex. HOMER1 is a postsynaptic scaffolding protein associated with glutamate receptors and offers a potential postsynaptic marker. In the present investigation the distribution of HOMER1 in close apposition to synaptic ribbons was determined to evaluate its efficacy as a reliable marker for synapse quantification in adult vestibular epithelia.
Methods: Peripheral vestibular epithelia from chinchillas and mice were harvested and processed using immunohistochemical methods routine for our laboratory. Primary antibodies targeting CtBP2, HOMER1, TUBB3, and calretinin (CALB2) to label the synaptic ribbon, postsynaptic receptor complexes, calyces and parent axons, and striolar calyces. Specimens were incubated for 72 hours, washed in PBS, and then immunoprocessed with appropriate secondary antibodies conjugated to unique fluorophores. Specimens were imaged using a 63X Plan-apochromat objective on a LSM 880 confocal microscope. Raw Airyscan image stacks from the crista central and peripheral zones, and utricular striola and extrastriola, were deconvolved (Huygens Deconvolution, SVI Inc.) and analyzed using custom MATLAB scripts. The image analyses included strategies for automatic detection and segmentation of CtBP2+ and HOMER1+ puncta, and segmentation of TUBB3+ calyces and parent axons. In addition, CtBP2+ puncta inside (type I hair cells) and outside (type II hair cells) labeled calyces were also distinguished.
Results: We found that 93.9% of CtBP2-positive puncta associated with HOMER1 or TUBB3. 48.9% of these puncta were found inside of the calyx of Type I hair cells, while 51.1% were associated with Type II boutons or lateral face synapses. Across the epithelia, 12.1% of Type I synapses were associated solely with HOMER1,

26.2% were associated with only TUBB3 and 61.7% were associated with both HOMER1 and TUBB3. In Type II synapses, 15% of synaptic ribbons associated with only HOMER1, 8.4% associated with only TUBB3, and 76.6% were associated with both HOMER1 and TUBB3. These results demonstrate a high probability of a synaptic ribbon associating with HOMER1 in 73.7% of Type I synapses and 91% of Type II synapses, leading to an average of 82.5% association with HOMER1 throughout the epithelia.
Conclusions: The findings of this study present HOMER1, in conjunction with TUBB3, as an effective and reliable marker of the postsynaptic density in vestibular afferents of both Type I and Type II hair cells. With limited evidence of common postsynaptic markers in the vestibular periphery, HOMER1 has solidified its role as a candidate for further use and research.

SU46. Manifestations of Critical Dynamical Behavior in an Isolated Segment of the Mammalian Cochlea

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Category: Inner Ear: Cochlear Mechanics
Background: The mammalian cochlea harbors an active process characterized by amplification of mechanical inputs, sharp frequency selectivity, compressive nonlinearity, and spontaneous otoacoutic emission. The individual hair cells of nonmammal tetrapods display correlates of all four processes, which emerge from the operation of their hair bundles in a critical dynamical regime near a Hopf bifurcation. It remains uncertain whether mammalian hair cells function in a similar manner, however, and whether criticality accounts for the active process of the mammalian cochlea.
Methods: In order to examine the properties of mammalian hair cells in the absence of traveling waves, we investigated the responses of cochleae excised from Mongolian gerbils (Meriones unguiculatus) 3-4 weeks of age. The experimental protocol involved extraction of the cochlea from the temporal bone, followed by isolation of the second turn of the cochlear duct. Subsequently, the bone covering the scala vestibuli's floor and the scala tympani's ceiling was meticulously removed, exposing 50-250 μm of the organ of Corti. Reissner's membrane was excised and cyanoacrylate glue was applied to the ends of the exposed partition to achieve electrical and ionic isolation.
The isolated cochlear segment was carefully positioned within a custom-made experimental chamber that enabled the apical surfaces of the organ of Corti to be immersed in artificial endolymph while the basolateral surfaces remained in contact with artificial perilymph. Physiological temperatures of 36-38 °C were maintained and a constant endocochlear potential of 100 mV was applied.

The experimental chamber housed two speakers and a microphone. The acoustic stimulus propagated into a tightly sealed compartment to stimulate the excised partition. We assessed the response of the cochlear segment through electrophysiological recordings of microphonic signals and conducted concurrent microscopic observations with optical coherence tomography.

Results: By using frequency sweeps as well as complex zwuis tones, we found that the microphonic response was tuned to frequencies of 1-4 kHz that corresponded to the exposed segment of the organ of Corti. The phase accumulation of the response over the corresponding frequency range amounted to only a fraction of a cycle, indicating that the traveling wave was largely suppressed. The microphonic response to single tones at the best frequency grew as the one-third power of the stimulus, a power-law relationship characteristic of critical oscillators. Furthermore, simultaneous stimulation at two frequencies near the characteristic frequency elicited combination tones whose linear growth with the level of stimulation also accorded with criticality. Control experiments with no endocochlear potential resulted in decreased microphonic responses with a linear relationship to the stimulus magnitude and the absence of combination tones.
Conclusions: These results suggest that hair cells of the mammalian cochlea operate in a critical regime similar to that of the cells in nonmammalian tetrapods.

SU47. Outer Hair Cells (OHCs) Produce Cochlear Amplification via a Cortilymph Fluid Space Impedance That is Wavelength Dependent

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Category: Inner Ear: Cochlear Mechanics

Background: It had been thought that cochlear amplification was produced by OHC forces acting on the basilar membrane (BM) through stiff Deiters cells (DCs). However, optical coherent tomography (OCT) measurements of motion within the organ of Corti (OoC) don't fit with this hypothesis: (1) at low frequencies the transverse motion at the OHC-DC junction (ODJ) is much larger than BM motion (or reticular-lamina (RL) motion), which shows that DCs are not stiff. (2) OHC motions, discerned from RL and ODJ measurements, are at the wrong phase to amplify BM motion. Recent OCT measurements have led to the hypothesis that OHCs produce cochlear amplification by changing the OoC cross-sectional area so that RL and tectorial-membrane (TM) motion, acting on scala media, increase traveling-wave pressure (Altoè et al., 2022; Guinan 2022). We seek to understand how OHC motion changes OoC area near the best frequency (BF) but not at low frequencies.

Methods: Using our OCT system in the 40-50 kHz region of anesthetized, sensitive gerbils, we measured RL and ODJ transverse motions at each OHC row. For seven animals, we calculated Rohc, the ratio of OHC top-to-bottom motions for the third-row OHC: Rohc=RL/ODJ. Starting from the Altoè et al. TOY model, we made a simple heuristic model of a transverse cut through the OoC, the TOoC model, in which OHCs were a displacement source and impedances were pure springs. In the TOoC model, the OHC bottom drives DC and BM impedances in series, and the OHC top drives a transverse RL+TM impedance in parallel with an effectively transverse cortilymph-space (CS) impedance (Zcs). Zcs comes from OHC contractions/expansions cyclically forcing perilymph out of the peri-OHC space into the tunnels. Perilymph flows from where OHC contract to where OHCs expand, a distance of ½ the wavelength, lambda, of the traveling wave. From this, the TOoC-model Zcs was set proportional to lambda.

Results: The TOoC-model Rohc was similar to the experimental Rohc, which was less than unity at low frequencies and increased with frequency becoming ~20 dB near BF.

Conclusions: The TOoC model shows that the Rohc pattern of internal OoC motions can be produced by an OHCs load impedance that is determined by the distance cortilymph flows along the tunnels, i.e., is controlled by lambda. An impedance at the OoC top that controls OHC contractions and OoC area is consistent with the hypothesis that BM cochlear amplification is produced by RL+TM motion increasing scala-media traveling-wave pressure. Having the OHC load be from driving the peri-OHC cortilymph, a load that decreases as the traveling-wave lambda decreases, allows wide-band OHC motility to produce traveling-wave and BM cochlear amplification that is focused where lambda is short, i.e., near BF. [Supported by R01 DC07910 from NIDCD (to SP)]

SU48. Transport of Therapeutic Agents Along the Cochlea by Acoustic Steady Streaming – an FEM Simulation Study

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Category: Inner Ear: Cochlear Mechanics

Background: With the recent development of inner ear therapies, pharmaceutical agents will soon become clinically available. But the application of the drug to the inner ear still faces challenges. The blood-labyrinth barrier makes systemic administration for many compounds impossible, and trans-tympanic application on or through the round window is the only option. Once inside the cochlea, they still need to be transported apically to the target tissue.

Methods: Steady streaming is a non-linear acoustic effect that has the potential to facilitate the transport of therapeutic agents once inside the scala tympani. The steady flow driven by acoustic stimulation can be quantified by the non-linear term of the Navier-Stoke equation. Streaming eddies were observed already decades ago in physical cochlear models, and more recently simulated in simple two-dimensional finite-element models (FEM). Using the FEM package COMSOL Multiphysics and applying the perturbation technique, which hugely reduces computational cost, we were able to simulate streaming in a three-dimensional model.

Results: Streaming velocity increases quadratically with sound pressure level. As two anchor point, we observed for a 1-kHz tone traveling wave with 100 nm partition displacement (~80 dB SPL) at its peak a streaming velocity of ~0.01 mm/min. Increasing partition displacement to 1000 nm (~100 dB SPL) caused an astonishing streaming velocity of ~1 mm/min.
We will discuss the difference between our 2D and 3D streaming fields, demonstrate how multiple tones can elongate the streaming eddy along the length of the cochlea and how particle tracing can be used to follow the streaming path that the compounds take.

Conclusions: Our simulations show that steady streaming could be a feasible method of transporting pharmaceutical agents deep into the cochlear spiral. Since the transport speed increases quadratically with sound pressure level, a compromise must be found between required streaming duration and potential cochlear damage. Although the model geometry used in our 3D simulations is just a simple box, advancing simulations to 3D is an essential step to studying steady streaming inside realistically shaped cochlear models (e.g., coiled).

SU49. Can the Spontaneous Otoacoustic Emissions Predicted by Coherent Reflection Models be Represented by Individual Van der Pol Oscillators?

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Category: Inner Ear: Cochlear Mechanics
Background: Sounds generated by the inner ear in the absence of external stimuli are called spontaneous otoacoustic emissions (SOAEs). Previous experimental studies of mammalian SOAEs in isolation have demonstrated that they can be modeled as individual Van der Pol-Rayleigh (V-R) oscillators in the presence of noise and when a tone is introduced. The goal of the current study is to evaluate the effectiveness of V-R oscillators in modeling SOAEs generated by coherent reflection models (Shera, 2003).
Methods: Two coherent reflection models of the mammalian cochlea (Ku and Elliot, 2007; Bowling et al., 2015) are implemented in MATLAB with cochlear roughness being modeled as uncorrelated, additive, spatial perturbation in organ of Corti parameters. Noise is modeled as internal, uncorrelated, temporal forcing on the basilar membrane. The V-R model's parameters are adjusted so that its limit cycle oscillation matches the properties of the SOAE predicted by the CR models. Both the tuned V-R models and original models are then studied in two parts: (1) the effect of an external tone on the amplitude of SOAEs; (2) the effect of noise on the properties of SOAEs (amplitude, bandwidth, quality factor, amplitude modulation) without external noise.
Results: The introduction of noise broadens the SOAE peaks and progressively transforms the amplitude distribution from bimodal (indicative of an active oscillator) to unimodal (gaussian noise dominates the amplitude distribution). The spectra and amplitude distribution of the coherent reflection models with noise are qualitatively similar to experimental SOAE recordings. Further, the introduction of an external tone suppresses SOAEs. The key predictions of the coherent reflection models are found to behave similarly to a single V-R oscillator.
Conclusions: V-R models are a good benchmark to use for future study and comparison of different SOAE models of different fidelity.

SU50. Srf is Required for Supporting Cell Cytoskeletal Architecture and Hearing

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Category: Inner Ear: Cochlear Mechanics
Background: The cochlea transforms the mechanical signal that enters the ear into an electrical signal. the sensory epithelium plays a key role during this process. The actin cytoskeleton is fundamental to establishing the architecture of different cell types of sensory epithelium, not only hair cells but also supporting cells. Serum response factor is a highly conserved and ubiquitously expressed transcription factor that belongs to the MADS-box protein family. Through cofactor Mrtfs, Srf activates the expression of target genes involved in actin cytoskeletal activities.

Methods: Immunocytochemistry was used to detect the expression of proteins interested in this study. Auditory brainstem response and Distortion product otoacoustic emissions recording were used to evaluate the hearing in control and Srf cKO mice. Scanning electron microscopy was used to investigate the morphology of hair cells and supporting cells. Statistical analysis was performed using GraphPad Prism 8.

Results: In this study, we showed that sox2 positive supporting cell deletion of Srf leads to hair cell loss and deformed sensory epithelium. Srf fl/fl; sox2-creER+ mice showed a weak appearance and died during development. In order to circumvent this limitation, we utilized mice expressing the recombinase Cre under the control of the Fgfr3 promoter, permitting deletion of floxed Srf in the pillar and Deiters' cells through tamoxifen induction. Srf fl/fl; fgfr3-icreER+ mice showed less intensity of F-actin in the pillar and Deiters' cells. These mice developed mild hearing loss at P30 and became worse at P60. Distortion product otoacoustic emissions recording of Srf fl/fl; fgfr3-icreER+ mice was elevated significantly compared to control at P30. Further analysis using scanning electron microscopy revealed that the Deiters' cell phalangeal processes in Srf fl/fl; fgfr3-icreER+ mice displayed increased tortuosity.

Conclusions: Srf is required for supporting cell cytoskeletal architecture and hearing.

SU51. Reevaluating Cochlear Fluid Viscosity's Influence on Basilar Membrane Motion: A Finite Element Study

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Category: Inner Ear: Cochlear Mechanics

Background: This study reevaluates the impact of cochlear fluid viscosity on basilar membrane (BM) motion. Previous studies employing mathematical models suggested that increased viscosity leads to a significant attenuation of sound waves on the BM beyond the best frequency (BF) position.

Methods: We developed a finite-element tapered box cochlear model to investigate the effects of cochlear fluid viscosity on BM motion. Our simulations aimed to identify potential disparities with prior research and elucidate viscosity's role in shaping BM behavior.

Results: The outcomes of our finite element simulations confirm that cochlear fluid viscosity indeed exerts a substantial influence on BM motion, aligning with the initial findings of mathematical model-based research. Our simulations demonstrate a marked reduction in sound wave amplitudes beyond the BF position attributed to increased viscosity.

Conclusions: This study underscores the significance of accounting for cochlear fluid viscosity in BM motion analysis. In contrast to certain previous finite element model-based investigations that failed to detect the viscosity effect, our simulations provide compelling evidence supporting the pivotal role of viscosity in BM motion beyond the BF position.

SU52. Enhanced Cochlear Amplification in Response to Hearing Loss is Mediated by the Medial Olivocochlear Efferent Pathway

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Category: Inner Ear: Cochlear Mechanics

Background: Central feedback onto outer hair cells (OHCs) through the medial olivary cochlear (MOC) efferents is thought to control cochlear gain by suppressing responses to nuisance stimuli. Here, we tested the hypothesis that brain state modulates cochlear amplification. We also assessed the role of the MOC efferent pathway in states of profound hearing loss using VGLUT3-/- mice. Alpha9-/- mice, in which the MOC efferent pathway is non-functional, were used as controls in all of these experiments.

Methods: Using a miniature version of our 1-D Optical Coherence Tomography (OCT) device, we recorded sound-evoked vibrations from the organ of Corti within the apical turn of the cochlea in awake wild type (WT,

CBA/CaJ) and Alpha9-/- mice through the ear canal. Pupillometry, a proxy for brain state, was tracked simultaneously and we correlated spontaneous changes in pupil diameter with cochlear vibratory responses. In separate experiments we recorded auditory brain stem responses (ABR) or local field potentials (LFP) in the inferior colliculus (IC). Further experiments assessed cochlear vibrometry in anesthetized WT, Alpha9-/-, VGLUT3-/-, and Alpha9-/-xVGLUT3-/- mice using our standard 1-D OCT system.

Results: In WT (n=6) and Alpha9-/- mice (n=6), there were no obvious changes in tuning curve magnitude or phase responses over a wide range of sound stimulus frequencies and levels regardless of pupil diameter. We calculated the gain of cochlear amplification, the best frequency (BF), and the sharpness of frequency tuning (Q10dB), and found no correlation with pupil diameter. Removal of cortical input to the MOC efferent pathway by general anesthesia also did not alter tuning curves or these assessments of cochlear amplification in either WT or Alpha9-/- mice. Consistent with the lack of pupil-indexed state dependence in the OCT data, all five waves of the ABR and early positive LFP responses in IC were not state dependent, whereas later-wave IC LFPs responses did depend on state.

We then studied whether MOC efferents alter cochlear amplification in mouse models of permanent hearing loss by crossing Alpha9-/- with VGLUT3-/- mice. We studied only homozygous (+/+ or -/-) genotypes. Cochlear gain was equivalent in WT (n=9) and Alpha9-/- (n=9) littermates. However, deaf VGLUT3-/- (n=8) littermates had increased cochlear amplification. The increase in cochlear amplification was not present in Alpha9-/-xVGLUT3-/- littermates (n=9).

Conclusions: Our studies indicate that MOC efferents do not dynamically alter cochlear gain as brain state changes. However, they are necessary for cochlear gain to increase in chronic states of hearing loss. These data suggest that efferent-mediated increases in cochlear amplification may be one way the brain partially compensates for hearing loss. This process may also underlie some of the associated symptoms of hearing loss, such as hyperacusis.

The research is supported by NIH DC014450, DC017741, DC013774, DC019700, and EB017113.

SU53. Refining Cochlear Impedance Representation in a Finite-Element Human Ear Model

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Category: Inner Ear: Cochlear Mechanics

Background: The examination of intracochlear hydraulic pressure, along with various impedances like cochlear input and scalae differential impedances in human ears, is essential for understanding sound transmission within the inner ear. Although cochlear input impedance and differential impedance mainly show a viscous response, many past finite-element (FE) ear models didn't adequately represent this, and the assumptions of tube-like acoustics and fluid viscosity not fully explain this behavior. The interplay of imperfect viscosity resistance with inertia reactance at lower frequencies adds to the complexity. In this study, our objective is to develop an FE human ear model that aligns with experimental impedance data.

Methods: In this study, we integrated human middle and inner ear models (both of which have been previously validated and published) using image-registration techniques. This integrated model enables precise observations of viscosity effects within the frequency-dependent boundary layers. To explore fluid dynamics in the cochlea and evaluate several impedances pertinent to hearing science, we conducted forward air-conduction (from the ear canal to the cochlea), reverse stimuli applied at the round window, and bone conduction stimuli. These simulations were executed in the frequency domain and were accompanied by an extensive sensitivity analysis, concentrating on various parameters such as the cochlear partition damping, mass, stiffness, and fluid viscosity.

Results: The preliminary results involved calibrating and adjusting the model to align with the findings of the previously independent models. The initial parameter assessment suggests that the viscosity of cochlear fluid exerts limited impact on the cochlea impedance. In contrast, the characteristics of the cochlear partition significantly affect the overall impedance of the cochlea. Nevertheless, the preliminary results of model did not exhibit the expected dominance of viscosity in maintaining a steady impedance magnitude.

Conclusions: This model provides a valuable tool for investigating how various cochlear parameters influence its definitions of impedances, an endeavor difficult to execute in experiments. We plan to further examine

other parameters to identify the mechanism responsible for the viscous-dominant behavior of the cochlea. Subsequently, we'll employ the model to deepen our understanding of the mechanics in the middle ear and cochlea during air-conduction forward, round-window-initiated reverse, and bone conduction stimuli.
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SU54. S398 in Prestin is Critically Important for Cl- Binding

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Category: Inner Ear: Membranes and Fluids
Background: Prestin (Slc26a5) is a member of the Slc26 family of membrane transporters that also functions as the membrane motor of OHCs. Prestin's electromotility is dependent on the presence of intracellular Cl- that functions as an allosteric modulator or extrinsic voltage. In particular, the S398E mutation in the structural Cl- binding site abolishes sensitivity to salicylate thus suggesting loss of Cl- binding.
Methods: We used all-atom explicit solvent molecular dynamics (MD) simulations of gerbil prestin (PDB 7SUN) in a lipid membrane at physiological Cl- concentrations. We analyzed binding of Cl- ions to the putative binding site, including the four residues F137, S396, L397, and S398 that were shown to be important for binding this ion in its inside open contracted state. In addition to wildtype protein, we also simulated a model of the S398E mutant, in which serine 398 was changed to a negatively charged glutamate residue. Binding probabilities were calculated under different voltage conditions (–150 mV, 0 mV, +150 mV) over 2 μs simulations.
Results: Substitution of S398 with a glutamate residue results in a loss of binding of Cl- to all four coordinating residues that bind to Cl- in its inside open contracted structure. We previously established that S398E was insensitive to intracellular Cl- and salicylate using measures of gating charge movement (NLC) with whole cell patch clamp experiments (F137A did not have measurable NLC or was significantly right shifted, although it did have a measurable current in the presence of SCN). Comparison with mutations in the other residues important for binding to Cl- and electrophysiological validation of this model are ongoing.
Conclusions: These data establish that S398 is critical for binding to chloride in prestin's inside open contracted state.

SU55. Prestin and Slc26a9 Show Distinctly Different Responses to Voltage Change in Molecular Dynamic Simulations

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Category: Inner Ear: Membranes and Fluids
Background: Prestin (Slc26a5), a member of the SLC26 family of transporters, is responsible for sound amplification by outer hair cells (OHC) in the inner ear. The compact arrangement of prestin within the OHC membrane allows for coordinated conformational changes, resulting in relatively modest variations in surface area when responding to depolarization signals. Although structures with sulfate and salicylate were purported to show an expanded conformation of prestin (Bavi et al., 2021), it is not clear that these structures represent the true physiological expanded state. Furthermore, the detailed conformational transition between compact and expanded states remains unclear.
Methods: To investigate the response of prestin to external membrane voltage, molecular dynamics (MD) simulations of gerbil prestin 7SUN (Butan et al., Nature Comm., 2022), starting in a contracted state, were run on Anton2 with external electric fields corresponding to membrane potential differences of +150 mV, 0 mV, and -150 mV at 296K and 333K. We analyzed the cross-sectional area in the membrane, membrane thickness and a newly defined "gate opening distance". Additional simulations were performed for the related SLC26 transporters Slc26a9, which does not show voltage-dependent electromotility, under varying external electric fields (0 mV and -150 mV) at 296K and 333K temperatures using the GROMACS 2021 MD package.

Results: Prestin shows an expansion on the inner cytoplasmic half of the protein in response to a voltage change to -150 mV. The transmembrane helix 6 (TM6) rotates outwardly away from TM12 together with outward rotation of TM1 particularly in its cytoplasmic half after about 2 μs in simulation and expands the gate distance from 29 Å to 36 Å, followed by a lipid insertion into the protein. In contrast, we see less or no changes in prestin in response to +150 mV or 0 mV. On the other hand, Slc26a9 did not show any significant changes in area and conformations at -150 mV or 0 mV in 3 μs of simulation. Unlike in prestin, TM6 and TM1 of Slc26a9 remained stable throughout the simulation. Whole cell patch clamp data of mutations in these helices confirm the role of some of these charged residues at the inner membrane, with additional experiments ongoing.
Conclusions: These data support the validity of the intrinsic voltage dependent model of prestin electromotility and suggest that electromotility occurs by differential expansion of the inner and outer leaflets of the membrane. These data have implications to the membrane bending model of prestin.

SU56. Preclinical Development of SENS-501 as a Treatment for the Autosomal Recessive Non-Syndromic Deafness 9 (DFNB9) Using an Adeno Associated Vector-Based Gene Therapy

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Category: Gene Therapy
Background: Congenital sensorineural defects heavily impact patients' life and their ability to communicate with others. Among this vast family of communicating disorders, non-syndromic autosomal recessive deafness 9 (DFNB9) is one of the most common form of congenital deafness, accounting for up to 8% of cases. This severe-to-profound auditory neuropathy is caused by biallelic loss of function in the OTOFERLIN gene (OTOF), which encodes for a calcium sensor protein involved in neurotransmitter release at presynaptic level between inner sensory hair cells (IHC) and spiral ganglion neurons. So far, the only medical solution is cochlear implantation, which improves hearing to some degree, but still has a lot of limitations.
Methods: To address this unmet medical need, we developed SENS-501, a dual-AAV (adeno associated virus) hybrid approach using two different recombinant vectors each containing one half of the OTOF cDNA. This strategy was tested on congenitally deaf DFNB9 mutant mice by injecting SENS-501 in the inner ear through the round window membrane. Hearing improvement was evaluated with multiple auditory and behavioral tests.
Results: Auditory brainstem response (ABR) recordings showed significant lowering of the thresholds along the auditive spectrum after intra-cochlear injection, with durable (one year) improvement of hearing in a dose-responsive manner. A startle reflex behavior test was performed to confirm the ability of treated DFNB9 mutant mice to integrate sound similarly to wild-type mice. We were able to detect restoration of their startle reflex ability when exposed to randomized sudden loud noises. We also evaluated vestibular function of treated mice by analyzing their exploratory behavior in an open-field arena. Data indicated that our approach did not show any significant increase in circling behavior when compared to control mice. We performed a similar set of experiments in non-human primates (NHP) after SENS-501 intracochlear injections using a surgical approach and our proprietary medical delivery device approved for clinical trial. Surgery and SENS-501 injection did not affect ABR thresholds, similar to what was observed in mice. Immunohistochemistry experiments were performed in both mice and NHP, demonstrating effective recombination and selective expression in IHCs of the full-length therapeutic protein and its flag-tagged counterpart. A 3-month GLP toxicology and biodistribution study following a single intra-cochlear injection was conducted in NHP with two doses of SENS-501. Biodistribution data indicated that the vast majority of the vectors remained in the inner ear. SENS-501 shedding in fluids showed a limited dissemination of the vectors with decreasing levels over time. The two doses of SENS-501 were well tolerated and no clinical or adverse SENS-501-related effects in the inner ear or outside the inner ear tissue in any evaluation-including immunology were found.

Conclusions: Altogether, our nonclinical pharmacology, biodistribution, and safety studies support the clinical development of SENS-501 for hearing loss due to genetic OTOFERLIN protein deficiency.

SU57. Surgical Approach for a Safe Intra-Cochlear Injection of AAVs in Macaca Fascicularis

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Category: Gene Therapy
Background: More than half of the congenital non-syndromic deafness cases have a genetic cause that affect specific inner ear cell populations, including sensory hair cells, supporting cells and spiral ganglion neurons, all of which play an important role in the process of hearing. Adeno-associated virus (AAVs)-based gene transfer shows great potential for the treatment of hearing loss, as they provide stable gene expression over long period of time and are safe to use. Furthermore, AAV tropism and transgene expression patterns can be fine-tuned through AAV capsid and promotor engineering. To accelerate translation to humans, we now report the feasibility and efficiency of a trans-canal approach for an intracochlear injection through the round window (RW) for vector delivery in non-human primates (NHPs).
Methods: Two to three years old NHPs were included in this study. AAV8-eGFP expressing the enhanced green fluorescent protein (eGFP) was delivered in the cochlea of NHPs by either a round window injection (RWI) or a RWI combined with a laser-induced fenestration of the oval window membrane, also known as stapedotomy. A dedicated medical delivery device developed for clinical use was employed. Safety was assessed in a GLP-like setting. Auditory Brainstem Response (ABRs) and Distortion Product Otoacoustic Emission (DPOAE) measurements were performed to assess the hearing function of NHPs before and three weeks after the surgical procedure. Whole mount cochleae were analyzed three weeks post-surgery to evaluate inner ear morphology and AAV8-eGFP tropism.
Results: No clinical, local or systemic findings were reported at the end of the study indicating that the surgical procedure was well tolerated and associated with a good healing process in the outer, middle and inner ears. The two surgical approaches tested resulted in limited increase of ABR thresholds and decrease of DPOAE amplitudes, which remained contained within the normal hearing threshold range of NHPs. The stapedotomy procedure greatly enhanced the AAV8-eGFP transduction of inner hair cells (IHCs) of the organ of Corti, in particular, as compared to the RWI procedure. An apico-basal gradient of transduction of IHCs was observed as well as transduction of supporting cells of the greater and lateral epithelial ridges. Most importantly, the intracochlear injection had no impact on the spiral ganglion neuron or IHC survival.
Conclusions: This study demonstrated the safety and the feasibility of intracochlear injections in NHPs using our proprietary medical delivery device. Hearing thresholds were generally considered not clinically significant three weeks post-injection given that outer, middle and inner ears were still in a healing state. Thus, AAV8-eGFP efficiently transduces the IHCs of NHPs, in particular, and should be considered as an AAV capsid for inner ear gene therapy in humans. These data motivate future translational studies to evaluate gene therapy for human hearing disorders.

SU58. Gene Therapy Targeting Auditory and Vestibular Systems in Clic5 Mutant Mice

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Category: Gene Therapy
Background: The auditory and vestibular systems, essential for hearing, balance, and spatial orientation, depend on intricate interactions between genes and regulatory elements. Pathogenic mutations in genes crucial for both systems can lead to dual sensory impairments, resulting in hearing loss and vestibular dysfunction. While gene therapy emerged as a promising solution for improving auditory function, few models have been tested to treat both hearing and vestibular anomalies. Previously, utricle injection has proven to be the effective

method for transducing all six sensory organs within the inner ear of neonatal mice (Lee et al. 2020). Here, we aim to restore both vestibular and auditory functions in the Clic5 mutant mice using the utricle injection route.

Methods: Firstly, we studied the auditory and vestibular function in Clic5 c.680T greater than C mouse model. Auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs) were used to monitor the auditory capacity of this model, followed by behavioral assays at 4, 8, and 12 weeks to evaluate circling behavior, balance, and orientation. Hair bundle structural abnormalities were explored by confocal microscopy. Next, to assess the therapeutic potential, we generated two synthetic AAV2/9-PHP.B vectors: self-complementary AAV (scAAV) and single-stranded AAV (ssAAV). Following injection into the Clic5 -/- mouse utricle at P0, hearing, and vestibular functions were assessed.

Results: Clic5c.680T greater than C mice exhibit progressive hearing loss and vestibular dysfunction, including circling behavior, head bobbing, and an inability to swim. In both auditory and vestibular systems, hair cells exhibited elongated and disordered hair bundles, accompanied by the absence of some hair cells. Next, using in-vivo AAV gene delivery via the utricle route, we observed vestibular function recovery and improved hearing, with some cases showing auditory brainstem response thresholds close to those of wild-type mice for each of the AAVs vectors.

Conclusions: Our results show that AAV gene therapy using utricle injection improved the auditory and vestibular function in the Clic5c.680T greater than C mutant mice and will contribute to advancing translational research to treat hearing and balance disorders in humans in the future. Research supported by the U.S.-Israel Binational Science Foundation.

SU59. In Vivo Adenine Base Editing Ameliorates Auditory Function in a Mouse Model of Dominant Deafness

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Category: Gene Therapy

Background: Gene therapy has a great potential to cure hereditary hearing loss. The efficacy of gene replacement on dominant genetic hearing loss is limited. Adenine base editor (ABE) precisely catalyzes A▪T to G▪C conversion without requiring double-stranded DNA breaks (DSBs). Here, we utilized SpRY-ABE8e(V106W) to ameliorate auditory function in the Kcnq4G229D mouse model, which harbors a dominant pathogenic mutation (c.G683A).

Methods: We constructed plasmids, and screened different combinations of ABE8e and guide RNA (gRNA) in vitro. To reduce RNA off-target effect, ABE8e was optimized to ABE8e(V106W) variant. Base editor was divided into two halves by Npu trans-splicing split intein and packaged by adeno-associated virus (AAV) vector PHP.eB. Kcnq4+/G229D and Kcnq4G229D/G229D mice were injected via round window at postnatal day 1-2 (P1-2), and subsequently were evaluated by auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE) at the age of 4 to 12 weeks. The survival of hair cells was observed by immunohistochemistry. Base editing efficiency was analyzed by high-throughput sequencing (HTS).

Results: To repair the mutation, we screened SpRY-ABE8e and NG-ABE8e together with different gRNAs in vitro, and observed a highest editing efficiency from the combination of SpRY-ABE8e-g3. Split SpRY-ABE8e(V106W)-g3 showed 42 ± 2.8% editing, compared to 54 ± 1.0% editing from full-length SpRY-ABE8e(V106W)-g3. Dual SpRY-ABE8e(V106W)-g3 AAVs were injected into the inner ears of Kcnq4G229D mice at P1-2, and improved the hearing of Kcnq4+/G229D and Kcnq4G229D/G229D mice at the age of 4 to 12 weeks. At 8 weeks, we observed the survival of hair cells and found that hair cells of Kcnq4G229D mice were much more than that of untreated mice. Analysis of editing efficiency showed that the Kcnq4 c.G683A point mutation was reversed to wild-type sequence (c.G683G) with 21 ± 7.3% editing in Kcnq4+/G229D mice as well as to 21 ± 3.8% editing in Kcnq4G229D/G229D mice.

Conclusions: Adenine base editor repaired pathogenic mutation A▪T to wild-type sequence G▪C in deafness gene and ameliorated auditory function in a mouse model of dominant deafness, giving new insights into treatment of hereditary deafness and clinical translation in the future.

SU60. Gene Therapy With AAV.ie Rescues Auditory Function in a Mouse Model of DFNB111 Hearing Loss

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Category: Gene Therapy

Background: Many advances have been made in gene therapy for treating hereditary hearing loss in recent years. However, previous studies focused on deafness genes predominantly expressed in single-cell types. Approximately 17.3% of mild-to-moderate hearing loss is caused by mutations in MPLZ2, a gene underlying DFNB111, which is widely expressed in different inner ear cells, including supporting Deiters cells (DCs), inner hair cells (IHCs), outer hair cells (OHCs), and the stria vascularis (SV). Here, we utilized gene replacement therapy mediated by AAV-ie with high transduction efficiency in diverse types of inner ear cells for the Mpzl2-/- mouse model.

Methods: The Mpzl2-/- mouse model was generated by targeting the exon 2 of Mpzl2. The CMV-AAV-ie-Mpzl2 system was injected into the cochlea of Mpzl2-/- mice at postnatal(P)1-P3 via the round window membrane at a titer of 1.0×1013 vg/ml. The auditory function was measured by ABR and DPOAE thresholds at click and 4-32 kHz at 4 weeks, 8 weeks, 12 weeks, and 5 months after injection. The cochlear phenotypes were evaluated by immunofluorescent staining at 12 weeks.

Results: Mpzl2-/- mice had moderate and slowly progressive hearing loss and exhibited OHCs and DCs degeneration, without obvious changes in the number of IHCs and other supporting cells, and SV thickness, compared to age-matched Mpzl2+/+ mice. We found that AAV-ie-Mpzl2 administration significantly lowered the ABR and DPOAE thresholds of Mpzl2-/- mice at all tested frequencies for at least 5 months. The ABR thresholds in Mpzl2-/- injected ears were ameliorated by 7–19, 8-16, 15-23, and 12-28 dB SPL across all frequencies at 4 weeks, 8 weeks, 12 weeks, and 5 months, respectively, with some frequencies recovered to wild type levels. In addition, the number of OHCs and DCs in Mpzl2-/- injected ears were both significantly increased than that in untreated Mpzl2-/- mice in three turns of cochleae.

Conclusions: This is the first study that demonstrated the utility of gene replacement therapy for Mpzl2-related hearing loss, which raises hopes for DFNB111 patients and provides a reference for gene therapy to treat genetic deafness where the pathogenic gene is expressed in diverse subtypes of cochlear cells.

SU61. Restoration of Auditory Function With Otoferlin Gene Transfer Therapy in Nonsense (Q828X) and Missense (R1934Q and Deaf5) Models of OTOF Deficiency

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Category: Gene Therapy

Background: Otoferlin is a calcium sensor protein expressed in the inner hair cells and is important for proper synaptic transmission between inner hair cells and the afferent fibers of the spiral ganglion. Biallelic loss of function mutations in the OTOF gene lead to congenital severe-to-profound auditory neuropathy spectrum disorder (ANSD) in both humans (DFNB9) and in mice. DFNB9 is believed to be causal in 2–3 % of individuals born with bilateral sensorineural hearing loss. In human patients, numerous variants of OTOF-related hearing loss (DFNB9) have been identified. We previously demonstrated that OTOF gene transfer therapy can restore auditory function measured by Auditory Brainstem Response (ABR) in Q828X nonsense mutant mice. Here, we compared the efficacy of OTOF gene transfer in additional mouse models carrying missense variants that lead to presence of mutant otoferlin to evaluate its efficacy in a wider range of OTOF variants.

Methods: Three different mouse models of OTOF-related deafness were treated with dual hybrid AAV vectors encoding OTOF. Q828X is a mouse model of a cognate variant (p.Q829X) characterized in Spanish and Latin American populations (Migliosi et al. 2002; Rodríguez-Ballesteros et al. 2008). R1934Q mutant

mice were generated to model DFNB9 due to a homozygosity of human p.R1939Q variant that serves as a founder allele among East Asian populations (Kim et al. 2018). Lastly, Deaf5 is a mouse model harboring an ENU-induced missense variant resulting in a single base substitution in exon 10 of the mouse otoferlin gene (Longo-Guess et al., 2007). For all three groups, male and female mice were administered with dual AAV-Otof cDNA vectors around 4–6 weeks of age. Cochlear function was assessed at 4 weeks after administration by measurement of ABR to tone bursts over 4-32 kHz. Transgene expression was evaluated by anti-myc tag antibody staining of cochlea wholemount preparation to distinguish it from endogenous Otoferlin.
Results: In all three DFNB9 mouse models with different variants of Otof, the majority of mice showed restoration of auditory function as measured by improvement in ABR thresholds compared to vehicle- treated control mice over the entire tested range of the stimulus (4–32 kHz). In all groups, over 50% of animals achieved ABR thresholds that fell within the range observed from wild type mice. Treatment-induced Otoferlin expression using anti-myc tag staining confirmed successful transduction in all three groups.
Conclusions: These results demonstrate that an OTOF gene transfer therapy with dual hybrid AAV can successfully restore cochlear function in various otoferlin-deficient situations in murine models and, further, is likely to provide benefit for DFNB9 patients in wider populations in the on-going phase 1/2 clinical trial of DB-OTO in pediatric patients with profound, congenital hearing loss caused by mutations of the otoferlin gene.

SU62. Novel Antisense Therapy to Durably Treat USH2A Patients

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Category: Gene Therapy
Background: Usher syndrome (USH) is the most common inherited genetic disease of combined deaf-blindness. Mutations in USH2A account for half of USH cases. The size of USH2A (~15.6Kb) prevents the development of classic gene augmentation therapy mediated by adeno-associated virus (AAV)-based vectors, because it largely exceeds the capacity of single or even dual AAVs. Thus, we took advantage of an antisense oligonucleotide (ASO) strategy to correct hearing and vision loss in patients living with USH2A. We target an out-of-frame mutation, an USH2A founder mutation identified in patients at Boston Children's Hospital, and a major cause of USH in the French-Canadian population. To restore the proper reading frame, we developed a strategy leading to dual-exon skip of human USH2A. Our hypothesis is that in-frame deletion of two exons in human will result in expression of slightly shorter yet still functional USH2A protein.
Methods: In silico analysis was performed using Alphafold2 to predict the 3D structure of both wild-type and exon skipped USH2A protein. We designed ASO sequences based on the bioinformatic analysis using interactive genome viewers. Candidate ASOs were synthetized with full phosphorothioate backbones and 2'-O-(2-methoxyethyl) ribose sugar modifications. For testing, ASOs were transfected into the retinoblastoma-derived RB1 cell line at a concentration of 10-200nM. We performed RT-PCR to validate ASO-induced USH2A dual-exon skipping. To validate this approach, two Ush2a zebrafish mutant models were generated using CRISPR-Cas9 and sgRNA to mimic (1) the patient's mutation or the (2) dual exon skipping strategy. Phenotype analysis was performed to assess vision (OKR), hearing (acoustical startle reflex), and balance (swimming behaviors) along with histology to assess inner ear and retinal structures.
Results: Our strategy leads to an in-frame internal deletion of 105 amino acids out of 5202 amino acids. This deletion excises a single fibronectin domain. In silico analysis demonstrates the preservation of protein folding properties suggesting that protein function would be preserve. Our data demonstrate successful single and dual-exon skip with different combinations and dosages of ASOs. We have generated two zebrafish models with truncating mutations in ush2a that correspond to the pathogenic human variant of interest. Animals homozygous for either of these alleles are viable, fertile and display normal startle response and vestibular behavior, consistent with published zebrafish USH2A models. Experiments assessing histology and function of the retina are ongoing as well as production and validation of the dual exon skip model.
Conclusions: ASOs have great promise as a platform to treat genetic disorders, including Usher syndrome specifically. 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.

SU63. Preventing Autosomal-Dominant Hearing Loss in Bth Mice With CRISPR/CasRx-Based RNA Editing

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Category: Gene Therapy

Background: CRISPR/RfxCas13d (CasRx) editing system can specifically and precisely cleave single-strand RNAs, which is a promising treatment for various disorders by downregulation of related gene expression. TMC1 is the sixth most commonly inherited deafness gene, the TMC1 point mutation (c.1253T greater than A; p.M418K) in humans, which is identical to the Tmc1 mutation (c.1235T greater than A; p.M412K) in Beethoven (Bth) mice. There is still a lack of studies using the CRISPR/Cas13 RNA editing system for hereditary deafness therapy. To explore the potential therapeutic effects of CRISPR/Cas13, we tested this RNA-editing approach on Bth mice and successfully downregulated the expression of the Tmc1 Bth transcript preventing progressive hearing loss.

Methods: The mouse model with the humanized homologous mutation (Tmc1 c.1235T greater than A; p.M412K) was constructed. The different combinations of CasRx and sgRNAs were screened. AAV-PHP.eB based CasRx was microinjected into the inner ear of neonatal mice through the round window membrane. The auditory functions were assessed by ABR and DPOAE. Immunostaining was used to observe the survival of hair cells and hair bundles. Electrophysiology was performed to analyze the mechanoelectrical transduction current. RNA-Seq was performed to analyze the transcriptome data of editing efficiency and off-targets.

Results: We first screened 30 sgRNAs in cell cultures and found that CasRx with sgRNA3 reduced the Tmc1Bth transcript by 90.8%, and the Tmc1 wild-type transcript (Tmc1+) by 44.3% in vitro. We then injected a newly developed AAV vector (AAV-PHP.eB) based CasRx into the inner ears of neonatal Bth mice, and we found that Tmc1Bth was reduced by 70.2% in 2 weeks with few off-target effects in the whole transcriptome in vivo. Consistently, we found improved hair cell survival, rescued hair bundle degeneration, and reduced mechanoelectrical transduction current. Importantly, the hearing performance, measured in both ABR and DPOAE thresholds, was improved significantly in all ages over 8 weeks.

Conclusions: We validated the CRISPR/CasRx-based RNA editing strategy in treating autosomal-dominant hearing loss, paving the way for its further application in many other hereditary diseases in hearing and beyond.

SU64. Evaluation of Cannabidiol as a Therapeutic for Noise-Induced Hearing Loss

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Category: Inner Ear: Damage and Protection

Background: The neurons that innervate inner hair cells within the murine cochlea are known to be both genetically and physiologically heterogeneous and can be divided into three subtypes: type 1A, 1B, and 1C. Prior research from our laboratory found that type 1A neurons, which are considered relatively protected from noise trauma, upregulate the pro-survival ATF family of transcription factors in response to permanent threshold shift (PTS)-inducing noise trauma, which is the most severe form of noise-induced hearing loss (NIHL). We have identified several compounds that could be used to pharmacologically induce the ATF transcription factors in neurons. One such compound is cannabidiol (CBD), a known ATF agonist. We investigate whether administration of CBD can upregulate the ATF transcription factors and extend neuroprotection to the more vulnerable SGN subtypes of 1B and 1C neurons and reduce hearing loss following a PTS-inducing noise exposure.

Methods: To assess the physiologic impact of CBD treatment in vivo, mice of both sexes were randomly assigned to either the treatment or control group (n=24, 12 per group) and underwent baseline auditory brainstem response (ABR) testing at 9 weeks of age. At 10 weeks of age, mice were administered either a

single dose of CBD (60mg/kg) or vehicle via intraperitoneal (IP) injection 1 hour before exposure to a PTS-inducing noise (8-16 kHz, 105 dB SPL, 2 hours). Repeat ABR testing was performed 24 hours and 1-week following the noise exposure to assess the acute hearing loss and permanent threshold shift, respectively.

To determine whether CBD can modulate ATF mRNA expression within the murine cochlea, 10-week-old adult female mice (n=4, 2 per group) were divided into either an experimental or control group, then administered a cannabidiol or vehicle solution, respectively. Twenty-four hours following IP injection, the cochleae from both groups were collected and prepared for fluorescent in situ hybridization to assess the spatiotemporal changes of Atf3, Atf4, and their targets Gadd45a and Ddit3.

Results: Auditory brainstem response testing performed 24 hours and 1-week after noise exposure showed no significant difference in threshold shifts between CBD and vehicle treated mice in either sex. However, pre-treatment with CBD was found to provide statistically significant protection in wave I amplitude 24 hours following PTS noise exposure in both male and female mice when compared to vehicle treated males and females (p less than 0.01). Immunohistochemical analysis found no differences in expression of Atf3, Atf4, Ddit3, and Gadd45a transcripts between CBD and vehicle treated mice.

Conclusions: Pre-treatment with a single dose of CBD prior to traumatic noise exposure showed significant protection of wave I amplitudes in both male and female mice compared to vehicle treated mice, particularly at higher noise intensities. Additional experiments are required to further assess the effects of CBD within the auditory system.

SU65. CX3CR1 Fate Mapping Reveals Heterogeneity in Cochlear Macrophages and Blood Circulating CCR2-Expressing Infiltrated Macrophages Promote Hair Cell and Neuron Survival After Acoustic Trauma

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Category: Inner Ear: Damage and Protection

Background: Any cochlear trauma causes inflammation, activates the resident macrophages (RM) and recruits the blood-circulating monocytes and monocyte-derived macrophages (Mo/Mo-M), but their specific functions in the injured cochlea are unknown. It is well established that chemokine fractalkine receptor (CX3CR1), present on cochlear macrophages, influences the density of those macrophages and promotes the survival of spiral ganglion neurons (SGNs) in injured cochlea. As CX3CR1 is expressed on both RM and Mo-M, it remains unclear if CX3CR1-expressing RM and Mo-M are distinct and differentially promotes neuron survival after cochlear injury. We developed a CX3CR1 fate mapping model wherein CX3CR1-expressing RM and Mo-M are endogenously labeled with different fluorescent reporters to define the heterogeneity in cochlear macrophages regarding their origin, spatiotemporal distribution, morphology, fate, states, and function following acoustic trauma.

Methods: Tamoxifen inducible CX3CR1YFP−CreERT2R/YFP−CreERT2 mouse was crossed with Rosa-lsl-tdTomato (R26RFP) reporter mouse line. The progeny CX3CR1YFP−CreERT2/wt:R26RFP were injected with either tamoxifen (75 mg/kg, twice) or vehicle (corn oil) at ~1 month of age and euthanized at various time points after injection to determine the Cre recombination efficiency and turnover rate of resident cochlear macrophages. After 60 days of tamoxifen injection, cohort of CX3CR1YFP−CreER/wt:R26RFP mice were exposed to a noise level of 112 dB SPL at 8-16 kHz octave band for 2 hours to examine the ontogeny, distribution, morphology and fate of RM and Mo-M. To determine the precise role of RM and Mo-M in SGN survival, mice lacking RM due to PLX5622 (CSF-1R oral inhibitor) treatment or CCR2 (expressed on Mo-M) knockout mice were similarly noise exposed. Following noise exposure, mice were subjected to ABRs, DPOAEs, euthanized and tissues were collected at different time points for flow cytometry, multilabel immunohistochemistry and confocal microscopy.

Results: After 60 days of tamoxifen injection, long-lived cochlear RM were YFP+ RFP+ with 98 ± 1.7% recombinant efficiency and short-lived blood-circulating CX3CR1 lineage (Mo/Mo-M) were YFP+ RFP- with 2.5 ± 1.1% recombinant efficiency. After acoustic trauma, morphologically similar RM and Mo-M were

observed in the Rosenthal's canal, lateral wall and Organ of Corti, whereas the sham exposed mice showed the presence of only RM. Both RM and Mo-M were Ki67 positive, suggesting that the increase in macrophage numbers in the noise-damaged cochlea is a contribution of both macrophage subtypes. Additionally, CX3CR1-blood infiltrated macrophages expressed CCR2, absence of which was associated with increased loss of sensory hair cells and SGNs after acoustic trauma. Fibrinogen probing showed its presence inside the cochlea after acoustic trauma, suggesting a leaky vasculature.
Conclusions: With the newly developed CX3CR1 fate mapping tool the data imply that macrophages in noise-damaged cochlea are heterogeneous in terms of their ontogeny, distribution, and fate. Also, blood-infiltrated macrophages may promote the survival of cochlear sensory cells after acoustic trauma.

SU66. REV-ERBα Agonist SR9009 Ameliorates Noise-Induced Hearing Loss and Reduces Cochlear Macrophage Infiltration

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Category: Inner Ear: Damage and Protection
Background: Noise-induced hearing loss (NIHL) is one of the most common causes of acquired hearing loss in modern society. The possible mechanism includes inflammatory responses in the cochlea after acoustic trauma. Previous studies report the effectiveness of REV-ERBα agonist SR9009 in attenuating inflammatory responses in multiple organs. We thus aim to see whether S9009 could mitigate NIHL and the associated cochlear inflammatory responses in mice.
Methods: We treated CBA/CaJ mice with REV-ERBα agonist SR9009 and exposed the mice to broad-band noise for 2 hours. Auditory brainstem response (ABR) thresholds were accessed before and 3 weeks after noise exposure. The number of infiltrating Iba1+ macrophages into the cochlea was evaluated.
Results: Treatment with SR9009 mitigated the ABR threshold shifts induced by noise exposure, particularly at lower frequencies. It also reduced the noise-induced decrement in ABR wave I amplitude. Additionally, SR9009 decreased the infiltration of Iba1+ macrophages into the cochlea following noise exposure.
Conclusions: Our results suggest that SR9009 has the potential to ameliorate NIHL, presumably through the attenuation of cochlear inflammatory responses. These findings could propel the advancement of REV-ERBα agonists in clinical trials, exploring their efficacy in reducing the severity of NIHL.

SU67. Effects of Selective Inner Hair Cell Loss and Cochlear Synaptopathy Loss on Psychophysical Intensity Increment Detection Tasks in the Chinchilla Animal Model

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Category: Inner Ear: Damage and Protection
Background: Auditory intensity coding is essential for perceiving and coding complex acoustic stimuli, such as speech. These signals are considered complex in part due to rapid fluctuations along the intensity and temporal domains. Temporal processing ability is thought to be negatively impacted by age-related or noise-induced hearing loss. It has been suggested that deficits in temporal coding or intensity detection ability likely contribute to speech processing deficits in damaged ears. The current study aimed to evaluate chinchilla sensitivity to intensity differences with an intensity increment detection (IID) psychophysical task following carboplatin-induced selective inner hair cell (IHC) loss or noise-induced cochlear synaptopathy. The main hypothesis is that intensity coding deficits may be the result of peripheral damage, specifically related to IHC.
Methods: Young adult chinchillas were used for this study. Hearing sensitivity and cochlear non-linearities were assessed via Auditory Brainstem response (ABR) thresholds, distortion product otoacoustic emissions (DPOAEs), and psychophysical pure tone thresholds in quiet. Suprathreshold ABR wave 1 was also measured to assess cochlear output. Chinchillas were conditioned to respond to intermittent changes in intensity to an otherwise continuous reference narrowband noise. IID performance was assessed at 1-12 kHz and at three continuous reference noise levels, low (20 dB SPL), moderate (50 dB SPL), and high (70 dB SPL). The low-level noise was initially increased by 20 dB SPL, the moderate-level noise initially increased by 15 dB SPL

and the high-level noise initially increased by 10 dB SPL. An automated method of limits procedure was used to determine IID thresholds, intensity decreased by 0.5 dB SPL for correct responses and increased by 1 dB SPL for incorrect responses until the lowest intensity at which the animal achieved 66% correct was obtained. Following baseline testing, chinchillas received a single dose of 75 mg/kg of carboplatin or we were exposed to 89 dB octave band noise centered at 4 kHz for 24 hours. All measures were re-assessed after a four-week recovery period.
Results: Following carboplatin treatment there were no significant elevations of ABR or pure tone thresholds and no significant changes to DPOAE; results suggesting that hearing sensitivity remained unchanged. Consistent with previous studies, ABR wave 1 was reduced following IHC loss. Chinchilla IID thresholds were significantly elevated following carboplatin. Following the noise exposure, animals had a temporary threshold shift that recovered at 4-weeks post noise as measured by ABR thresholds and DPOAE amplitudes and a permanent reduction of wave 1 at high frequencies. Histological analysis confirmed cochlear synaptopathy. IID was negatively impacted following synaptopathic noise exposure with greater deficits at the high-intensity level.
Conclusions: These results suggest that IHC loss and damage may impact sensitivity to intensity changes and that this model could be used to study the effects of cochlear pathologies involving IHC.

SU68. CFTR Potentiator Ivacaftor Protects Against Noise-Induced Hair Cell Loss by Increasing Nrf2 and Reducing Oxidative Stress

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Category: Inner Ear: Damage and Protection
Background: Over-production of reactive oxygen species (ROS) in the inner ear can be triggered by a variety of pathological events identified in animal models after traumatic noise exposure. Our previous research found that inhibition of the AMP-activated protein kinase alpha subunit (AMPKα) protects against noise-induced cochlear hair cell loss and hearing loss (NIHL) by reducing ROS accumulation.
Methods: In this study, we have investigated a potential target of AMPKα and ROS, cystic fibrosis transmembrane conductance regulator (CFTR), and the protective effect against noise-induced hair cell loss of an FDA-approved CFTR potentiator, ivacaftor, in FVB/NJ mice, mouse explant cultures, and HEI-OC1 cells using a variety of methods, including auditory functional and immunohistochemistry measurements and flow cytometry.
Results: Noise exposure increases phosphorylation of CFTR at serine 737 (p-CFTR, S737), which reduces CFTR function, resulting in oxidative stress in cochlear sensory hair cells. Pretreatment with a single dose of ivacaftor maintains CFTR function by preventing noise-increased p-CFTR (S737). Furthermore, ivacaftor treatment increases nuclear factor E2-related factor 2 (Nrf2) expression, diminishes ROS formation, and attenuates NIHL. Additionally, inhibition of noise-induced AMPKα activation by compound C also diminishes p-CFTR (S737) expression. In line with these in-vivo results, administration of hydrogen peroxide to cochlear explants or HEI-OC1 cells increases p-CFTR (S737) expression and induces sensory hair cell or HEI-OC1 cell damage, while application of ivacaftor halts these effects. Meanwhile, ivacaftor increases Nrf2 expression and reduces ROS accumulation, whereas cotreatment with ML385, an Nrf2 inhibitor, abolishes the protective effects of ivacaftor against hydrogen-peroxide-induced HEI-OC1 cell death.
Conclusions: Our results indicate that noise-induced sensory hair cell damage is associated with p-CFTR. Ivacaftor has potential for prevention of NIHL by a single dose through maintaining CFTR function and increasing Nrf2 expression for support of redox homeostasis in sensory hair cells.

SU69. Glucocorticoid Treatment Protects Inner Hair Cell Ribbon Synapses From Degeneration Following Kainic Acid-Induced Excitotoxicity in Neonatal Mouse Cochlear Explants

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Category: Inner Ear: Damage and Protection
Background: Noise-induced cochlear synaptopathy is a result of an excessive release of excitotoxic glutamate from inner hair cells (IHCs). Such synaptopathy can lead to degradation of transmission of auditory information and hamper understanding of speech in noisy environments. During kainic acid (KA, glutamate analog)-induced excitotoxic trauma there is an upregulation of inflammatory genes in areas near inner cell synapses and spiral ganglion neurons (SGNs) (Wu et al., 2020). However, the precise role of inflammation in KA-induced cochlear excitotoxicity and ribbon synapse degeneration and/or repair is unknown. We hypothesized that inflammation plays a detrimental role, and inhibition of inflammation with glucocorticoid prevents the degeneration of IHC synapses during KA-induced excitotoxicity.
Methods: Neonatal (P2-P6) mouse cochlear explants were treated with KA (0.5 mM) for 2 hours to induce synaptopathy and neuropathy (Wang et al., 2011, modified). To examine the role of inflammation in synaptic degeneration, explants were co-treated with KA and dexamethasone (100 μM), an anti-inflammatory glucocorticoid or pre-treated with glucocorticoid receptor inhibitor, RU486 (200 μM) for 30 minutes followed by dexamethasone and KA co-treatment. To examine the effective dose of dexamethasone, explants were co-treated for 2-hours with KA and 0 μM to 1000 μM of dexamethasone. Treated explants were allowed to recover for 18 hours. At the end of the recovery period, explants were fixed with 4% paraformaldehyde for 15 minutes and immunolabeled for IHCs, presynaptic ribbons, postsynaptic densities, and afferent nerve fibers and imaged using confocal microscopy. Paired ribbon synapses per IHC were analyzed using IMARIS software.
Results: Treatment with KA resulted in ~ 64.6% loss of IHC ribbon synapses and degeneration of SGN peripheral dendrites when compared to vehicle treatment (p less than 0.0001, one-way ANOVA). However, co-treatment with dexamethasone displayed intact ribbon synapses per IHC that were comparable to the vehicle (control) treatment group (Veh=13.7 and Dex+KA=13.3) (p=0.7665, one-way ANOVA). Pharmacological inhibition of glucocorticoid receptors with RU486 reversed such protective effect of dexamethasone and showed significant synaptic and neuronal loss similar to kainic acid-alone treatment, suggesting that dexamethasone's protective effect is receptor specific. Based on the dose-response curve, the EC50 of dexamethasone in preserving 50% of inner hair cell synapses during KA-cochlear synaptopathy was found to be 1.279 μM. Last, glucocorticoid receptor (NR3C1) protein and mRNA levels (Milon et al., 2021) are elevated in cochlear macrophages after a synaptopathic noise trauma in mice.
Conclusions: These data suggest that inflammation promotes KA-induced synaptic and neuronal loss and anti-inflammatory drugs such as steroids can prevent cochlear synaptopathy and neuropathy. Using the dose dependency curve, 100 μM of dexamethasone is the optimal dose in preventing KA-induced cochlear excitotoxicity in explants. Upregulation of glucocorticoid receptors in cochlear macrophages following noise trauma may represent an endogenous mechanism for the protective effects of glucocorticoids and macrophages in noise-induced cochlear synaptopathy.

SU70. Patterns of Cochlear Neo-Ossification Following Cochlear Implantation in Mice

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Category: Inner Ear: Damage and Protection
Background: Cochlear implant (CI) outcomes are hampered by several issues, including post-implantation cochlear fibrosis and neo-ossification, which is present in up to 95% of cadaveric temporal bones in some series. Cochlear fibrosis and neo-ossification are clinically relevant problems: (1) The degree of neo-ossification surrounding a CI has been correlated with worse word recognition scores (2) Fibrotic tissue and neo-ossification may increase local electrode impedance of the CI, necessitating higher electric current stimulation, which reduces battery life and decreases dynamic range (3) The degree of neo-ossification correlates with loss of residual acoustic hearing after hybrid CI. The basic processes of cochlear neo-ossification after CI are not well understood (i.e. endochondral vs intramembranous ossification). Here, we describe the differential contributions of intramembranous and endochondral ossification toward cochlear neo-ossification after CI.
Methods: Mice were unilaterally implanted with non-functional silicone cochlear implants and followed either 10 (n=5) or 28 (n=5) days post-operatively until cochleae were harvested. Whole cochleae were imaged

with 3D X-ray Microscopy, before and after CI removal. Next, cochleae were decalcified and mid-modiolar section were prepared. A combination of Movat's pentachrome, safranin-O/fast green and hematoxylin and eosin stains were performed on adjacent sections to differentiate cartilage, osteoid and mineralized bone on microscopic images.
Results: Utilizing differential histologic staining of cartilage and bone, the patterns of intramembranous and endochondral neo-ossification that comprise post-CI intracochlear neo-ossification are described. Spatial patterns of neo-ossification are further characterized in reference to cochlear CI position using 3D X-ray Microscopy and correlations between radiologic tissue mineral density measurements and histologic presence of mineralized bone, osteoid and cartilage are made.
Conclusions: Robust neo-ossification was seen after cochlear implantation in mice, with this response appearing to result from a combination of de-novo scalar endochondral ossification as well as intramembranous ossification extending form the endosteum. Further work is needed to better elucidate the mechanisms of post-CI neo-ossification and identify the relevant cellular precursors.

SU71. Characterizing Translational Rodent Noise Exposures in a Novel Dynamic, Ambient Noise Delivery Chamber

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Category: Inner Ear: Damage and Protection
Background: Acoustic overexposure is known to cause mechanical and structural damage to the vital structures of the inner ear, leading to transient or permanent impairment of auditory and, in more intense exposures, vestibular function. In order to understand and mitigate damage associated with noise-induced hearing and vestibular loss, there is a pressing need to design and validate preclinical models in controlled environments. Therefore, to recapitulate translational exposures, we have developed an innovative rotating housing system for noise delivery to awake rodents. This system offers several distinct advantages over standard noise chambers utilized in pre-clinical models, including secure housing, eliminating anesthesia-related confounders of functional outcomes, and a reduction in exposure variability during awake noise. The main objective of this work is to characterize noise sensitivity profiles in rats following ambient exposure to a noise paradigm analogous to those which humans routinely encounter in daily life.
Methods: A circular housing chamber was designed and assembled using ¼" wire mesh fastened to a 12" acrylic plate, with two mesh dividers fastened to the main body forming four individual housing cells secured with a removable wire mesh lid. Four speakers were mounted above the chamber to deliver continuous ambient noise. The noise delivery system, or "carousel", was then calibrated and validated using a sound level meter (Convergence Instruments, [Quebec, Canada]) placed in four different positions within one cell for repeated trials to characterize noise dosage prior to commencement of noise exposures. Male Brown Norway rats (n=4) were exposed to broadband (4-16 kHz) noise at 110 dB for 1 hour to induce permanent sensorineural hearing loss (SNHL). Auditory brainstem responses (ABRs) and cervical vestibular evoked myogenic potentials (cVEMPs) were collected at baseline and at time points up to 28 days following noise. Hypothesis testing was conducted to examine longitudinal differences in auditory and vestibular function with age-matched controls (n=4). The University of Miami IACUC (#21-129-vvc ad01) approved all procedures.
Results: During the noise validation stage, we established that each cell received an average dose of 112.56 dB +/- 1.47. Auditory deficits consistent with permanent threshold shifts were experienced by all rats in the experimental group, exemplified by day 28 post-noise ABR thresholds. There was a significant difference between noise-exposed and control thresholds observed at 4, 8, 16, 24, and 32 kHz (p less than 0.01) at day 28. Similarly, cVEMP assessments in the noise-exposed group demonstrated temporary shifts in threshold, amplitude, and latency at both 1 kHz and 8 kHz with complete recovery by day 28 post-noise. Overall, ambient noise exposure in this novel chamber was well tolerated by all animals, regardless of group.
Conclusions: Our novel noise delivery system reliably and reproducibly induced auditory and vestibular deficits associated with acoustic overexposure in Brown Norway rats following ambient noise exposure.

SU72. Molecular and Morphological Changes in Hair Cell Stereocilia After Acoustic Trauma

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Category: Inner Ear: Damage and Protection

Background: Noise-induced hearing loss (NIHL) is the second most common cause of hearing loss after age-related HL. Prolonged exposure to loud noise causes various defects in both inner hair cells (IHCs) and outer hair cells (OHCs), such as disorganized stereocilia, HC degeneration, reduced ribbon synapses, and F-actin core disruption. However, the mechanical damage caused to the stereocilia by noise trauma has not been studied in detail. In this study, we systematically investigated changes in stereocilia morphology at the macro and molecular levels at multiple time points after noise trauma.

Methods: C57BL/6N mice aged 7-8 weeks were used. Temporary threshold shift (TTS) group was exposed to 2-20kHz white noise at 105dB SPL for 30 minutes, and permanent threshold shift (PTS) group to 2-20kHz white noise at 110dB SPL for 2 hours. Hearing function was assessed before, 2 hours, 1 day, and 2 weeks after noise exposure by measuring auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs). Stereocilia morphology was examined with scanning electron microscopy and immunofluorescence microscopy.

Results: In the TTS group, both ABR and DPOAE thresholds were significantly shifted 2 hours after noise exposure and gradually returned to pre-exposure levels. Stereocilia morphology was slightly disturbed 2 hours after noise exposure and returned to a normal state after 2 weeks. In contrast, in the PTS group, ABR and DPOAE thresholds were irreversibly shifted. There was also permanent damage in the stereocilia in both IHCs and OHCs. In the PTS-exposed OHCs, the tallest stereocilia were sprayed laterally and the tectorial membrane (TM) attachment links were not observed, indicating detachment of the tallest stereocilia from the TM. Intact tip-links were rarely observed due to the sprayed stereocilia. In addition, the cytoskeletal architecture of the cuticular plate was disrupted, as evidenced by abnormal spectrin ring and actin filament organization.
Surprisingly, PTS induced stereocilia fusion specifically in IHCs but not in OHCs. Proteins concentrated at the base of stereocilia (taper proteins) including CLIC5 (chloride intracellular channel 5 protein), PTPRQ (protein tyrosine phosphatase receptor Q), and TPRN (taperin) were shown to be associated with stereocilia fusion. In the PTS-exposed IHCs, all the taper proteins examined were not restricted to the taper region but were abnormally distributed along the stereocilia. Interestingly, these taper proteins were not simultaneously mislocalized but sequentially such that CLIC5 was mislocalized immediately after noise exposure, followed by PTPRQ 2 hours after noise exposure and TPRN 1 day after noise exposure.

Conclusions: These findings suggest that IHC stereocilia fusion is induced by mislocalization of the taper proteins in the PTS-exposed mice. These results provide valuable insights into the molecular mechanisms of NIHL and have potential implications for the development of future therapeutics.

SU73. A Study of Hearing Function and Histopathologic Changes in the Cochlea of the Type 2 Diabetes Model Mice

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Category: Inner Ear: Damage and Protection

Background: Sensorineural hearing loss is reported to be frequently associated with diabetic patients, however it has not been widely recognized as a complication. Hearing impairment occurs in the low- and mid-range, which is important for conversation, and thus significantly deteriorates QOL.

Methods: In order to elucidate the mechanism of the onset of hearing loss in diabetes, we conducted a study using Tsumura Suzuki Obese Diabetes (TSOD) mouse, a type 2 diabetes mellitus (T2DM) model. Body weight, blood glucose levels, and auditory brainstem responses (ABRs) were measured. The cochleae were excised and evaluated histopathologically.

Results: It was confirmed that TSOD mice developed deafness earlier than those of the control, and histopathologically, a significant reduction in the capillary distribution density was observed in the stria vascularis. This is considered to be a finding of diabetic microangiopathy in the inner ear. In order to prevent hearing loss caused by diabetes, caloric restriction and Kampo administration (Bofutsushosan and Daisaikoto)

were conducted to TSOD mice, resulting that the hearing loss was significantly ameliorated and the cochlear blood flow was maintained. On the other hand, the administration of metformin, drawing attention in the field of anti-aging medicine, was not effective in TSOD mice, while, in the presbycusis model DBA/2, the hearing loss was suppressed from the early stage in an interesting issue. Its mechanism was confirmed to have protective effects on cochlear hair cells and spiral ganglion cells. Additionally, sodium glucose co-transporter-2 inhibitors, which are antidiabetic drugs, suppressed the progression of diabetic hearing loss.
Conclusions: The background of T2DM is often associated with the metabolic syndrome caused by excessive accumulation of visceral fat. Various factors are involved in its pathogenesis in a complicated manner. Further studies are needed to clarify the mechanism of diabetic inner ear disorder.

SU74. Pathophysiological Assessment of Blast-Induced Perilymphatic Fistula

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Category: Inner Ear: Damage and Protection
Background: The risk of blast injuries is increasing for military personnel in wars and conflicts and civilians due to IEDs. The incidence of ear-related disorders such as perforation of the tympanic membrane, hearing threshold shift, tinnitus, and dizziness is high in blast injury.
Perilymphatic fistula (PLF) is a disorder of the inner ear physiology caused by leakage of the perilymph and can occur by various mechanisms. Cochlin-tomoprotein (CTP) was identified perilymph-specific and has been used for objectively diagnosing PLF.

PLF can occur due to barotrauma by external origin, including blast exposure. However, no literature exists on using CTP to diagnose blast-induced PLF in humans or experimental animals. Therefore, we explored identifying PLF by detecting CTP in the middle ear of a blast-exposed animal model and evaluating their effects on auditory function and cochlear tissue.

Methods: CBA/J mice (male, 8 weeks old, 10 animals (20 ears)) were used for the shock-tube-induced blast-exposed group. For the electrophysiological cochlear function assessment, ABR and DPOAE were measured before and 1 day, 1 month, and 2 months after blast exposure. At the same time, we observed the tympanic membrane by endoscope to investigate the perforation and its closure. PLF was identified by CTP detection in middle ear lavage fluid collected on days 0, 3, and 7 after blast exposure. CTP was detected by Western blotting using monoclonal anti-cochlin IgG2 as the primary antibody. Perilymph collected by round window perforation and recombinant human CTP (rhCTP) were used as positive controls, and middle ear lavage collected after myringotomy was used as a negative control. After the evaluation of ABR and DPOAE 2 months after blast exposure, mice were sacrificed, and the cochlear tissues, such as hair cells, synapses, and stereocilia, were evaluated by whole mount dissection and immunostaining. The mice were divided into 2 groups, PLF (+) and PLF (-), due to the CTP detection or not, respectively.
Results: Subjects fell from the stand when blast-exposed or with residual tympanic membrane perforation at the final endoscopic scan were excluded from this study. We detected the band equivalent to CTP from several ears, which means that PLF certainly exists. We compared the electrophysiological and histopathological change between PLF (+) and (-) groups. Furthermore, we investigate the correlation between the onset of PLF and the size of tympanic membrane perforation.
Conclusions: In an animal model of blast injury induced by a shock tube, CTP was detected in the middle ear lavage by Western blotting, confirming that blast injury causes PLF. Although PLF has not been focused on and explored as an ear-related disorder in blast injury, further elucidation of the pathology and the necessity for treatment should be considered.

SU75. Proteomics Study in Cochlea of Chronic Suppurative Otitis Media

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Category: Inner Ear: Damage and Protection
Background: Chronic suppurative otitis media (CSOM), is the leading cause of permanent hearing loss among children in the developing world. We have created a validated mouse model for Pseudomonas Aeruginosa (PA) CSOM and shows that it mimics the hearing loss seen in the human. that is, outer hair cell (OHC) loss in the cochlear basal turn. We identified that macrophages cause the hearing loss We aim to find the stimulus for these macrophages in the inner ear during CSOM.
Methods: An overall proteomics analysis compared the changes in the CSOM cochlea compared to the control cochlea. The cochleae from CSOM and control mice harvested after seven days of middle ear infection, and subjected to mass spectrometry using Orbitrap Eclipse Tribrid Mass Spectrometer. The overall protein profiles were analyzed using Byonic version 4.4.1. The alterations in the protein expressions were compared between CSOM cochleae and the control cochleae. The Student t-test was performed to determine the significant differences. The function and the pathway of the proteins were analyzed using the PANTHER and STRING databases.
Results: In total, 3230 proteins were identified. Out of these, only 9 proteins were matched uniquely with PA. More than 200 proteins were found in the CSOM cochlea but were absent in the control cochlea. Additionally, more than 200 proteins were significantly upregulated in CSOM compared to the control. The pathway analysis of these proteins revealed associations with both the upstream and downstream immunological responses.
Conclusions: The study supports the notion that molecules generated in CSOM may be responsible for triggering the macrophages in the inner ear leading to hair cell loss.

SU76. Multiple Mechanisms of Aminoglycoside Ototoxicity in Zebrafish Hair Cells Distinguished by Subcellular Localization of Action

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Category: Inner Ear: Damage and Protection
Background: The mechanosensory hair cells of the zebrafish lateral line has become a widely used model for understanding cellular pathways underlying aminoglycoside (AG) ototoxicity. The hair cells located on the surface of the zebrafish in clusters called neuromasts, are easily accessible for visualization. Like mammalian inner ear hair cells, they are also susceptible to damage from ototoxic chemicals. By varying time of exposure and AG concentration, previous studies from our group separated an acute mechanism that happens within minutes of AG exposure and a delayed mechanism that occurs hours after AG exposure. AG uptake and accumulation into vesicular compartments also differed between these two exposure paradigms. Neomycin uptake showed rapid cytoplasmic accumulation and gentamicin into endolysosomal compartments. In-depth studies revealed that neomycin-induced acute toxicity caused disruption of intercompartmental calcium flow resulting in mitochondrial collapse, ROS production, and cell death. However, it is unclear whether this cell death model can be generalized to other AGs, such as gentamicin or G418 and whether cellular distribution of AGs plays a role in the different cell death pathways.
Methods: To monitor intracellular calcium dynamics, we used high-resolution live fluorescent imaging of zebrafish lines expressing red and green calcium indicators targeted to cytoplasmic or mitochondrial compartments in hair cells. We also conjugated far-red fluorescent Bodipy-650 to G418 for super-resolution imaging and analysis of AG accumulation and distribution in hair cells using a machine-learning based algorithm. Dose response analyses were used to resolve temporal sensitivity of AGs and effects of endolysosomal perturbations.
Results: Gentamicin and G418 produce distinct temporal and dose response functions that are substantially delayed compared to neomycin. Hair cells undergoing G418-induced delayed cell death exhibit dramatically different Intracellular calcium dynamics from those undergoing acute death. Perturbation of endolysosomal compartments using lysosomotropic agent GPN results in accumulation of G418 into significantly fewer vesicles. This interference with G418 cellular compartmentalization is robustly protective against G418-induced delayed hair cell death but not neomycin-induced acute death.

Conclusions: Our experiments elucidate at least two different mechanisms by which AGs kill zebrafish hair cells. They are distinct temporally, in their intracellular calcium dynamics, and in AG distribution inside hair cells. The accumulation of AGs in endolysosomal compartments plays a key role in the delayed cell death pathway. Multiple distinct cell death pathways activated by different AGs have potentially important consequences for therapeutic approaches to prevent AG-induced ototoxicity.

SU77. Investigating Neuroprotective Bclw as a Preventative Measure Against Noise-Induced Hearing Loss

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Category: Inner Ear: Damage and Protection

Background: Global health organizations recently identified hearing loss as a major risk factor for age-related dementia and protecting one's hearing as a key factor in preventing cognitive decline. While the cellular mechanisms leading to hearing loss are not yet established, exposure to excessive noise clearly accelerates cochlear degeneration. Even temporary Noise Induced Hearing Loss (NIHL) can lead to permanent damage of the spiral ganglion neurons (SGNs) that transmit sound information from inner hair cells. This sensorineural damage likely manifests in humans as "Hidden Hearing Loss" that is undetected by hearing threshold tests but often reported as difficulty interpreting speech. This paradoxical phenomenon whereby patients can perceive sound but fail to discriminate nuances in speech could be linked to certain SGN subtypes being more susceptible to synaptopathy following noise exposure and with age. Thus, there is a need to develop a deeper understanding of how these subtypes respond to insult and to identify preventative measures that confer protection to those that are most vulnerable. Here we aim to achieve these goals by leveraging genetic tools to characterize patterns of SGN subtype loss,and testing the efficacy of neuroprotective pro-survival Bcl2 family member Bclw in preventing noise induced synaptopathy.

Methods: We take a multipronged approach to establish a comprehensive understanding of SGN subtype loss and its links to Bclw. Using Netring1 to drive Cre expression in a reporter mouse line, we measure the degree of SGN subtype synapse loss between 2 and 20 months of age as well as following noise exposure at 8 weeks of age. In parallel, we conditionally delete Runx1 in mice as a means of testing if SGN molecular identity predisposes Type 1B and 1C neurons to synaptopathy. To measure Bclw's potential as a preventative measure against NIHL, we virally overexpress this pro-survival gene and evaluate synapse function and morphology upon exposure to noise. Finally, we examine whether these subtypes are more vulnerable to an age-related reduction in Bclw expression, or if loss of Bclw preferentially impacts these SGNs.

Results: Our preliminary findings suggest we can quantify the pattern of subtype synapse loss using Netring1 to label SGN Type 1B and 1C synapses. We find in pilot studies that conditional deletion of Runx1 from SGNs may prevent reductions in ABR wave I amplitudes and loss of synapses following noise exposure. Initial Bclw viral overexpression shows promise for synapse protection. We will discuss results from follow up experiments and from in situ hybridization studies characterizing Bclw expression patterns throughout age in the cochlea.

Conclusions: Our work highlights the need to generate a deeper characterization of how noise impacts vulnerable SGN subtypes, how the mechanisms of this damage relate to age-related synaptopathy, and whether booting endogenous mechanisms such as Bclw provides therapeutic potential.

SU78. Biochemical and Bioenergetic Alterations in Cochlear Tissue: A Comparative Study in Wild Type and Alport Syndrome Knockout Mice Under Quiet and Noise Conditions

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Category: Inner Ear: Damage and Protection

Background: The study aims to elucidate the biochemical and bioenergetic alterations in cochlear tissues of wild type (WT) and Alport Syndrome Knockout (KO) mice under quiet and noise-induced metabolic stress

conditions. We hypothesize that noise exposure amplifies existing metabolic imbalances, potentially implicating mitochondrial dysfunction culminating in accelerated cochlear impairment.
Methods: 129Sv WT and Collagen 4α3 KO (Alport AR) mice, aged 9 weeks, were subjected to either quiet or noise-induced metabolic stress. Data were collected from quiet-reared mice and from noise-exposed mice at two time points post-noise: Immediately post-exposure (Day 0) and five days post-exposure (Day 5). Auditory Brainstem Response (ABR) and Endolymphatic Potential (EP) were measured. The stria vascularis from both cochlea of individual mice were microdissected and processed to conduct a comprehensive array of biochemical assays and bioenergetic calculations. These included measurements of Total Protein, AMP, ADP, ATP, NAD/NADH ratios, and Na+-K+- ATPase activity.
Results: Data reveal significant disparities in adenine nucleotide ratios, Adenylate Energy Charge (AEC) and redox states (NAD/NADH) between WT and KO mice, particularly under noise-induced metabolic stress. Noise-exposed KO mice exhibited maladaptive increases in ATP/ADP ratios and AEC, coupled with elevated Total Protein levels, suggesting fibrosis and continued heightened cellular stress. Notably, Na+-K+- ATPase activity was found to be decreased in KO mice, indicating impaired ion homeostasis and cellular excitability. These alterations were also reflected in the ABR and EP measurements, with KO mice showing a decrease in both parameters compared to WT mice, which demonstrated recovery by Day 5 post-noise exposure.
Conclusions: The study uncovers distinct metabolic and mitochondrial profiles in cochlear tissue of WT and KO mice, particularly in response to noise exposure as a metabolic stress. Mitochondrial dysfunction is hypothesized due to observed imbalances in adenine nucleotide ratios and redox states, which are critical for mitochondrial energy production and cellular adaptation to stress. The maladaptive response in KO mice, including altered Na+-K+- ATPase activity, suggests a compromised ability to adapt to metabolic stress, thereby accelerating cochlear dysfunction. These findings underscore the need for targeted therapeutic interventions, potentially focusing on metabolic modulators and mitochondrial enhancers, to mitigate accelerated cochlear impairment. Extension beyond the stria vascularis to include other critical cochlear tissues, such as the organ of Corti, will provide a more comprehensive understanding of cochlear metabolic dynamics during noise induced metabolic stress.

SU79. Physiologic Otoprotection via Renin-Angiotensin System Blockade in Noise-Induced Hearing Loss Model

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Category: Inner Ear: Damage and Protection
Background: Current treatments for hearing loss are limited to sound amplification or cochlear implant devices. Establishment of additional or complimentary medical therapies for hearing loss could have wide-reaching impact. Impairments in cochlear microcirculation and perfusion have been identified in patients with presbycusis and in preclinical models of noise induced hearing loss. Treatments targeting the renin angiotensin system have the potential for system-wide vasoprotective effects with the potential to protect microcirculation within the inner ear, such as in the stria vascularis and spiral ligament. Blockage of the angiotensin II type 1 receptor using losartan, a widely used and clinically-relevant angiotensin receptor blocker in humans, is proposed as a potential otoprotective therapy in those at risk for hearing loss.
Methods: CBA-J mice (age 9-10 weeks) were treated with standard chow vs losartan-infused chow (20mg/kg/day, equivalent to ~100mg daily dose in humans) for three days prior and two weeks after exposure to noise. Noise induced hearing loss protocol was comprised of a two-hour exposure to 8-16 kHz octave band noise (102.5dB for males, 105dB for females). Electrophysiology measures (ABR/DPOAEs) and serum samples were collected at days -1, 1, 7, 1 and 14 relative to noise exposure, followed by cochlear tissues harvesting.
Results: ABR auditory threshold shifts at 16 and 32 kHz were nearly completely prevented in the losartan treated cohort vs control group (p less than 0.01) at day 1 following noise exposure. Recovery of baseline threshold was observed in both groups by day 7. ABR wave 1 amplitude trends towards less pronounced

decrease at day 1 following noise exposure in Losartan treated cohort vs control group. There was no significant difference in DPOAE auditory threshold between treatment and control group at any timepoint.
Conclusions: These findings suggest that treatment with RAS blockers provide physiologic otoprotection from temporary noise-induced hearing loss, as losartan-treated mice exhibited very little hearing threshold shifts with ABR testing at certain frequencies following noise exposure compared to control subjects. This finding has significant clinical implications for selection of appropriate medication regimen among patients at risk for noise-induced hearing loss.

SU80. Disruption of F-Actin Within the Shafts of Stereocilia is a Key Difference Between Temporary and Permanent Noise-Induced Hearing Loss

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Category: Inner Ear: Damage and Protection
Background: Sound detection is initiated by deflection of rod-like mechanosensitive stereocilia protruding from the apical surface of the auditory hair cells. Shafts of the stereocilia are composed of highly ordered and stable core of parallel actin filaments, some of which extend inside the cell, into F-actin meshwork of the cuticular plate. During sound-induced deflections, stereocilia pivot around their bases, where F-actin forms rootlets that presumably allow sliding of actin filaments relative to each other. Structural integrity of the stereocilia bundles is essential for life-long mechanotransduction in non-regenerating mammalian auditory hair cells. However, stereocilia can be highly susceptible to overstimulation. Indeed, classical experiments by Liberman (1987) have established the damage to the stereocilia bundles as the hallmark of the permanent noise-induced hearing loss (NIHL). Yet, despite decades of NIHL studies, it is still unknown exactly which ultrastructural changes in the stereocilia bundles are evoked directly by mechanical overstimulation and which of them are associated with temporary (TTS) or permanent (PTS) shifts of hearing thresholds after noise exposure.
Methods: Auditory brainstem responses were used to determine the noise severity (intensity and duration), which would reliably produce TTS and PTS in 16-20 kHz frequency region in the adult (P25-P26) male and female C57BL/6 mice. Then, different groups of mice were exposed to “TTS” or “PTS” noise and euthanized immediately after noise exposure together with non-exposed control group and their cochleae were fixed. Then, the regions of the organs of Corti corresponding to 16-20 kHz frequencies were dissected, high pressure frozen, freeze-substituted, and low-temperature embedded into the resin for subsequent serial sectioning with focused-ion beam scanning electron microscopy (FIB-SEM). FIB-SEM images of stereocilia bundles in both inner and outer hair cells were obtained.
Results: Our data show that the immediate effects of noise exposure may include: i) disorganization of F-actin within stereocilia; ii) shrinkage of F-actin meshwork in the cuticular plate; and iii) expansion of the rootlets. However, disorganization of F-actin within the cuticular plate and expansion of the rootlets occurred in both TTS and PTS and increased with the severity of noise exposure. The only pathology that was consistently different between TTS and PTS is the disorganization of F-actin in the shaft of stereocilia, the region that is known to have minimal or no turnover in mammalian auditory hair cells.
Conclusions: We conclude that disorganization F-actin within the shafts of stereocilia differentiates between PTS and TTS.

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SU81. Envelope following Response and Auditory Brainstem Response as Potential Biomarkers of Central Compensation Following Selective Inner Hair Cell Loss

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Category: Inner Ear: Damage and Protection

Background: In animal models, cochlear damage has been shown to produce compensatory central gain from the auditory system. Despite the well-known association between peripheral acoustic injury and central compensation in animals, we lack sensitive correlates of central auditory gain that can be applied to humans in both clinical and research settings. Here, we assess the utility of using clinically feasible electrophysiological assays such as the envelope following response (EFR) and the auditory brainstem response (ABR) to assess changes in central auditory gain following selective inner hair cell (IHC) loss in the chinchilla animal model.
Methods: Young adult (2-3 years old) chinchillas were used in this study. Cochlear nonlinearity, hearing sensitivity, and auditory neural pathways were evaluated by assessing distortion product otoacoustic emissions (DPOAEs), ABR thresholds, ABR wave 1-5 amplitudes, and EFR amplitudes. EFR was elicited using amplitude modulated (AM) tones (AM depths: 100%, 80% and 20%; AM rates: 40 Hz and 88 Hz). All measures were obtained before and after selective IHC loss induced by a single dose of 75 mg/kg of carboplatin (i.p.). ABR and EFR were reassessed after a three-week recovery period. Post-exposure changes in ABR wave 5/1, 5/3 and 3/1 amplitude ratios and EFR amplitudes were evaluated as potential markers of central compensation following selective IHC loss.
Results: Histological results showed 60-80% loss of IHC with little-to-no loss of outer hair cells. Carboplatin treatment yielded a significant reduction in ABR wave 1 amplitude with no change in DPOAE amplitudes or ABR thresholds. Following IHC loss, we observed a significant increase in ABR wave 3/1, 5/1 and 3/5 amplitude ratios, a significant reduction in fast-rate EFR amplitudes, and no significant change in slow-rate EFR amplitudes.
Conclusions: Enhanced central auditory gain secondary to selective IHC loss can be detected via far-field ABR and EFR recordings in the chinchilla animal model. These results suggest that central compensation following reduced peripheral sensory input may be elucidated via electrophysiological assays that are accessible to clinical settings.

SU82. Effects of Angiotensin Receptor Blockade on Cochlear Synaptopathy Following Noise Overexposure

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Category: Inner Ear: Damage and Protection
Background: Noise induced hearing loss (NIHL) is a major public health concern, with chronic and acute noise overexposures resulting in progressive damage to cochlear hair cells, the stria vascularis, and the spiral ganglion. Cochlear synaptopathy may be concurrent with or temporally distinct from loss of hair cells or neurons, and the mechanisms of synaptopatphy in NIHL are uncertain at this time. Relatively normal audiograms can mask potential widespread synaptopathy that can precipitate eventual neuronal degeneration and hearing loss. This “hidden hearing loss” indicates a necessity for early intervention. Losartan is an angiotensin 1 receptor (AT1R) antagonist that exerts substantial protective effects in chronic inflammatory and vasculopathic conditions including renal, pulmonary, cardiac, and neurovascular/neurodegenerative diseases. In light of this therapeutic's proposed anti-inflammatory and known vascular modulatory effects, losartan is a promising candidate in investigating protection against NIHL.
Methods: CBA/J mice (n=14) age 9-10 weeks were randomly assigned to receive standard chow or losartan-infused chow (20mg/kg/day) beginning 3 days prior to exposure to 2 hours of 8-16k octave band noise at 102.5 dB (males) or 105 dB (females) to induce a transient threshold shift NIHL phenotype. Auditory brainstem responses (ABR) were obtained at baseline and at 1, 7, and 14 days after noise exposure. Cochleae were then harvested at 14 days following noise exposure for whole mount and immunostained with antibodies against CtBP2 (pre-synaptic ribbon), PSD95 (post synaptic density protein), and Myo7a (inner and outer hair cells). Samples were imaged with confocal microscopy. Synapses, defined as CtBP2 puncta with PSD95 puncta within 0.5 micrometers, and inner hair cells (IHC) at the basal turn of the cochlea, were quantified and compared between groups.
Results: Transient ABR threshold shifts (TTS) at 1 day after noise exposure for the control group were approximately 16 dB (at 16 kHz) and 18 dB (at 32 kHz) and approximately 5 dB (at 16 kHz) and 1 dB (at 32

kHz) for the losartan-treated group. In both groups, the threshold resolved to baseline levels by 7 and 14 days. At the basal turn, there was a trend towards increased average number of synapses per IHC in losartan-treated animals (6.23, n=4) relative to the control group (4.83, n=4), though this did not reach statistical significance (p=0.12).
Conclusions: Robust synaptopathy was likely not achieved using this relatively mild noise exposure protocol which induced only a small TTS. However, the identified tendency toward preservation of IHC synapses is promising and warrants further investigation.

SU83. A Novel Therapeutic Strategy of Mitochondrial Transplantation Into Cochlear Cells for the Treatment of Hearing Loss

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Category: Inner Ear: Damage and Protection
Background: Hearing loss is a prevalent and disabling condition with no treatment currently available. A recent approach of mitochondrial therapy (mitotherapy) by means of transplantation of exogenous healthy mitochondria into damaged organs (e.g., the heart) has shown promising results in mitigating mitochondria-related disorders. Remarkably, despite the essential role of mitochondria in hearing, this novel strategy has never been tested for the treatment of hearing loss. More importantly, whether cochlear cells take up exogenous mitochondria and the consequences on cell bioenergetics has never been tested.
Methods: Exogenous mitochondria were isolated from HEI-OC1 cells, labeled with a pHrodo Red fluorescent dye, and co-incubated with a new set of HEI-OC1 cells for 24H. Following internalization, cellular growth, oxidative-stress response, and bioenergetics of the recipient cells were assessed by using standard techniques.
Results: We, for the first time, successfully transplanted exogenous mitochondria from HEI-OC1 auditory cells into a new set of HEI-OC1 cells through co-incubation. We next tested the potential toxicity of transplanted mitochondria in auditory cells. We found that co-incubating recipient cells with increasing doses of exogenous mitochondria leads to increased mitochondrial uptake by the recipient cells while no toxic effect was observed on cell growth. Lastly, we found that transplanted exogenous mitochondria boost the spare respiratory capacity in recipient HEI-OC1 cells and protect cells from H2O2-induced cell loss.
Conclusions: Given that HEI-OC1 cells are derived from mouse auditory cells and are considered as a progenitor for sensory and supporting cells of the organ of Corti, these findings provide the first milestone for the feasibility of mito-therapy in auditory cells. If shown to be successful in animals and ultimately in humans, this novel therapy has prominent potential for the treatment of sensorineural hearing loss.

SU84. TTR is Not Required for Hearing.

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Category: Gene Therapy
Background: Transthyretin amyloidosis is a rare, fatal disease caused by the aggregation of the transthyretin (TTR) protein. TTR aggregation can give rise to amyloid fibrils, leading to the formation of plaques. It is estimated that approximately 25% of the world population over 80 years old have cardiac amyloid deposits composed of wildtype TTR (Westermark et al., 1990). Mutations of TTR, including p.V30M, p.L55P, and p.V122I result in increased aggregation, earlier onset, and severity of disease. TTR plaques affect several organ systems leading to neurological or cardiac symptoms. Different mutations in TTR are generally associated with pathology of different organ systems. TTR is produced in the liver, brain, eye and ear (Finsterer et al., 2019; Kolla et al., 2020) where it is secreted into the serum, CSF, vitreous and likely the endolymph or perilymph, respectively.
Methods: RNA scope was used to determine where TTR is expressed in the cochlea. ABRs were recorded at 8 kHz, 16 kHz, and 32 kHz at decreasing sound intensity levels from 90 to 15 dB, on TTR KO and wildtype mice monthly from the age of 8 weeks until 83 week of age. Analysis was done on ABR waveforms to determine thresholds, peak 1 amplitude, and peak 1 latency. DPOAE was taken at 83 weeks.

Results: Patients with both wild-type and hereditary TTR amyloidosis have been shown to have greater hearing loss than would be expected just due to aging (Bartier et al., 2019; Bequingon et al., 2017). It is unclear whether this impairment is due to a problem with TTR function within the ear or another mechanism.
In this experiment we evaluated whether TTR was an important factor in hearing by examining how it affects hearing in mice. First, we confirmed that TTR is indeed expressed in the inner ear of mice via RNA scope. We found that it is expressed exclusively in Reissner's membrane (RM) and approximately 40% of RM cells express TTR in neonatal and adult mice. We then generated a TTR knockout mouse with the TTR gene completely removed. Auditory Brainstem Response (ABR) measurements were taken longitudinally in the TTR KO mouse model to assess whether TTR is necessary for normal hearing function over the lifespan of the mouse. No discernible significant difference was observed between TTR KO and TTR wildtype mice in ABR thresholds, Wave I amplitude or Wave I latency over 74 months, suggesting that TTR is not necessary for normal hearing function or development.

Conclusions: These results have implications for treatment of TTR amyloidosis, particularly in gene therapies that involve deletion of the TTR gene, suggesting that a loss of TTR will not affect hearing.

SU85. Comparing Round Window Membrane Permeability Enhancers: An Animal Study

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Category: Inner Ear: Drug Delivery
Background: Dexamethasone (DEX) is a widely used drug for the treatment of acute inner ear disease. Intratympanic DEX (IT-DEX) injection offers the advantage of minimal systemic side effects and high inner ear penetration. However, a significant limitation of IT-DEX is its inability to effectively penetrate the round window membrane (RWM), leading to drug elimination. In this study, we investigated the safety and efficacy of adjuvant agents known to enhance RWM permeability.
Methods: Three injectable agents (Histamine 0.01 g/ml, 3% hypertonic saline, sodium caprate 1.94 mg/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. RWM samples were also collected on the day of IT injection and one week later for transmission electron microscopy (TEM) examination.
Results: The highest perilymph concentration of DEX was achieved when injected after 3% hypertonic saline. Additionally, structural changes in the RWM were observed in the order of histamine, hypertonic saline, and sodium caprate, with significant recovery seen after one week.
Conclusions: 3% hypertonic saline induced transient RWM structural changes, improving the intratympanic penetration of IT-DEX and was proved to be relatively safe. Administering 3% hypertonic saline intratympanically 15 minutes before IT-DEX administration is expected to enhance therapeutic outcomes.

SU86. The Transcription Factor Pou4f1 Regulates Spiral Ganglion Positioning and the Timing of Hair Cell Differentiation

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Category: Development: Cellular/Systems
Background: Pou4f1 is a transcription factor known to be involved in early spiral ganglion (SG) development. It is initially expressed broadly by immature spiral ganglion neurons (SGNs) but later becomes restricted to just the SGN 1C subgroup. Germline knockout (KO) of Pou4f1 leads to previously described defects in SGN survival and axonal patterning, and is lethal past P0. However, the effects of Pou4f1 deletion on other aspects of cochlear formation, such as hair cell development and SGN positioning and patterning, have not been fully assessed.

Methods: To assess changes in cochlear development in the absence of Pou4f1 we collected cochleae from Pou4f1 KO mice at embryonic day (E)16 and postnatal day 0 (P0). Wildtype littermates were collected as controls. Tissue was immuno-stained with markers for hair cells, support cells and neurons. To assess changes in gene expression in the absence of Pou4f1, KO and wildtype SGNs were dissected at E14 and analyzed using bulk RNA sequencing (RNASeq).
Results: At P0, Pou4f1 KO mice displayed SGN defects consistent with previous results, in particular, a loss of basal SGNs and substantial axon pathfinding defects in the apical regions. In E16 Pou4f1 KO cochleae four rows of Myosin7a+ hair cells were present along the entire length of the cochlear spiral. In contrast, in wild type controls Myosin7a staining was limited to the basal region. This result suggests that, in the absence of Pou4f1, HCs differentiate prematurely. This phenotype is reminiscent of the effects of deletion of Sonic hedgehog (Shh) from the spiral ganglion. Consistent with this conclusion, RNASeq results from E14 SGNs confirmed a signification reduction of Shh expression in Pou4f1 KOs indicating that Pou4f1 regulates SGN Shh expression, which in turn regulates the timing of HC differentiation.
In E16 Pou4f1 KO cochleae we also identified defects in SGN positioning. In wildtype cochlea SGN cell bodies are located in an organized spiral medial to the sensory epithelium. However, in cochleae from Pou4f1 KOs, SGNs appeared in ectopic locations both laterally towards the sensory epithelium, or medially throughout the modiolar region. This suggests the loss of a guidance cue which normally restricts migrating SGNs to the region which will become Rosenthal's canal.

Conclusions: These findings show that Pou4f1 acts as a regulator of multiple developmental processes, not only in the SG, but, indirectly, in cochlear sensory cells. Future work will focus on examining the role of Shh, and other Pou4f1 targets, in HC and SGN development.

SU87. Varying Mechanical Forces Drive Sensory Epithelium Formation

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Category: Development: Cellular/Systems
Background: The mechanical cues of the external microenvironment have been recognized as essential clues driving cell behavior. Although intracellular signals modulate cell fate during sensory epithelium development is well-understood, the driving force of sensory epithelium formation remains elusive.
Methods: We constructed a stiffness-adjustable gelatin methacryloyl (GelMA)-HA-Arg-Gly-Asp (RGD) hydrogel system for the culture of cochlear organoids. By investigating the biological behaviors of cochlear progenitor cells (CPCs) in the designed mechanical environment of the hybrid hydrogel, we tuned the mechanical parameters of the hydrogels to define the optimal conditions for CPC-derived organoid expansion and differentiation. To correlate the developmental process of the cochlear epithelium with the stages of cochlear organoid formation in the stiffness-shifted hydrogel system, we further employed atomic force microscopy (AFM) to measure the stiffness of the stromal area connected to the epithelium. Using shRNA or inhibitors, we intervened in a series of molecules, including ITAG3, F-actin, YAP, PIEZO2, ERK1/2, and KLF2, to clarify their role and relationships in the development of the inner ear.
Results: In the stiffness-tunable hydrogel system, the mechanical force from hydrogels driving cochlear organoid formation mimicked the dynamic extracellular matrix (ECM) force shaping sensory epithelium formation in vivo. As the driving force, moderate ECM stiffness activated the expansion of cochlear progenitor cells (CPC)-derived epithelial organoids by modulating the integrin α3 (ITGA3)/F-actin cytoskeleton/YAP cascades. While higher stiffness induced the transition of CPCs into sensory hair cells (HCs) through increasing the intracellular Ca2+ signaling mediated by PIEZO2, and subsequently activating KLF2 to accomplish the cell specification.
Conclusions: This study opens avenues for defining alternative culture systems to better manipulate stem/progenitor cell fate through modulation of the mechanical properties of the system. Moreover, the mechanism through which mechanical forces regulate cell fate provides potential targets for regenerative medicine.

SU88. Illuminating the Depths of Auditory Cortex: The Role of Subplate Neurons in Developing Circuits

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Category: Development: Cellular/Systems

Background: To form circuits that process information, make decisions, and execute complex behaviors, billions of brain cells must establish and refine trillions of appropriate connections during development. A major step in this process involves pioneering axons from the thalamus traversing to layer 4 (L4), cementing the ability of the periphery to communicate with the cortex. Surprisingly, thalamic axons do not proceed directly to L4, but rather synapse first onto subplate neurons (SPNs) located beneath L6. In the visual system, early ablation of SPNs disrupts ocular dominance column formation, suggesting that SPNs exert a powerful influence over early large-scale network activity and connectivity, however, the role of SPNs in shaping auditory cortical circuits has remained underexplored, prompting questions about how or if these neurons are involved in shaping emergent tonotopic organization.

Methods: To explore the role of the SPNs in cortical development, we generated a new mouse model using CRISPR/Cas9-mediated insertion of CreER into the endogenous Cplx3 locus, allowing for unprecedented access to SPNs. In order to understand what types of activity these cells display before hearing onset, we crossed this line to a Cre-dependent GCaMP6s line and used two-photon imaging in awake (non-anesthetized) mouse pups.

Results: We observed highly coordinated spontaneous activity among groups of SPNs that we hypothesized could reflect activity generated within the cochlea, as previous experiments demonstrated that early sensory-independent activity propagates form the cochlea to the cortex at this age (PND 7). To address this, we simultaneously imaged auditory SPNs and midbrain neurons using widefield imaging and observed a high degree of correlation between the two areas, suggesting that the periphery drives SPN activity at this time. Further, robust responses to pure tone stimuli could be seen in SPNs using widefield and two-photon imaging in mice after hearing onset, providing additional evidence that peripheral activity can engage SPNs and that activity is organized along the tonotopic axis.

Conclusions: Our results indicate that SPNs receive organized thalamic input during a time period where thalamocortical axons are just reaching layer IV, suggesting that SPNs may play an instructive role in organizing layer IV inputs. Ongoing experiments seek to alter SPNs during this critical developmental period by either genetic ablation (Cre-dependent DTA) or silencing (Cre-dependent tetanus toxin) strategies to understand how these neurons contribute to cortical development. We hope that the proposed studies lead to fundamental knowledge of how these early circuits coalesce into their mature state so that we can better understand early developmental auditory processing disorders (APDs) in children, whose etiology and mechanisms remain unknown.

SU89. A Spatial Transcriptomic Atlas of the Developing Inner Ear at Single-Cell Resolution

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Category: Development: Cellular/Systems

Background: The inner ear develops from the otocyst, an ellipsoid structure consisting of a layer of apparently homogeneous epithelial cells. Remarkably, most of the regions destined to form the sensory and non-sensory components associated with the semicircular canals and coiled cochlea are already specified at this stage. However, it is not yet fully understood how regional fate specification occurs in the early otocyst. To dissect this process, it is essential to have detailed gene expression atlases at each stage of development. Single-cell RNA sequencing (scRNA-seq) datasets are available at the otocyst stages, but they do not provide spatial information. While high-throughput spatial transcriptomics techniques are rapidly advancing, achieving single-cell resolution remains a challenge. In situ hybridization (ISH) images are relatively easy to produce and are readily available in large quantities in developmental biology laboratories. In this study, by combining scRNA-seq datasets with ISH images, we developed a tool that generates a three-dimensional atlas of the otocyst transcriptome at single-cell resolution.

Methods: ISH images for marker genes were selected based on known spatial expression patterns in the otocyst at E9.5, E10.5 and E11.5. These included sensory markers such as Lfng, Fgf10, and Sox2, and non-sensory markers such as Tbx1 and Lmx1a. Each ISH image set was subjected to a workflow consisting of preprocessing, segmentation, and serial section registration steps to create a 3D model of the otocyst shape at each stage. ISH signal intensity measurements were then superimposed on the models to generate spatial gene expression profiles for the markers. The profiles were integrated by mapping all otocyst models onto one reference model using 3D registration. The integrated spatial gene expression profile was used as marker input for virtual reconstruction of scRNA-seq datasets of the otocyst.
Results: We assessed the accuracy of the spatial reconstruction of scRNA-seq datasets using several metrics. We first examined genes with known expression patterns and confirmed that the relative expression domains were closely reflected in the reconstructed otocyst atlas. To demonstrate its use as a tool for discovery, we analyzed the reconstructed atlas to identify genes that were previously unknown yet potentially important for inner ear development, whose expression in the otocyst were validated by ISH. We are currently creating an online resource where the atlas can be interactively viewed and explored. We are also analyzing single nuclear multiome (RNA-seq + ATAC-seq) data generated from multiple otocyst stages using our platform to identify potential regulators that contribute to regional fate specification.
Conclusions: We present a workflow for reconstructing spatial information from scRNA-seq datasets by exploiting the abundance of ISH images. The reconstructed 3D gene expression atlas of the otocyst will be a valuable tool to explore and investigate the process of inner ear development.

SU90. 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: In most tissues, the proliferation of progenitor cells and the specification of terminally differentiated cell types coincide during development. A similar pattern is observed in the vestibular sensory organs, where hair cells are produced throughout development in the growing macula. However, in the organ of Corti, the proliferation and differentiation of progenitor cells are strictly separated both spatially and temporally. A rapid apical-to-basal wave of transcriptional upregulation of Cdkn1b, encoding p27kip1, was shown to control this unique pattern of cell cycle exit in the cochlea. Despite the necessity for this complex patterning, the mechanism regulating the expression of Cdkn1b and initiating cell-cycle exit in the developing organ of Corti remains unknown.
Methods: Here, we propose that distinct transcriptional regulators are utilized in the organ of Corti to enforce the abrupt upregulation of the Cdkn1b gene and early cell cycle exit. To test this idea, we compared the gene regulatory networks (GRNs) employed in the 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 and utilized Scenic+, which allows the identification of putative tissue-specific transcription factors, enhancers, and downstream targets. To assay enhancer activity, these elements 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.
Results: We identified several putative cochlear-specific transcription factors and Cdkn1b enhancers that may drive the developmental wave of p27kip1 upregulation and early cell cycle exit. The activity of the identified enhancers was confirmed to be restricted to cochlear supporting cells in vivo. Additionally, 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 in the process of validating the transcription factors predicted to interact with each such element to drive Cdkn1b 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 essential for proper auditory system development and, ultimately, for normal hearing. Despite its importance, how this developmental patterning is controlled remains unknown. This study aims to identify the transcription factors and gene regulatory elements uniquely utilized in the developing cochlea to establish the postmitotic prosensory domain and to maintain lifelong mitotic quiescence in the organ of Corti.

SU91. Differential Regulation of Hair Cell Actin Cytoskeleton Mediated by SRF and MRTFB

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Category: Development: Cellular/Systems

Background: The MRTF-SRF pathway has been extensively studied for its crucial role in driving the expression of a large number of genes involved in actin cytoskeleton of various cell types. However, the specific contribution of MRTF-SRF in hair cells remains unknown.

Methods: Immunocytochemistry, SEM, TEM, ABR and DPOAE tests, Whole-cell patch-clamp recording, FACS-based hair cell RNA-seq and In vivo AAV gene delivery

Results: In this study, we showed that hair cell-specific deletion of Srf or Mrtfb, but not Mrtfa, leads to similar defects in the development of stereocilia dimensions and the maintenance of cuticular plate integrity. We used FACS-based hair cell RNA-seq analysis to investigate the mechanistic underpinnings of the changes observed in Srf and Mrtfb mutants, respectively. Interestingly, the transcriptome analysis revealed distinct profiles of genes regulated by Srf and Mrtfb, suggesting different transcriptional regulation mechanisms of actin cytoskeleton activities mediated by Srf and Mrtfb. Exogenous delivery of calponin 2 using Adeno-associated virus transduction in Srf mutants partially rescued the impairments of stereocilia dimensions and the F-actin intensity of cuticular plate, suggesting the involvement of Cnn2, as an Srf downstream target, in regulating the hair bundle morphology and cuticular plate actin cytoskeleton organization.

Conclusions: Our study uncovers, for the first time, the unexpected differential transcriptional regulation of actin cytoskeleton mediated by Srf and Mrtfb in hair cells, and also demonstrates the critical role of SRF-CNN2 in modulating actin dynamics of the stereocilia and cuticular plate, providing new insights into the molecular mechanism underlying hair cell development and maintenance.

SU92. Open Board

SU93. Degeneration of Supporting Cell Primary Cilia Within the Mouse Cochlea

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Category: Development: Cellular/Systems

Background: The cochlear and vestibular epithelium contain mechanosensory hair cells mediating the detection of hearing and balance, respectively. The process of mechanotransduction relies on the deflection of apical F-actin-based protrusions, the so-called stereocilia. The correct arrangement of the developing stereocilia and the planar cell polarity is mediated by one true primary cilium that is called kinocilium. The mammalian cochlea hair cell kinocilium degenerates between P8 - P12 while the vestibular kinocilium persists. Beside sensory hair cells, non-sensory supporting cells contain one primary cilium as well. Even if it is believed that primary cilia of epithelial non-sensory supporting cells persist, the development and time course of supporting cell primary cilia was not featured yet.

Methods: To constitute the development of primary cilia in the inner ear, we first performed immunohistochemical staining of the mouse cochlea and vestibulum of different ages using common markers of primary cilia function and development including acetylated tubulin, ARL13B and IFT140. The results were analyzed using confocal laser scanning microscopy. We additionally performed scanning electron microscopy (SEM) on cochlea samples of immature (P0-P8) and mature (P30+) mice.

Results: Right after birth (P0/1) all markers stain cochlear and vestibular hair cell kinocilia and supporting cell primary cilia. While all markers provide a positive ciliary staining in the vestibulum for P8, P12 and P30, no ARL13B- or IFT140-positive cochlear primary cilia and kinocilia are visible in P8 or older mice. Beside the known degeneration of cochlear hair cell kinocilia starting from P8 on, the tubulin-staining indicates a similar loss of supporting cell primary cilia in the cochlea between P8 - P12. SEM images show a loss of cochlear supporting cell primary cilia within the cochlea of mature mice.

Conclusions: We conclude that the primary cilia of cochlear supporting cells degenerate between P8 - P12 as the essential ciliary proteins IFT140, ARL13B and acetylated tubulin are not abundant anymore that comes along with the results of SEM.

SU94. GDF6 is the Master Regulator of Cochlear Duct Elongation

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Category: Development: Cellular/Systems

Background: Cochlear identity is specified in a ventral region of the otocyst by extrinsic SHH signaling originating from the ventral midline. In the absence of SHH, cochlear formation is completely absent. However, once the cochlear identity is established, SHH does not appear to be required for cochlear elongation, as the cochlea, although abnormal, can still elongate without SHH. It is currently unclear what the intrinsic signal is that drives the elongation of the ventral otic region with cochlear identity.

Epithelial-mesenchymal interactions play a crucial role in organ formation. Cochlear elongation also appears to require these interactions, as evidenced by the severely shortened cochlear duct in the absence of genes expressed in the periotic mesenchyme (POM), including Sox9, Tbx1, and Fgfr1/2. However, the cellular heterogeneity of the POM and the cochlear epithelial-POM interactions required for cochlear elongation are not well understood.

In the mutant otocysts, where cochlear formation is completely absent (Smo cKO), Gdf6 expression was greatly reduced (Muthu et al., 2019). GDF6 belongs to the TGF-β family ligands and is known to regulate mesenchymal differentiation. We observed that Gdf6 is specifically expressed in the posteroventral domain of the otocyst from the time the cochlear primordium emerges from the ventral otocyst. In this study, we tested the hypothesis that GDF6 plays an essential role in cochlear elongation by mediating otic epithelial-POM interaction.

Methods: We used Gdf6 KO mice, whole-mount immunostaining, in-situ hybridization, and 10X Genomics multiome kit (snRNA-seq + snATAC-seq). The resulting DEG (Differentially Expressed Genes) and DAR (Differentially Accessible Regions) were analyzed using Seurat 4.9 in R and MultiVelo and SCENIC+ in Python.

Results: In Gdf6 KO mice, the cochlear duct was shortened to about 10% of the control length. Despite the severe shortening, hair cell organization appeared, with only a few ectopic rows of hair cells. Furthermore, the mediolateral patterning of the cochlear duct showed no significant differences between control and Gdf6 KO otocysts. Using single nucleus multi-omics analysis, we found that the epithelial cells of Gdf6 KO otocysts lack the cell population that later forms the middle and apical regions of the cochlea, accompanied by an increase in saccular non-sensory cells. Our results also showed that a previously unidentified POM population adjacent to the posteroventral region, where Gdf6 is normally expressed, disappeared in Gdf6 KO. We are currently analyzing the multi-omics data obtained from different stages of cochlear development to uncover the upstream and downstream mediators of GDF6 signaling.

Conclusions: Based on our analysis, we propose that epithelial GDF6 signaling acts as a key regulator of cochlear elongation, possibly by controlling POM differentiation to optimize cochlear epithelial branching and elongation.

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SU95. Netrin-G1 Regulates Spiral Ganglion Neuron Axon Terminal Morphology

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Category: Development: Cellular/Systems

Background: Proper encoding of sound information relies on precise connectivity between spiral ganglion neurons (SGNs) and their pre- and post-synaptic targets. In the periphery, SGN peripheral processes extend through the habenula perforata and form synapses with inner hair cells (IHCs) in a subtype-specific manner. While a few axon guidance molecules have emerged as regulators of gross SGN wiring, little is known about the molecules that orient and fine-tune the intricately organized SGN peripheral processes beyond the habenula. In recent years, Ntng1 has emerged as a robust marker of adult Type 1b and 1c SGNs. Netrin-G1 (encoded by Ntng1) is a synaptic cell adhesion molecule that has been shown to regulate synaptic specificity and restrict in-growing axons to specific laminar domains in other systems. It does so through interactions with binding partner NGL-1 (encoded by Lrrc4c). In this study, we investigated the roles of Netrin-G1 and NGL-1 in SGN circuit wiring and function.
Methods: HCR in situ hybridization was performed to detect Ntng1 mRNA levels at ages P1, P6, P12, and P30. Ntng1-/- and Lrrc4c-/- mouse lines were used for phenotypic analysis of SGN morphology and synaptic contacts. Wholemount immunohistochemistry was performed on cochleae using antibodies against Netrin-G1, synapse markers, cytoskeletal markers, and SGN subtype marker Calretinin, and imaged using confocal microscopy. Auditory Brainstem Response (ABR) measurements were recorded to assess auditory function in mutant and control littermates.
Results: We first sought to determine whether Ntng1 is expressed in SGNs at the same time as peripheral process refinement. Indeed, we found that Ntng1 mRNA was present in developing SGNs throughout postnatal development and maintained in adulthood, where expression was restricted to Type 1b and 1c SGNs (Calb2 medium and low). Intriguingly, depletion of Netrin-G1 protein in adult Ntng1-/- animals resulted in severe balloon-like swellings in putative Type 1b and 1c, but not 1a, SGN peripheral processes directly below IHCs. These swellings appeared shortly after the onset of hearing and became progressively more pronounced into adulthood. Despite this severe morphological phenotype, Ntng1-/- animals still formed synapses with IHCs, which were visualized using antibodies against synaptic markers. ABR measurements indicate Ntng1-/- animals can detect sound, but further analysis is needed to determine whether their auditory responses are fully normal. Surprisingly, depletion of NGL-1 in adult Lrrc4c-/- animals results in only mild, infrequent swellings of SGN peripheral processes, suggesting Netrin-G1 may interact with an unknown binding partner to shape terminal morphology.
Conclusions: Our preliminary findings suggest that Netrin-G1 is required for maintenance of SGN Type 1b and 1c terminal morphology after the onset of hearing. In the future, we plan to determine whether this phenotype is experience dependent, and whether other aspects of subtype-specific connectivity or function are altered both in the periphery and in the cochlear nucleus.

SU96. Ccer2: An Upregulated Gene During Mammalian Hair Cells Development

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Category: Development: Cellular/Systems
Background: Atoh1, a helix loop helix transcription factor, is necessary and sufficient for sensory hair cell formation and differentiation. These specialized sensory cells detect sounds and movements in the auditory and vestibular systems, respectively. To discover the genes downstream of Atoh1 and involved in hair cell development, we profiled the transcriptome of Atoh1-induced ectopic hair cells.
Methods: We electroporated embryonic (E) day 13 mouse cochlear explants with an Atoh1-GFP reporter construct or an empty GFP vector as control. At this stage of development, overexpression of Atoh1 results in a 100% conversion of electroporated cells into hair cells. To identify the earliest genes regulated by Atoh1 overexpression, we used fluorescence-activated cell sorting (FACS) and sorted the cells overexpressing GFP 24 hours after electroporation. We extracted RNA from Atoh1-GFP and control-GFP cells and performed bulk RNA-sequencing (RNA-seq).
Results: We found over 800 differentially expressed genes (~700 upregulated and ~100 downregulated), and our bioinformatic analysis detected several known hair cell genes (e.g., Gfi1, Jag2, Dll1) in Atoh1-overexpressing cells. Furthermore, we identified Ccer2 (coiled-coil glutamate-rich protein 2), a novel gene that was significantly upregulated (6-fold change). CCER2 is an uncharacterized protein with no published information about its structure, localization, or function. We confirmed the expression of CCER2 in

endogenous cochlear and vestibular hair cells and assessed that it is one of the earliest markers expressed during hair cell development. We investigated its spatiotemporal expression during mouse cochlear and vestibular development and found that in the cochlea, CCER2 has a developmental base-to-apex gradient and is transiently expressed starting at E13 up to postnatal day 6, following the spatiotemporal expression of Atoh1. The protein is expressed embryonically and throughout adult stages in the balance organs (utricle and saccule). We analyzed the function of Ccer2 in hearing and balance by generating Ccer2 mutant mice (FVB/NJ background) using CRISPR/Cas9 technology. We performed hearing and balance tests using auditory brainstem response (ABR), distortion product otoacoustic emission (DPOAE), and rotarod test. We also carried out BioID (proximity-dependent biotin identification)-based proteomic analysis to investigate CCER2's interactome and used automated image analysis and machine learning to shed light on its subcellular localization.
Conclusions: Our transcriptomic analysis is the first RNA-seq study to profile Atoh1 downstream targets in the early stages of hair cell differentiation. Bioinformatic analysis led to the discovery of Ccer2, a novel and specific marker for inner ear sensory hair cells and differentiation.

SU97. PCP-Autocount: A Novel ImageJ Plug-In for Automated Quantification of Planar Cell Polarity

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Category: Development: Cellular/Systems
Background: During development, planes of cells give rise to complex tissues and organs. Critically, cells must be properly orientated in these planes, a feature known as planar cell polarity (PCP). While PCP is a common attribute in many tissues, the highly organized orientation of hair cells is especially prominent, and is critical for normal auditory and vestibular functioning. To study PCP genes and their function investigators must measure cell orientations, which can be a time-consuming process.
Methods: To facilitate PCP data collection, an ImageJ plug-in was coded to analyze binary images and identify "chunks" of white pixels that contain "caves" of infiltrated black pixels. Cell orientation angles are then measured by generating vectors connecting the centers of mass of each chunk and its cave. Cochleae (P4) and utricles (E17.5) from wildtype mice were immunostained for β-Spectrin and imaged on a confocal microscope. Images were preprocessed using existing ImageJ functionality to enhance contrast, make binary, and reduce noise. For E17.5 utricle images, one investigator manually measured hair cell polarity as the angle resulting from a line bisecting the cuticular plate (chunk) and fonticulus (cave) of each cell. Sample areas from multiple striolar and extrastriolar locations were regionally matched across 4 WT utricles. A separate investigator ran the PCP Autocount (PCPA) plug-in on binary images created from the same sampling boxes and angle 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 0-5 reflecting angle measurement accuracy, where 0 = perfect measurement, 1 = less than 10° incorrect, 2 = 11-40° incorrect, 3 = 41-90° incorrect, 4 = 91-180° incorrect, and 5 = incorrectly identifying a mass of pixels as a cell. PCPA was also tested against a variety of images copied from publications examining PCP in various tissues and across various species.
Results: When sample boxes from E17.5 utricles were manually quantified then compared against PCPA's angle measurements, the average difference between human quantification and PCPA quantification per box ranged from 0.88-5° with a mean difference of 0.71°. When P4 cochlear images were analyzed via PCPA and subsequent angle measurements from the apical, middle, and basal turns of the cochleae were evaluated by an independent investigator, 95.36%, 96.52%, and 97.95% of cells received a score of 0. Qualitative examination of example images of Drosophila ommatidia, mouse ependymal cells, and mouse radial progenitors revealed a high level of accuracy for PCPA across a variety of stains, tissue types, and species.
Conclusions: PCP Autocount is an ImageJ plugin with graphical user interface that accurately measures cell orientations across a number of staining procedures and sample types including β-Spectrin immunolabeled cochlear and vestibular tissues.

SU98. Neurotrophin Signaling Supports the Development of Neurons in the Avian Cochlear Nucleus

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Category: Development: Cellular/Systems

Background: Neurotrophins are growth factor proteins that mediate normal development by using spatial and temporal signaling gradients. While neurotrophins are critical for promoting peripheral auditory development, less is known about how they affect central auditory structures. Within the chicken cochlear nucleus magnocellularis (NM), an analogous structure to the mammalian anteroventral cochlear nucleus, signaling between brain-derived neurotrophic factor (BDNF) and its high-affinity receptor TrkB is spatiotemporally regulated. While we know that BDNF-TrkB signaling is present during early embryonic development, we do not know how it mediates appropriate neuronal maturity, or if it affects NM neurons in a tonotopically or developmentally distinct manner.

Methods: We exogenously applied BDNF on NM neurons ex vivo and studied the neurons' intrinsic properties using whole-cell patch clamp electrophysiology. We collected data at two developmental time points: one before hearing onset (E13) and one after hearing onset during embryonic maturity (E20-21). We also recorded from neurons in both high-frequency and low-frequency regions to test the spatial effects of neurotrophin signaling. Additionally, we used in ovo electroporation to genetically manipulate NM neurons to express YFP (for control groups) or TrkB-eGFP (for experimental groups).

Results: BDNF application significantly reduced outward potassium currents and increased aberrant neuronal excitability for high frequency NM neurons earlier in development, when TrkB expression is endogenously high. High frequency neurons showed significantly stronger effects than low frequency neurons before hearing onset, suggesting a spatial specificity of BDNF-TrkB signaling. Little to no changes were seen to mature NM neurons, which express virtually no TrkB receptor at this stage in development. We also report preliminary data demonstrating the intrinsic neuronal effects of genetically prolonging endogenous BDNF-TrkB signaling throughout development within NM.

Conclusions: Overall, our results demonstrate that endogenous BDNF-TrkB signaling causes neuronal alterations within NM that promotes normal development of a mature auditory system. When TrkB is expressed normally, BDNF strongly affects the intrinsic properties of high-frequency neurons early in development. This lies in parallel with our previous report that suggests that Neurotrophin-3 (NT-3) signaling strongly affects the development of low-frequency NM neurons during the same developmental period. We thus propose a spatial gradient of neurotrophin signaling, where high-frequency neurons are responsive to BDNF while low-frequency neurons are responsive to NT-3 before hearing onset. Elucidating the effects of exogenous neurotrophins on the auditory system is not only an essential step to infer the effects of neurotrophins in vivo, but it has vital consequences for the use of neurotrophins as therapeutics for auditory-related disorders.

SU99. Open Board

SU100. Molecular Mapping of the Cochlea Using the Xenium In Situ Platform

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Category: Genetics A: Genomics and Gene Regulation

Background: The mammalian inner ear consists of over 20 different cell types that are spatially and transcriptionally distinct. The advent of single cell sequencing provides valuable insight into the transcriptional profile of the mammalian cochlea at a single cell resolution. Bioinformatic analysis is capable of separating cell types into dimensionally reduced clusters based on expression data and cell type identify is determined using molecular markers. The Xenium in situ platform by 10X Genomics provides a new, high throughput method capable of detecting up to 300 molecular probes, simultaneously. It is particularly useful for validation of scRNA-seq experimental results. However, to simplify the data analysis, it is best if the probe set includes molecular markers for the various inner ear cell types, to simplify the data analysis. In this study, we use the gene Expression Analysis Resource (gEAR) to carefully curate a list of molecular markers specific

to major cell types within the mammalian cochlea. We then apply this custom panel to mouse cochlear cryosections and use the Xenium analyzer to test the capabilities of this high throughput in situ hybridization system in the auditory field and test our marker gene list.
Methods: A gene list was generated using multiple P1 to Adult scRNASeq datasets acquired from the gEAR platform, and a custom panel was ordered from 10X Genomics. The mean transcript per cell chosen for each marker gene was between 0.1 and 100 transcripts. Inner ears from C57BL/6 J mice were harvested and fixed at 2 days, 7 days, and 1 month of age. The tissue was frozen, cryo-sectioned, and applied to Xenium slides. The probes were hybridized and amplified by following a standardized workflow. The Xenium in situ analyzer was used for interpretation and imaging of samples.
Results: Major cell types within the cochlea were accurately identified at all three timepoints based on our chosen molecular markers. We further show the resolution, sensitivity, and specificity of this approach.
Conclusions: Our custom panel accurately identifies the cell types from the organ of Corti to the lateral wall, and it is applicable to postnatal and adult mice. The small number of marker probes used leaves ample room within the custom panel to investigate differentially expressed genes for future transcriptional studies.

SU101. A Jack of All Trades: POU3F4 Orchestrates Diverse Transcriptional Programs in the Developing Cochlea

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Category: Genetics A: Genomics and Gene Regulation
Background: The cochlea consists of diverse cellular populations working in harmony to convert mechanical stimuli into electrical signals for the perception of sound. One such cell type are otic mesenchyme cells (OMCs), which are a specialized type of neural crest and cranial paraxial mesoderm that express multiple unique transcription factors (e.g., Pou3f4), all of which are known deafness genes, highlighting the importance of OMCs in auditory function. During inner ear development, OMCs surround and influence the development of many key cochlear structures, including the organ of Corti, spiral ganglion neurons (SGNs), and stria vascularis. We have recently shown that OMCs are not homogenous but are transcriptionally, spatially, and functionally distinct well before they terminal differentiate into the fibrocytes of the lateral wall and spiral limbus, modiolar osteoblasts, and specialized tympanic border cells of the basilar membrane. Interestingly, mutations of the OMC-specific transcription factor Pou3f4 are associated with DFNX2, the most common form of X-linked deafness. Loss of Pou3f4 results in numerous cochlear defects, seemingly affecting each of the OMC subpopulations differentially. Here, we hypothesized that the transcriptional changes downstream of POU3F4 in each of the four OMC subpopulations are unique, resulting in the multifaceted cochlear phenotype observed in Pou3f4-KO mice.
Methods: OMC enriched single-cell RNA-sequencing (scRNA-seq) and single-cell ATAC-sequencing (scATAC-seq) datasets were generated from Pou3f4 wildtype and mutant whole cochleae at embryonic day (E) 15 and postnatal day (P) 7. The transcriptional divergences between Pou3f4 wildtype and mutant datasets were deciphered with Seurat and CellChat. The epigenetic changes caused by loss of Pou3f4 were evaluated using ArchR and Signac. Finally, these results were validated through in-situ hybridization and/or immunohistochemistry.
Results: We show that loss of Pou3f4 leads to an OMC subtype-specific response, with each OMC subtype displaying a unique chromatin landscape and sharing only a few differentially expressed genes. Finally, using cell-cell communication analyses, we describe potential mis-regulated signaling cascades in Pou3f4-KO mice responsible for the defects observed in the surrounding cell types such as the SGNs and stria vascularis.
Conclusions: In conclusion, the otic mesenchyme specific transcription factor POU3f4 supports four distinct transcriptional cascades in the regionally and molecularly distinct mesenchymal domains during cochlear development.

SU102. gEAR v2.0 - A Redesigned Interface for Better User Experience, Plus New Features

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Category: Genetics B: General

Background: Development on the gEAR Portal began almost 10 years ago, starting with just 5 datasets and anatomical cartoons for each we wanted to colorize based on their expression patterns. Since then, the gEAR has grown to house over 1,100 datasets with thousands of registered users. Throughout the years we have made minor updates to the interface and continuously added new tools and features, but the overall interface has remained similar.

With a goal of modernizing the interface and improving the user experience, students at the University of Maryland College Park took on the gEAR Portal as the focus of their capstone course within the Master of Science program in Human-Computer Interaction (HCIM). Here we report on the complete UI redesign that is the product of this work, and the new functional features we have added in the last year.

Methods: The HCIM group did extensive evaluations of many pages of the gEAR Portal, proposed new user-focused designs for them all, and followed each up with rounds of user testing with members of the hearing research community. The product of these were new designs (using Figma) for each page, which are being implemented by gEAR engineers for release at ARO.

Results: Each redesigned page of the new gEAR Portal is being completely re-implemented following the designs of the HCIM and user feedback. On the technical side, we are dropping our old frameworks (Bootstrap, JQuery, etc) and writing the new pages with Bulma and pure Javascript (ES6), with a focus on making the gEAR usable on all browsers/platforms, even down to portable devices.

Other recent feature additions include improved plotting configuration, automated portal health checks, improved permalinking ability, better gene cart integration (now can save computed marker genes as a gene cart, or use carts in the compare tool) and many bug fixes.

Conclusions: We hope the gEAR Portal continues to be a useful resource for the hearing community.

SU103. Bulk RNA-Sequencing Reveals the Spatial-Temporal Transcriptional Dynamics of Hearing Loss in a Mouse Model of Congenital CMV Infection

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Category: Genetics A: Genomics and Gene Regulation

Background: Congenital cytomegalovirus (CMV) infection is the single leading cause of non-genetic congenital hearing loss. We and others have shown that spiral ganglion neuron (SGN) count is moderately decreased in CMV-infected mice at postnatal day 32 (P32) and inversely correlates with the severity of hearing loss. However, a comprehensive understanding of the underlying mechanism of CMV-related hearing loss remains elusive. Here, we implemented bulk RNA-sequencing (RNA-seq) to investigate transcriptional signatures of cochlear damage in a mouse model of congenital CMV infection.

Methods: C57BL/6 mice were infected with murine CMV (MCMV) via intra-peritoneal injection at P0, and cochleae were collected at P7, P14, and P24 for RNA-seq. Modiolus and lateral wall tissues were dissected and separately processed at P7 and P14. Auditory brainstem response (ABR) thresholds were obtained from CMV-infected P24 mice before cochlear harvest. RNA-seq data were analyzed in R using the tools DESeq2, GOSeq, and WGCNA.

Results: Many immunity-related genes were up-regulated in CMV-infected cochlear tissues at P7. Functional enrichment analysis also revealed a tissue-specific down-regulation of gene ontology (GO) terms related to neuronal projection, synaptic regulation, and neurotransmitter receptor activity with CMV infection in the P7 modiolus. Conversely, Tubb3, Syp, Nefm, and Foxa3 expression were unchanged, suggesting stable SGN counts at P7. Cell cycle-related genes were also down-regulated in the P7 modiolus in a tissue-specific manner. As the cochlear sensory epithelium and SGNs undergo terminal mitosis between embryonic days 13 (E13) and 15 in the mouse, the results suggest a negative impact of congenital CMV infection on the cellular division

of a hitherto unimplicated modiolus cell type. At P14, the CMV+ modiolus samples appeared to comprise two transcriptionally distinct clusters characterized by the relative expression of many immunity-related genes, with the more immune-active CMV+ cluster additionally distinguished by widespread down-regulation of neuron-related GO terms. We hypothesize that the high-immune/low-neuron P14 modiolus samples represent a nascent phenotype of CMV-related hearing loss. Elevated ABR thresholds were significantly associated with immunity-related gene over-expression in CMV+ mice at P24.
Conclusions: Results support a pattern of immune-mediated SGN loss in CMV-related hearing loss. However, we also identified dysregulated transcriptional signatures not attributable to neurosensory or immunologic cells. Immunohistochemistry and/or single-cell transcriptomics will provide further insight.

SU104. Chromatin Remodeling Protein CHD7 Regulates Anti-Sense Transcription of Neurogenic Factor Sox11 During Neuronal Differentiation

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[1]*Rutgers University,* [2]*Sampled*

Category: Genetics A: Genomics and Gene Regulation
Background: Chromodomain-helicase-DNA-binding protein 7 (CHD7) is a nucleosome remodeler expressed in spiral ganglion neurons and other cochlear cell types. Pathogenic variants of CHD7 are associated with CHARGE syndrome, a congenital disorder associated with hearing loss. CHD7 is recruited to enhancers and promoters and functions to control cell-type-specific gene expression. We showed that CHD7 binds the 3' untranslated regions (UTRs), where CHD7 function is unknown. The 3' UTRs occasionally contain promoters oriented in the opposite direction of a coding gene and are used as a start site to initiate anti-sense transcription. Here, we use immortalized otic progenitors (iMOPs) as a cellular system to clarify CHD7's function in regulating anti-sense transcription at the Sox11 gene. Sox11 is a crucial gene for inner ear neurogenesis. In addition, a Chd7 conditional knockout mouse line was used to confirm the anti-sense transcription of Sox11.
Methods: Stable iMOP cell lines containing scrambled shRNA, Chd7 shRNA knockdown (KD), dCAS9, dCAS9-KRAB, and dCAS9-KRAB-MeCP2 were generated by lentiviral transduction and selection. The cells were used for morphological and molecular analysis. Cells were processed for either chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) to determine CHD7 binding sites or used for immunostaining. In vivo, Chd7 was inducibly deleted using a conditional knockout line (Chd7cKO; Ngn1CreERT2; Ai9). Tissue samples were processed for single-molecule fluorescence in situ hybridization (smFISH) and immunocytochemistry.
Results: Control iMOP cells displayed neuronal morphology upon neuronal differentiation and were reminiscent of spiral ganglion neurons. However, iMOP-derived neurons from Chd7 KD iMOPs showed shorter neurites than control cells. The results suggest impaired neuronal differentiation and neurite outgrowth in cells with reduced Chd7 levels. Chromatin immunoprecipitation (ChIP) sequencing was used to identify CHD7 binding sites in iMOP cells. Like previous studies, CHD7 binding was primarily observed at promoters and enhancers. Interestingly, a significant portion of the total binding sites was found within 3'UTR regions. We utilized the CRISPR-Cas9 inhibition (CRISPRi) system to prevent binding to the 3' regions of the Sox11 gene by either steric hindrance (dCAS9), transcriptional repression (dCAS-KRAB) or heterochromatin formation (dCAS-KRAB-MeCP2). Blocking the 3' region of Sox11 resulted in iMOP-derived neurons with short neurites, similar to Chd7 knockdown. CRISPRi at the 5' region of Sox11 served as a positive control. Finally, deletion of Chd7 in vivo appeared to reduce sense and anti-sense transcripts for Sox11 based on smFISH labeling in developing SGNs.
Conclusions: Taken together, CHD7 binding to the 3' UTR region of genes suggests a vital function during neuronal differentiation. In vivo, deletion of CHD7 suggests involvement in Sox11 sense and anti-sense transcription. Overall, the function of CHD7 at the 3'UTRs may offer a different layer of epigenetic regulation for gene expression.

SU105. Determining the Critical Window of Expression of Rfx1/3 in Hair Cell Development and Maintenance

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Category: Genetics B: General

Background: Our laboratory has previously shown that Rfx1 and Rfx3 are highly expressed in early postnatal hair cells and that loss of Rfx1 or Rfx3 alone does not affect hearing function. In contrast, conditional deletion of both Rfx1 and Rfx3 (Rfx1/3;Gfi1-Cre) from hair cells in mice at embryonic (E) day 16.5 exhibited significantly elevated hearing thresholds and loss of outer hair cells shortly after the onset of hearing. This previous work established that Rfx1/3 are essential for hearing function and outer hair cell maintenance during the late embryonic period. We are further studying the critical window of expression of Rfx1/3 in hair cell development and maintenance in this project.

Methods: Fluorescent in situ hybridization (RNAscopeTM) on C57BL/6 mice during embryonic and early postnatal timepoints was performed to better understand the expression pattern of Rfx1/3 in early hair cell development. To further investigate the critical window of expression of Rfx1 and Rfx3 necessary for hair cell maintenance and auditory function, we created two Rfx1/3 conditional knockout (cKO) mouse models, Rfx1/3;Myo15-Cre, to conditionally knockout Rfx1/3 at postnatal (P) day 0, and Rfx1/3;Prestin-CreERT2, to conditionally knockout Rfx1/3 beginning at P8 by tamoxifen injections at P8-P10. 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) at P16, P21, P30 and P45 (Rfx1/3;Myo15-Cre) and P30 (Rfx1/3;Prestin-CreERT2) to measure auditory function, followed by histological analyses. Additionally, histological analyses were conducted earlier than P16 in the Rfx1/3;Myo15-Cre mice to investigate the onset of hair cell loss.

Results: Rfx1 and Rfx3 were expressed in hair cells at both embryonic and early postnatal timepoints. Rfx1/3;Myo15-Cre homozygous mutant mice have significantly elevated ABR and DPOAE thresholds as early as P16 compared to wildtype littermates, with DPOAE thresholds undetectable beginning at P30. Outer hair cell loss is present at P12 in Rfx1/3;Myo15-Cre homozygous mutant mice with a basal-to-apical gradient pattern. Rfx1/3;Prestin-CreERT2 homozygous mutant mice did not show statically significant difference in ABR and DPOAEs when compared to wild type mice.

Conclusions: In conclusion, our study established that both embryonic and early postnatal expression of Rfx1/3 are necessary for outer hair cell function and maintenance. Future directions will investigate the role of Rfx1/3 in hair bundle formation during earlier embryonic hair cell development and investigate the regulatory network downstream of RFX1/3 in cochlear hair cell development.

SU106. Exploring the Role of FOXJ1 in the Auditory System

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Category: Genetics B: General

Background: Forkhead Box J1(FOXJ1) is a transcription factor with an evolutionarily conserved role in ciliogenesis in various tissue types. Previous RNA-sequencing experiments have shown that Foxj1 is highly and specifically expressed in the developing cochlear and vestibular hair cells. However, the role of FOXJ1 has not been explored in regard to inner ear function or hair cell and hair bundle development and maintenance. Due to its high expression in developing hair cells, known role in ciliogenesis in other systems, and known interactions with other transcription factors, RFX, that have an established role in the inner ear, we hypothesize that FOXJ1 is necessary for hair cell function and hair bundle development in the inner ear. Here, we investigate the role of FOXJ1 in the auditory system.

Methods: We validated the expression of Foxj1 in the inner ear using RNAscopeTM fluorescent in-situ hybridization in C57BL/6J wildtype mice. To study the role of Foxj1 within the auditory and vestibular system, we used a conditional knockout mouse line Foxj1flox/flox; Gfi1-Cre, hereafter referred to as Foxj1 conditional knock out (cKO), to knock out Foxj1 specifically in hair cells during development, beginning at embryonic day (E) 16.5. To measure auditory function, auditory brainstem response (ABR) testing at 8, 16, 24, and 32 kHz frequencies was performed on 1-month-old Foxj1 cKO and control littermates.

Immunohistochemistry was performed on 1-month-old cochleae to examine the morphology of inner and outer hair cells, including the stereocilia bundles.
Results: Foxj1 is expressed in vestibular hair cells in the utricle and saccule, vestibular ganglion neurons, and spiral ganglion neurons in 1-month-old C57Bl/6J mice, with no expression in cochlear hair cells at this timepoint. There was no significant difference in ABR thresholds between Foxj1 cKO mice and their wildtype littermates at 1-month-old. Correspondingly, 1-month-old cochlear tissue did not show morphological changes in the Foxj1 cKO in comparison to their wild-type littermate controls.
Conclusions: Deletion of Foxj1 from hair cells at E16.5 did not result in changes in auditory function at 1-month-old mice. Normal cochlear hair cells and stereocilia morphology indicate that expression of Foxj1 beyond the E16.5 timepoint is not necessary for cochlear hair cell development and maintenance. As there is a high expression of Foxj1 in vestibular hair cells, even in mature post-natal hair cells, follow up studies will include Vestibular Sensory Evoked Potential testing of Foxj1 cKO and control littermates to examine vestibular function as well as histological analysis of vestibular hair cell morphology.

SU107. Reduced Expression Level of KCNE1 is Sufficient to Maintain Hearing

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Category: Genetics B: General
Background: Kcne1, a regulatory subunit of a potassium (K+) channel, and Kcnj10, an inwardly rectifying K+ channel, are expressed in marginal and intermediate cells respectively of the stria vascularis, where they regulate the movement on K+ ions into the endolymph. Mutations in KCNE1 are associated with Jervell and Lange-Neilson Syndrome (JLNS) Type 2 which causes profound bilateral deafness that begins in early childhood, and prolongation of the QT interval. Mutations in KCNJ10 are associated with epilepsy, ataxia, sensorineural deafness and renal tubulopathy (EAST) syndrome. It is currently unclear whether it is possible to restore the hearing loss associated with JLNS or EAST syndrome using gene therapy or other treatments. Here, we aim to characterise the auditory phenotype in two mutant mice carrying novel alleles in these genes (Kcne1tm1a and Kcnj10tm1a).
Methods: All mice are on a C57BL/6NTac background which has been repaired for the Cdh23ahl variant. Kcne1tm1a and Kcnj10tm1a mutant alleles carry a promoter-driven cassette designed to interrupt normal gene transcription. A longitudinal set of auditory brainstem response recordings were used to characterise hearing impairment in Kcne1tm1a/tm1a mice and Kcnj10tm1/tm1a mice compared to their respective wildtype and heterozygote littermate controls (N = 6 per genotype). Endocochlear potential recordings were also performed in Kcne1tm1a/tm1a and Kcnj10tm1/tm1a mice compared to wildtype littermate controls at 8 weeks of age to investigate the function of the stria vascularis in vivo (N = 4 per genotype). Subsequently, whole inner ear tissue was collected at 4 weeks of age to quantify any changes to the level of expression of Kcne1 and Kcnj10 using digital droplet PCR (N = 6 per genotype).
Results: Surprisingly, auditory brainstem response recordings have shown that both Kcne1tm1a/tm1a and Kcnj10tm1a/tm1a mice do not show hearing loss between 4 weeks and 6 months of age. To further investigate strial function, we have also shown that the endocochlear potential in Kcne1tm1a/tm1a and Kcnj10tm1a/tm1a mice is comparable to their wildtype littermate controls at 8 weeks of age. Subsequently, preliminary data from digital droplet PCR has shown that Kcne1 mRNA in the inner ear of 4 week old Kcne1tm1a/tm1a mice is reduced to around a quarter of that in wildtype littermate controls. Data collection for the level of Kcnj10 expression in the inner ear is ongoing.
Conclusions: Together, data suggests that a reduced level of expression of Kcne1 may be sufficient to preserve normal hearing, and a partial restoration of KCNE1 expression in humans with JLNS may be enough to rescue auditory function. Work is ongoing to determine whether a partial expression of Kcnj10 is also sufficient to preserve normal hearing.

Thanks is given to Wellcome for funding this research.

SU108. FDXR Gene as a Cause of Adult-Onset Auditory Neuropathy Spectrum Disorder via a Mechanism of Mitochondrial Dysfunction

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Category: Genetics B: General

Background: FDXR gene encodes the mitochondrial ferredoxin reductase and it was previously reported to be associated with hearing loss and visual impairments, each representing characteristic auditory neuropathy (AN) and/or optic atrophy. Since the first report in 2017, only one more paper was added in the literature. However, auditory rehabilitation outcomes of this disease entity has not been investigated and the pathophysiologic mechanism is still not well elucidated. Here we report a hearing-impaired subject segregating with FDXR gene variant in a pedigree, who underwent cochlear implant with favorable outcome and we suggest possible pathophysiologic mechanism of adult-onset AN via mitochondrial dysfunction.

Methods: A 35-year-old woman visited clinic presenting with bilateral hearing loss, where left side hearing loss had progressed since her childhood and right side hearing loss developed 2 years ago. Clinically, patient showed AN characteristics and Auditory Neuropathy Spectrum Disorder (ANSD) was confirmed with absent ABR wave and present OAE. Exome sequencing was performed to identify the genetic cause of hearing loss and In vitro study was performed to provide the molecular evidence of pathophysiology.

Results: An exome sequencing identified novel homozygous FDXR variant (c.1096G greater than T), segregating with hearing loss in a family. Open-set sentence recognition test result improved from 38% preoperatively to 92% at postoperative 3months, which is expected to further improve afterward. To provide the molecular evidence for pathogenicity of the variant, mitochondrial functions between patient-derived lymphoblastoid cell lines (LCLs) and normal LCLs were investigated. Patient-derived LCLs showed decreased ATP level, increased ROS level, and decreased MMP level compared to normal LCLs. Interestingly, the administration of mitochondria isolated from umbilical cord mesenchymal stem cells (UC-MSC) rescued the mitochondrial dysfunction in the patient-derived LCLs, which was comparable to normal LCLs.

Conclusions: Taken together, FDXR-related adult-onset auditory neuropathy was identified first in Korean family and CI outcome was reported first in the literature, which was favorable. Mitochondrial experiment in patient-derived LCLs showed potential link between mitochondrial dysfunction and hearing loss. Furthermore, mitochondrial transplantation would be a potential therapy for hearing loss in this disease entity.

SU109. Identifying Genetic Underpinnings Using Whole Exome Sequencing and Phenotype-Genotype Correlations in Patients With BOR Syndrome

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Category: Genetics B: General

Background: Branchio-oto-renal syndrome (BOR syndrome) refers to a autosomal dominant syndrome of branchial anomalies, ear abnormalities, and kidney anomalies. We aim to adjust current monitoring strategy through enhancing genetic diagnostic rate using whole exome sequencing (WES) and analyzing phenotype-genotype correlation.

Methods: We recruited patients diagnosed with BOR syndrome who sought care at a tertiary hospital between January 2010 and December 2022. We collected comprehensive demographic information, clinical presentations, genetic diagnoses, and long-term trends in renal and auditory function. In cases where targeted region next-generation sequencing (NGS) failed to provide a molecular diagnosis, we used WES to identify

the disease-causing variant. We then investigated the correlation between genotype and disease presentation/prognosis.
Results: A total of 19 families with 21 subjects affected by BOR syndrome were included in this study. Targeted region NGS analysis identified disease-causing variants in the EYA1 gene (10, 48%) and the SIX5 (2, 10%), while molecular diagnosis was not attained in the rest of 9 patients (42%). Of the 9 undiagnosed cases of BOR syndrome, 5 underwent WES, which revealed disease-causing copy number loss identified in the EYA1 gene in 2 patients, and notably, PKD1 gene in another 2 patients.
As for auditory function, the majority exhibited sensorineural hearing loss (48%), with mild to moderate hearing impairment in 80% of cases. Only two patients (10%) met the criteria for cochlear implantation, resulting in significant improvements in sound perception and speech recognition were noted after surgery. Certain mutation on EYA1, including c.1615G greater than T (p.Glu539Ter), c.1540_1542del (p.Leu514del), c.1081C greater than T (p.Arg361Ter) and copy number loss, are correlated with moderate to severe hearing loss.

As for renal function, 90% of patients maintained renal function within the normal range. The two patients with abnormal renal functions presented with end stage renal disease and IgM nephropathy, respectively. Structural abnormalities were more common, including interstitial nephritis (19%), renal cysts (5%), abnormal kidney size (5%), kidney stones (5%), and hydronephrosis (5%). Therefore, renal ultrasound serves instead of blood tests as a screening tool for patients with BOR syndrome. On the contrary, The mutations found in patients with abnormal renal function included SIX5 c.1872dup (p.Ala625ArgfsTer15). Notably, copy number loss on PKD1 gene, associated a high probability of renal failure, were found in 2 patients of BOR syndrome. Regular renal function tests should performed based on high probability of impaired renal function in patients with these variants.

Conclusions: The presentation of BOR syndrome could be highly variable. A complete genetic testing, incorporating WES to identify both point mutations and copy number loss, helps with adjusting monitoring and therapeutic strategies.

SU110. High Prevalence of Syndromic Hearing Loss in Mexican Children With Severe to Profound Hearing Loss Undergoing Cochlear Implantation

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Category: Genetics B: General
Background: Recent advancements in next generation sequencing (NGS) allow for a greater understanding of genetic sensorineural hearing loss (SNHL) in pediatric patients. Representation in genetic testing research within different ethnicities has been investigated, in which Latino American children display lower diagnostic rates and significantly higher nondiagnostic or inconclusive results; despite similar prevalence rates of SNHL to their Asian and White counterparts. This study will further the need to expand genetic understanding of hearing loss genes within populations that are underrepresented and reduce ethnic disparities in diagnostic rates within the pediatric hearing loss field.
Methods: Mexican pediatric patients with severe to profound SNHL and that were candidates for cochlear implantation were recruited. Whole exome sequencing (WES) or targeted genomic enrichment and massively parallel sequencing (TGE + MPS) were performed, which utilizes a custom platform for analyzing non-syndromic and syndromic SNHL genes. Variant pathogenicity was established in according to criteria established by the American College of Medical Genetics, and conclusive variants were clinically confirmed via Sanger Sequencing.
Results: WES or TGE + MPS was completed for 42 probands. A genetic cause was identified for 42.9% of probands (n = 18). Of the 18 diagnosis made, 6 (33.3%) were nonsyndromic and 12 (66.7%) were syndromic or likely syndromic. Eight probands (66.7% of all syndromic diagnoses) were diagnosed with a syndromic form of hearing loss that mimics a nonsyndromic clinical presentation at a young age.
Conclusions: WES and TGE + MPS are effective diagnostic tools for pediatric SNHL, as well as identifying syndromic forms of SNHL that may not be phenotypically present at the point-of-care. A greater

understanding of genetics by gathering larger cohorts of underrepresented populations is necessary to improve the efficacy of genetic testing and timely medical intervention within these ethnically diverse populations.

SU111. Novel BCAP31 Variant Associated With Non-Syndromic Deafness Shows Mitochondrial Dysfunction and Higher Sensitivity to Cisplatin: Evidence for Amenability to Mitochondrial Replacement

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Category: Genetics B: General

Background: B-cell receptor-associated protein 31 (BAP31 or BCAP31) is an integral ER membrane protein, which involves the transport and quality control of transmembrane proteins. BAP31 is also important for the cross-talk of apoptotic signals between the ER and mitochondria. Due to its critical role in cellular physiology, the BAP31 dysfunction has been associated with numerous human diseases including deafness, dystonia, and central hypomyelination (DDCH) syndrome, cancer, metabolic syndrome, cystic fibrosis, and neurodegenerative diseases. Recently, we have found a novel in-frame insertion variant in the BCAP31 gene from a mid-sized family segregating only non-syndromic hearing loss in an X-linked, dominant fashion. Genetic evidence strongly suggests that this variant is the likely cause of hearing loss in this family. However, it is not known how this variant contributes to the hearing loss, which is an important issue to understand the molecular pathogenesis.

Methods: To address it, we compared the mitochondrial function between the patient-derived lymphoblastoid cell lines (LCLs) and normal LCLs.

Results: The patient derived LCLs showed the elevation in ROS, and the decrease in ATP and membrane potential intracellularly compared to normal LCLs. Furthermore, patient-derived LCLs demonstrated more pronounced cisplatin-induced cell death than did normal LCLs by confirming the increase in the expression of pro-apoptotic genes. Surprisingly, the administration of mitochondria (PN-101) isolated from umbilical cord mesenchymal stem cells (UC-MSC) was able to rescue the mitochondrial dysfunction in the BCAP31 mutant patient-derived LCLs just as was in the LCLs carrying Mitochondrial DNA 3243 A greater than G variant. The PN-101 application significantly alleviated cisplatin-induced cytotoxicity as well in the patient-derived LCLs.

Conclusions: Taken together, this study clearly showed that the pathogenesis of hearing loss due to BCAP31 variant may involve mitochondrial dysfunction and increased susceptibility to ototoxicity and also that the variants of this gene could cause non-syndromic hearing loss depending on the severity of the aberration in the above mechanisms. Meanwhile, we provide a possibility that allogenic normal mitochondrial administration could be a potential option for treating hearing loss that has a component due to mitochondrial dysfunction irrespective of deafness genes.

SU112. Differential Expression of miR-210-3p and miR-23a-3p in Sudden Sensorineural Hearing Loss Patients' Serum

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Category: Genetics A: Genomics and Gene Regulation

Background: The aetiology of idiopathic Sudden sensorineural hearing loss (SSNHL) is unknown. Recently researchers have identified differential expression of contrasting groups of microRNAs (miRNAs) in the serum or plasma of SSNHL patients. It is unclear if this reflects methodological, regional, or population specific variation in SSNHL associated miRNAs. Hence, we assessed the serum expression levels of miRNAs previously identified to be differentially expressed in SSNHL patients' plasma.

Methods: We collected serum from 17 consenting SSNHL adult patients (9 males and 8 females) and 19 controls (7 males and 12 females). After total RNA extraction and reverse transcription we undertook triplicate Quantitative real-time PCR analyses of miR-15a-5p, -18b-5p, -23a-3p, -143-3p, -183-5p, and -210-3p

previously identified to be differentially expressed in SSNHL patients' plasma . Cycle threshold (Ct) values were normalized to an internal control miR-191-5p. The inter-group mean normalized Ct values were compared using an Independent Sample t-test in SPSS. Inter-group mean age and distribution by sex was compared by Independent Sample t-test and Fishers exact tests respectively.
Results: The mean expression levels of miR-210-3p (5.03 ± SD 2.18) and (7.23 ± 2.30); and miR-23a-3p (-1.50 ± 2.64) and (1.65 ± 0.52) in SSNHL patients and controls respectively were significantly different (p = 0.006; and less than 0.001). There was no statistically significant difference in the mean expression levels of miR -15a-5p (-.46 ± 2.88 vs. -.06 ± 0.63), -18b-5p (3.67 ± 2.57 vs. 4.28 ± 0.56), -143-3p (2.07 ± 4.30 vs. 2.39 ± 0.80), -183-5p (4.64 ± 4.03 vs. 5.20 ± 1.23) in patient versus control groups, respectively. The mean age of the patient group (51.94 ± SD 14.3) years, and the controls (40.68 ± 11.3) years was not statistically different, and the groups did not vary significantly by sex.
Conclusions: This study found dysregulation of miR-210-3p and -23a-3p but not that of miR -15a-5p, -18b-5p, -143-3p, -183-5p in a sample of North American recruited SSNHL patients which is different to the reported dysregulation of all 6 miRs in the plasma of SSNHL patients recruited in Korea. This suggests that across patient populations there are some common miRNA findings that justifies further study of the role of miR-210-3p and -23a-3p in the development of SSNHL.

SU113. Precision Medicine and Gene Therapy for Hearing Impairment: Big Data, 3D Structure, and AI

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Category: Genetics B: General
Background: Precision medicine and gene therapy focus on treating genetic diseases based on genotype, and by targeting disease-causing variants with genetic modifications. The field of deafness is optimal for implementing precision medicine, as over 200 genes associated with hearing loss are known worldwide, with correlations between phenotype, genotype and specific populations. Gene replacement or variant gene editing could reverse the phenotype, which requires knowledge of the variant responsible for hearing loss. With the availability of electronic medical records (EMR), many patients are available for large-scale studies that include next-generation sequencing (NGS). While whole exome sequencing (WES) has rapidly advanced gene discovery, about half of inherited deafness still remains unsolved, partly since the pathogenicity of many genetic variants remains unknown. This poses a challenge for disease diagnosis and for the identification of future gene therapy candidates. Potential genetic variants are tested for pathogenicity using bioinformatic tools and cellular and animal models that allow evaluation of variants one-by-one. Our precision medicine project, based on a large-scale study, yielded thousands of variants discovered by in-depth analysis of WES of patients. We aim to leverage recent advancements in the field of protein structure determination to propose a novel Artificial Intelligence (AI)-based methodology for classifying disease-causing variants, based on features derived from the 3D structure of AlphaFold models.
Methods: Utilizing the Tipa Biobank of 60,635 samples from the Israeli Jewish population, including 3,807 hearing impaired, NGS was performed on 1038 deaf from the Biobank. We designed a unique pipeline for variant detection in a hearing-impaired population with very little background information. Following variant detection by bioinformatics mega-analysis, pathogenic variants are being evaluated. For novel variants, an AI-based methodology is being developed for the prediction of variants' pathogenicity, which employs machine learning algorithms trained on structural and evolutionary features, which are expected to capture the energetic and physico-chemical impact of disease mutations, thereby learning to predict unknown variants' functional impact at the protein level. Novel variants are being functionally investigated by CRISPR/Cas9 gene editing and gene therapy.
Results: Our bioinformatics meta-analysis pipeline for NGS 1038 samples yielded over 10% solved cases and an additional 20% of cases with inconclusive variants. Many variants detected are known and novel variants in genes previously associated with hearing loss. In addition, in over 10% of cases, homozygous variants were detected in novel genes.

Conclusions: Solving the etiology of hearing loss in the diverse Israeli Jewish population, using novel methods based on protein modeling and AI, and finding genotype-phenotype correlations, are the key for precision medicine for hearing loss, including diagnosis, prevention and treatment. This work can be applied world-wide as a model study.
Research Support: The Israel Precision Medicine Partnership Program (IPMP) and the National Institutes of Health/NIDCD R01DC011835.

SU114. Enriched Proliferation of Hair Cells in Otic Organoids Using the Notch Pathway Inhibitor and Wnt Agonist

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Category: Development: Cellular/Systems
Background: The otic organoid research is to restore and prevent hearing, and hair cells play a key role in hearing function. In the developing inner ear, activation of the Notch pathway inhibits the differentiation of precursor cells into hair cells. This inhibition of Notch signaling leads to enhanced hair cell proliferation. This study was an exploration that some signal regulators, the Notch pathway inhibitor (γ-secretase inhibitor, DAPT) and Wnt agonist (CHIR-99021), could promote the differentiation of hair cells from both mouse embryonic stem cells (ESCs) and human induced pluripotent stem cells (iPSCs).
Methods: The differentiation protocols we used in this study were based on the established ones suggested by Koehler et al (mouse ESCs) and Nie et al (human iPSCs). R1/E (ATCC) cells as mouse ESCs and mND2-0 (WiCell) cells as human iPSCs were used. It has been known to take about 30 days in mouse ESCs and 90 days in human iPSCs to the formation of otic organoids. In otic organoids derived from mouse ESCs, DAPT and Wnt agonist were co-treated at two periods, D12-18 (early) and D28-34 (late). In otic organoids generated from human iPSCs, they were treated at D15-18 (early) and D84-90 (late). The efficiency of the organoid was assessed by morphologic evaluation (Confocal and EM), immunohistochemistry (IHC), and qRT-PCR.
Results: According to the protocol, hair cell-like cells were successfully induced from both mouse ESCs and human iPSCs, showing stereocilia, hair cell markers and attached nerve endings. In otic organoids from mouse ESCs, the late period treatment (D28-34) was more effective in the proliferation of hair cells. In IHC, morphologically, early period treatment (D15-18) showed organoids with a consistently well-structured form. However, in qRT-PCR, the expression of the hair cell markers, Myo7a and Math1, was not different from those organoids without treatment. In otic organoids from human iPSCs, on the other hand, early treatment (D15-18) may be better; the level of hair cell markers was significantly higher when DAPT and CHIR-99021 were treated between D15-18, also with structurally stable form.
Conclusions: Otic organoids containing functional hair cells with electrophysiological properties could be successfully produced. Further treatment of DAPT+CHIR showed the possibility of otic organoid production with higher efficiency, which will enable the production of stable otic organoids that can be used for the purpose of organoid techniques. Particularly, hair cells that play a key role in the inner ear function might be obtained much faster via the treatment of DAPT and CHIR-99021 in otic organoids from human iPSCs.

SU115. Identifying and Characterizing Notch Target Genes Associated With Trans-Differentiation in the Mammalian Organ of Corti

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Category: Regeneration
Background: Major efforts in recent years are devoted to identifying therapeutic approaches for hearing disorders associated with damage to sensory hair cells (HCs). In recent years, a significant effort has been

made to develop treatments to induce transdifferentiating of HC and restore hearing. It is currently unclear what are the mechanisms underlying the loss of regenerative potential and what factors promote trans-differentiation of supporting cells (SCs) into HCs. Here we aim to understand the mechanisms of normal trans-differentiation during early stages as well as identify direct Notch targets associated with this trans-differentiation.
Methods: We use a cochlear explant assay to identify genes and enhancers in cochlear cells that start as SCs and trans-differentiate into HCs following broad inhibition of Notch or of specific ligands (Dll1, Jag2, Jag1). We use explants from LFNG-eGFP or LFNG-eGFP-Atoh1-mCherry mice to identify cells that started as SCs and trans-differentiated into HCS. Explants after treatments are being analyzed using both RNA-seq and CUT and RUN on the transcription factor Rbpj. To check how different treatments affect trans-differentiation following a single HC death, we are using laser ablation experiments, followed by live imaging during different stages of development. Finally, to test whether identified target genes can promote trans-differentiation, we are employing AAV-CRISPR-ON to induce expression of specific targets following damage from ototoxic sisomicin treatment in explant assays.
Results: Our preliminary results show the different dynamics of HC trans-differentiation following inhibition of Notch or its ligands. Both Dll1 and Jag2 showed stronger effect of HC trans-differentiation and disorganization than Jag1 inhibition at neonatal stages. Our RNA-seq data from SCs validate the upregulation of HC markers and different effects on Notch activity upon inhibition of Notch or its ligands. Live imaging after inducing local HC death at embryonic stages showed regeneration ability, where this ability is totally lost at neonatal stages. Finally, we have successfully calibrated the AAV-CRISPR-ON system by targeting Atoh1 expression.
Conclusions: These results highlight the complexity by which Notch and its ligands regulate trans-differentiation of SCs into HCs. Our RNA-seq data identified potential target genes that may have a role in trans-differentiation. Combining these results with CRISPRa and live imaging will provide detailed knowledge about the mechanism and the potential targets that can contribute to regenerative treatments for hearing.

SU116. Long-Term Results after Cell Transplantation to the Inner Ear Using the Inner Ear Catheter in Cochlear Implant Recipients

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Category: Regeneration
Background: Cell transplantation to the inner ear has been performed in experimental settings mainly for cell replacement therapy. We have investigated the anti-inflammatory and neuroprotective potential of autologous mononuclear cells in preclinical experiments. As a consequence, we have applied autologous mononuclear cells to patients undergoing cochlear implantation to prevent inflammation and protect residual cells.
Methods: For the application of the cells, the inner ear catheter (MED-EL, Innsbruck, Austria) has been inserted into the cochlea via the round window. The cell suspension was instilled slowly into the cochlea. Thereafter, cochlear implantation was performed in a standard manner. Routine follow-up investigations were performed and the impedances as well as hearing performance with the implant were analysed up to 5 years after cell transplantation. The results were compared to a control group from a previous study.
Results: Impedances were slightly increased in patients receiving autologous cell transplantation. Hearing performance with the implant was within the expected range and comparable to the control group. No adverse effects were observed after autologous cell transplantation to the inner ear.
Conclusions: Based on the results, autologous cell transplantation to the inner ear using a catheter during cochlear implantation seems a feasible and safe procedure. Specifically after a long term follow-up, there were no pathological increases in impedance values.

SU117. From Facultative Stem Cells to Hair Cells: Understanding Avian Hair Cell Regeneration

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Category: Regeneration

Background: The sensory hair cells of the mammalian cochlea are required for sound perception but are not regenerated following cellular damage. Hair cell loss results in deafness, which affects millions of people worldwide. In contrast, hair cells in the basilar papilla, the avian hearing organ, can regenerate following damage through direct transdifferentiation or proliferation of supporting cells, followed by new hair cell differentiation.

Methods: We employed single-cell RNA sequencing to investigate transcriptomic changes in the chicken basilar papilla at different time points following hair cell ablation with the aminoglycoside antibiotic sisomicin. Specific hair cell and supporting cell populations were identified using previously validated markers.

Results: Our transcriptomic analysis focused on supporting cells in the medial region of the basilar papilla, which preferentially undergo the proliferative mode of hair cell regeneration. We aimed to identify candidate genes involved in regulating cell cycle re-entry. Our strategy emphasized genes that are downregulated in medial region supporting cells when dying hair cells are still present but apoptotic death is imminent. Further curation of the identified genes revealed potential signaling pathways, such as Notch, which showed downregulation as early as 12 hours post-sisomicin treatment.

Conclusions: We hypothesize that these genes may play a role in permissive pathways that prevent supporting cells from re-entering the cell cycle in the undamaged basilar papilla. Our future work aims to validate the role of these candidate genes through pharmacological modulation and lentiviral manipulation. We envision that our results could provide insight into the mechanisms underlying hair cell regeneration in the chicken basilar papilla and identify therapeutic avenues for hair cell regeneration in humans.

SU118. Urolithin A Counteracts Mitophagy Decline and Attenuates Auditory Cell Senescence

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Category: Aging

Background: Aging of sensory organs is associated with a decline in mitochondrial function and the accumulation of dysfunctional mitochondria. Impaired mitophagy blocks the turnover of dysfunctional mitochondria and leads to their accumulation. Urolithin A (UA) induces mitophagy in various mammalian cells. This study was aimed at investigating the effect of the mitophagy activator, UA, on premature senescent auditory cells.

Methods: Low-dose H2O2 was used to induce premature senescence in auditory cells and cochlear explants. Senescence-associated p53, p21, and β-galactosidase activity were investigated in H2O2-treated cells and cochlear explants upon UA pre-treatment. Mitophagy-associated molecules, mitophagosomes, and mitophagolysosomes were examined in H2O2-treated cells upon UA pre-treatment. The effects of UA on ATP content, mitochondrial DNA (mtDNA) integrity, and mitochondrial membrane potential were investigated in H2O2-induced senescent HEI-OC1 cells.

Results: The levels of cellular senescence-associated p53 and p21 significantly increased in H2O2-induced senescent HEI-OC1 cells and cochlear explants. However, the levels of mitophagy-related molecules significantly decreased. UA significantly decreased the expression of senescence-associated p53 and p21, and increased the expression of mitophagy-related proteins, in H2O2-induced senescent cells and cochlear explants. The percentage of β-galactosidase-stained senescent cells also reduced in H2O2-treated cells and cochlear explants upon UA pre-treatment. The formation of mitophagosomes and mitophagolysosomes was restored upon UA pre-treatment of H2O2-induced senescent cells. The knockdown of mitophagy-related genes (Parkin and Bnip3) resulted in annulment of UA-induced anti-senescent activity. UA significantly increased the ATP content, mitochondrial DNA (mtDNA) integrity, and mitochondrial membrane potential in senescent HEI-OC1 cells.

Conclusions: This study proved that UA induces mitophagy and prevents premature senescence in auditory cells. The activation of mitophagy using UA can be a potential preventive strategy for patients with age-related hearing loss.

SU119. Age-Related Cellular and Molecular Changes Associated With Vestibular Sensory Epithelium

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Category: Aging

Background: In adults older than 70 years, there is a 40% decrease in hair cell (HC) density in the cristae of the canals, compared to 24% in the saccule, and 21% in the utricle. In addition to HC loss, morphological and functional changes have also been demonstrated in the remaining HCs. The damaging impact of such age-related vestibular sensory decline manifests itself in an exponential increase in geriatric dizziness and a subsequent higher prevalence of injurious falls leading to Presbyastasis. While many studies have focused on age-related hearing loss and cochlear HC degeneration, age-related vestibular HC degeneration and their contribution to age-related vestibular disorders remain elusive. Hence, in the current study, we focused on our study on molecular and cellular changes associated with vestibular HC and supporting cell (SC) degeneration during physiological aging. Our goal is to identify the underlying mechanisms that contribute to vestibular HC and SC aging and uncover the driving factors of age-related changes in vestibular sensory epithelium.

Methods: CBA/J mice aged between 2.5 and 23 months after birth were used for our experiments. We measured vestibular evoked potential (VsEP) at the system level to determine changes in vestibular function. At the cellular level, we examined changes in HC and SC morphology and ultrastructure using immunostaining (combined with confocal microscopy) and scanning electron microscopy. At the molecular level, we used single-cell RNA-sequencing (scRNA-seq) to examine changes in transcriptomes of HCs and SCs during aging. For comparison, we also examined changes in transcriptomes of cochlear HCs and SCs. For scRNA-seq, vestibular sensory end organs of saccule, utricle, and crista as well as sensory epithelia from the organ of corti were isolated. Each age group contains an equal number of males and females and a total of 100 mice were used to obtain 5 biological replicates for scRNA-seq. Droplet-based scRNA-seq was performed using the 10x Genomics chromium platform and raw data was processed by Cell Ranger to obtain count matrices. Downstream quality control, analysis, and visualization were performed in R using the Seurat package (4.3.0.1). mRNA expression was validated by RNAscope.

Results: Our results demonstrate numerous differentially expressed genes between young and old vestibular hair cells and supporting cells indicating age-related transcriptomic changes in genes related to metabolism, ubiquitination, proteostasis, stereocilia, and cytoskeletal functions. Our gene enrichment analysis further confirmed various up- and down-regulated biological processes in response to aging. Inlining with our age-related transcriptomic changes, we observed morphological and functional changes in the vestibular sensory epithelium.

Conclusions: Overall, our findings highlight age-related alterations of the vestibule at both cellular and molecular levels. This sheds light on potential underlying mechanisms and driving factors of vestibular aging which may be conducive to developing targeted treatment strategies and delay the onset of Presbyastasis.

SU120. Auditory Nerve Function and Cortical White-Matter Structure Predict Age-Related Deficits in Speech Communication

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Category: Aging

Background: Myelin supports action potential propagation velocity, which in turn determines arrival time, vital for integration of signals. As we age, myelin degrades and is partially regenerated, resulting in a buildup of defects in myelin sheathes, altering signal timing. These myelin defects can dramatically impact the auditory system, which relies on precise signal timing. Auditory nerve (AN) and the central auditory system,

including primary auditory cortex (ACtx) and arcuate fasciculus (AF; a large white matter tract connecting temporal and frontal cortex,), exhibit age-related myelin defects. Disruption of signal timing is expected to yield difficulties in speech comprehension in challenging listening environments. We hypothesized that age-related deficits of AN, ACtx, and AF myelin integrity would contribute, either independently or interactively, to perceptual deficits when identifying time-compressed speech (TCS). Additionally, we examined the extent to which age-related loss of AN myelin contributes to a loss of myelin or decreased white matter (WM) integrity in auditory ACtx and AF.
Methods: Experiments included a group of 39 older (55-83) and 28 younger (18-30) adults. AN myelin integrity was estimated functionally using the phase-locking value (PLV) calculated from the compound action potential (CAP) N1 component, previously found to be related to AN structure. Myelin in the central auditory system was assessed from radial diffusivity (RD) calculated from diffusion imaging, the higher values of which are associated with poorer myelin structure. Axial diffusivity and fractional anisotropy were also calculated and served as alternative metrics of WM structure. Speech recognition was assessed with a TCS task. Relationships between CAP PLV, FA, TCS, and age were assessed using linear regression model testing.
Results: Estimates of myelin (CAP PLV) and WM (FA_ACtx, FA_AF) integrity and TCS identification decreased with age. Estimates of myelin from the AN and WM from the cortex were positively associated with TCS. Supporting our hypothesis, CAP PLV and each of the central ROI measures of WM independently predict TCS. Importantly, CAP PLV and FA_ACtx appear to mediate the relationship between age and TCS. However, CAP PLV does not predict central WM structure (FA_ACtx or FA_AF).
Conclusions: Estimates of AN myelin and central WM independently predict TCS performance and can account for age-group differences in TCS. Due to the temporal precision required for auditory processing, signal timing disruptions caused by myelin defects are likely to negatively impact hearing and communication. Understanding how peripheral and central myelin uniquely contribute to age-related auditory processing deficits will help inform targeted interventions for improving speech comprehension in older adults and guide the development of diagnostic tools aimed at early identification of individuals at risk for age-associated auditory impairments.

SU121. Exploring the Mechanisms of Age-Related Hearing Loss Using Transcriptomics

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Category: Aging
Background: Age-related hearing loss (ARHL) results from complex progressive alterations of the auditory system. This pathology has psychological and medical comorbidities, including social isolation, frailty, depression, and cognitive decline. In the US, the prevalence of hearing impairments doubles every decade of life starting at around age 50. Whereas about 15% of middle-aged individuals (50 to 59 years old) are affected, the prevalence reaches 63.1% for those 70 and older. Despite being the most prevalent sensory deficit in older adults, and having enormous societal and economic impact, no therapies to prevent or slow ARHL exist. This is, in part, because the cellular and molecular mechanisms of ARHL remain poorly understood.
Methods: We are using mice to probe the molecular mechanisms of ARHL. As in humans, mice in many strains start showing ARHL in middle-age (around 1 year of age). To characterize the initial molecular changes in early-stage ARHL, we used bulk RNA sequencing to identify inner ear genes whose expression levels change between young adult (8-week-old) and middle age (62-week-old). For sequencing, total RNA was extracted from otic capsules from four animals at each age.
Results: Principal component analysis of the eight datasets revealed a clear segregation between young and middle-aged mice. Differentially expressed genes were identified and used for downstream analysis, including Gene Ontology (GO) analysis, and pathway analysis (IPA and KEGG). We identify age-related alterations in many biological processes, including synapse organization (e.g., neurotransmission, and synaptic vesicle transport), synaptogenesis, cellular localization (e.g., synaptic membrane and neuron to neuron synapses), molecular function (e.g., ion channels and regulation of membrane potential), inflammation, and calcium signaling.

Conclusions: Furthermore, analysis of VGF expression, a gene downstream of neurotrophin signaling, validated our prior RT-qPCR studies showing that neurotrophin-3 (Ntf3) expression and function is downregulated during aging. This, together with our demonstration that increasing Ntf3 expression in middle-aged mice rapidly increases ABR peak 1 amplitudes and prevents further age-related inner hair cell synaptopathy, suggest that the analysis of differential gene expression between young and middle-aged animals could identify new targets for therapies to prevent, reduce, or reverse ARHL.
Funding: R01DC000188-42.

SU122. Changes in Cortical Directional Connectivity During Difficult Listening in Younger and Older Adults

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Category: Aging
Background: Speech comprehension in noisy environments is crucial for daily communication, yet becomes more challenging with age, even for healthy aging. Understanding the changes in concurrent cortical connectivity under difficult listening situations and with aging can offer further insights into the neural mechanisms underlying age-related hearing impairments. Granger causality is a typically used measure of connectivity in functional magnetic resonance imaging (fMRI) studies. However, limited temporal resolution of fMRI restricts the ability to capture higher frequency neural interactions crucial for complex speech processing. While magnetoencephalography (MEG) can capture neural interactions at the millisecond scale, its limited spatial resolution poses challenges in conventional connectivity analyses. A recently proposed cortical connectivity analysis methodology, network localized Granger causality (NLGC), can extract Granger causal interactions in MEG data without the need for any intermediate source-localization step.
Methods: In this study, NLGC framework is applied to MEG recordings obtained from younger and older adults while performing a speech listening task under varying background noise levels (quiet, 0 dB and +6 dB). The analysis focuses on directional cortical connectivity patterns within and between the frontal, temporal, and parietal lobes, specifically in the delta and theta frequency bands. Furthermore, the nature of the directional links was classified in terms of facilitative, suppressive, or sharpening, based on their temporal relationships from source to target, enabling a more nuanced understanding of the functional roles of these connections, providing insights into excitatory and inhibitory connections at macroscopic scales. This one-shot approach also effectively addresses challenges related to false alarms and localization errors, providing a robust assessment of cortical connectivity.
Results: The results demonstrate significant age- and condition-related connectivity differences, particularly in the theta band. In younger adults, increasing background noise level leads to a shift from predominantly temporal-to-frontal (bottom-up) connections for speech in quiet to dominantly frontal-to-temporal (top-down) connections in speech in noise conditions. In contrast, older adults exhibit bidirectional information flow between frontal and temporal cortices regardless of the background noise level. Furthermore, in theta band, while younger listeners show a consistent pattern in the nature of their cortical links for different listening conditions, older listeners display a transition from predominantly facilitative links to predominantly sharpening links as the background noise level increases, suggesting an excitation/inhibition imbalance with aging.
Conclusions: Taken together, application of NLGC to study age-related changes in the brain provides a network-level cortical functional connectivity account of auditory processing as a function of age, listening difficulty, frequency band, and spatial distribution of the connections.

SU123. Novelty Detection in an Auditory Oddball Task on TgF344-AD Rats

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Category: Aging

Background: Age-related hearing loss is a widespread disability, affecting communication and social participation in the older population. Recent research highlights hearing loss as a significant risk factor for dementia, such as Alzheimer's disease (AD; Griffiths et al., 2020, https://doi.org/10.1016/j.neuron.2020.08.003).

Deviance detection, a fundamental element of the predictive coding theory in sensory perception, enables the brain to distinguish between common and unexpected sounds. This process relies on predicting environmental cues and highlighting stimuli that deviate from these predictions. As individuals age, peripheral processing accuracy diminishes, leading to a greater dependence on predictions over sensory input. The reliability of auditory predictions may be significantly impaired in dementia (Wolpe et al., 2016, https://doi.org/10.1038/ncomms13034). AD patients show affected temporo-parietal areas, which may lead to a worse adaptation to auditory stimuli. Consequently, aging alters the predictive process both cognitively and behaviorally.

In previous studies, we observed that rats can detect frequency deviant tones in a context-dependent manner, indicating a memory-based adaptation in auditory neurons that is shaped by stimuli saliency and behavioral relevance.

Methods: We trained 20 freely moving rats (16-months-old, TgF344-AD) from both sexes, to discriminate deviant tones in an oddball paradigm. TgF344-AD rats manifest many age-dependent AD related pathologies including amyloid plaques, neurofibrillary tangles with hyperphosphorylated tau, and neuronal loss (Cohen et al., 2013, https://doi.org/10.1523/JNEUROSCI.3672-12.2013). We divided the animals into two groups, based on the pair of frequencies presented as standard and oddball in the different tasks (4.8-6.7 and 8.0-11.3 kHz). This allowed us to study the relation between AD hallmarks and behavior, potentially associated to auditory deficits in our Alzheimer's disease model.

Various iterations of the oddball paradigm were presented to evaluate the animals' discrimination ability. We varied the interstimulus interval (1.5, 2 and 4 seconds) and tested the influence of frequency contrast relative to the standard tone (0.5, 0.75, 1.0 and 1.25 octaves between tones). We also presented a modified oddball paradigm where the frequency contrast between standard and deviant tone randomly varied between 9 possibilities. Adaptation to the standard tone was assessed under different standard/deviant probability ratios (90/10 and 70/30 %). Animals' responses were evaluated using the d’ index.

Results: During training, sex-based differences in temperament and task learning speed were apparent. When we applied the behavioral protocols, we observed differences between the young, healthy animals and the aged, AD ones. These disparities were mainly influenced by Alzheimer's disease-related lesion development and distribution, leading to considerable individual variability.

Conclusions: Age and dementia affect the detection of deviant stimuli differently, depending on disease progression and sex.

SU124. Neural Representation and Auditory Perception of Temporal Fine Structure Are Impaired by Age but not by Cochlear Synaptopathy

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Category: Aging

Background: Age-related loss of cochlear ribbon synapses results in silencing of the associated auditory-nerve (AN) fibers, and may lead to compromised temporal fine structure (TFS) perception (Bramhall et al., 2019, Hear Res 377: 88-103). In gerbils, a synapse loss comparable to that observed in old individuals can be pharmacologically induced, with ouabain, in younger, normal-hearing individuals (Bourien et al., 2014, J Neurophysiol 112: 1024-1039). Here, we tested the representation of TFS by single AN fibers, in normal-hearing and ouabain-treated young-adult, and old (≥ 36 months) gerbils. We used the TFS1 test (Moore and

Sek, 2009, Int J Audiology 48:161[1]171), comparing neuronal responses and behavioral discrimination sensitivity to harmonic and inharmonic tone complexes that differed in TFS but had similar envelopes.
Methods: Ouabain treatment followed the protocol of Bourien et al. (2014). Behavioral testing, using a Go/NoGo paradigm (details in Steenken et al., 2022, Eur J Neurosci 56: 4060-4085), started two weeks after treatment. Single-unit recordings were conducted in anaesthetized gerbils. For behavior and neural recordings, TFS1 stimuli comprised of three harmonic tone complexes with different combinations of center frequency (1600 Hz or 3200 Hz) and fundamental frequency (200 Hz or 400 Hz). Inharmonic tone complexes were derived by shifting all frequency components of the harmonic stimulus upwards by the same amount. Each stimulus was presented for 400 ms at 68 dB SPL. All-order inter-spike interval histograms from the responses of AN fibers were transformed into vector strength (VS) spectra. Neural representation of TFS and envelope was quantified by comparing peak VS at harmonic and inharmonic frequency component locations. Cochleae were labelled with antibodies against MyosinVIIa, CtBP2, and GluA2 to visualize inner hair cells, presynaptic ribbon synapses, and postsynaptic structures, respectively. Synapses were counted at cochlear locations corresponding to 2 kHz.
Results: Synapse loss was significant, and showed a similar extent, in old and ouabain-treated gerbils. GLMM ANOVAs revealed that old gerbils had compromised perception of TFS1 stimuli, compared to young-adult and ouabain-treated gerbils. In single AN fibers, TFS representation was similar in all gerbil groups. However, envelope representation was enhanced in old gerbils' AN fibers, likely due to enhanced VS when fibers are stimulated close to their threshold (Joris and Yin, 1992, J Acoust Soc Am 91:215-232)).
Conclusions: These results show conclusively that while old gerbils had difficulties with TFS perception, cochlear synaptopathy was not a main cause of that. Furthermore, TFS representation by the remaining AN fibers of old gerbils, was not degraded, confirming earlier findings (Heeringa et al., 2020, J Neurosci 40, 343–354). However, the enhanced envelope representation at typically lower sensation levels in old age might confound the perception of complex stimuli.

SU125. Age-Related Changes in the Physiological and Molecular Markers of Central Gain Assessed in the Mongolian Gerbil

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Category: Aging
Background: Aging is associated with a decreased ability to process complex sounds in challenging listening conditions, even in individuals with normal hearing thresholds. One contributing factor could be progressive age-related cochlear deafferentation. Recent studies suggest that cochlear deafferentation is often accompanied by compensatory plasticity, or a relative increase of activity in central auditory structures. Although increased central 'gain' may benefit listening in quiet, it is likely maladaptive for listening in noise, due to a non-selective increase in representations of both target and maskers. Changes in gain are thought to occur due to an imbalance between excitatory and inhibitory neurotransmission in the central auditory pathway. However, the relationship between physiological measures of central gain and cellular level excitatory and inhibitory (E/I) imbalances remains unclear. Here, we use Mongolian gerbils (Meriones unguiculatus) to examine age-related changes in central gain and their relationship to E/I imbalances in the auditory cortex, assessed using fluorescent mRNA in situ hybridization (FISH) of GABAergic and glutamatergic receptor subunits.
Methods: Scalp-recorded phase-locked neural responses to the stimulus envelope of amplitude modulated tones (envelope following responses, EFRs) were used to assess physiological markers of central gain in an age-graded series of Mongolian gerbils. EFR stimuli varied in amplitude modulation rates to emphasize peripheral vs. central generators. Central gain was calculated as a ratio of EFRs from cortical and peripheral generators. FISH was used to characterize mRNA expression dynamics of inhibitory GABA receptor subunits (Gabra1, which encodes the α1 subunit of GABAAR) and excitatory AMPA receptor subunits (Gria2, which encodes GluA2) from the auditory cortex of the same gerbils. The ratio of the average number of Gria2 mRNA fluorescent puncta and Gabra1 mRNA puncta per cell was denoted as the E/I ratio.
Results: Hearing thresholds measured by ABRs showed minimal changes with age. EFRs to fast AM frequencies (greater than 500Hz) showed age-related decreases while EFRs to slower AM frequencies

(~40Hz) were unchanged or enhanced. Physiological markers of central gain showed a relative increase of ~8dB despite no changes in hearing thresholds. Feasibility studies establish that FISH can be performed in gerbil auditory cortices, and molecular markers of E/I balance assessed with FISH are altered with age, driven by a relative decrease in mRNA associated with GABAergic neurotransmission. Preliminary results suggest that these E/I ratios are correlated with physiological markers of central gain.
Conclusions: These results suggest that aging is associated with reduced peripheral neural coding and increased relative neural activity from the auditory cortex. This central gain was accompanied by changes in the E/I balance assessed in the auditory cortex. These results link molecular changes in the auditory cortex to scalp-recorded electrophysiology and may facilitate the development of precision diagnostic tests and therapeutic interventions for specific targeting of E/I imbalance.

SU126. Comodulation Masking Release in Neurons of the Inferior Colliculus of Young and Old Mongolian Gerbils

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Category: Aging
Background: Elderly human subjects often experience perceptual difficulties processing signals in background noise with a complex acoustic spectro-temporal structure that is due to multiple sources being active at the same time. The spectro-temporal structure of the masking noise, however, can aid the detection of target signals. A well-established paradigm in human psychoacoustics testing this is the comodulation masking release (CMR) paradigm. It compares tone-detection thresholds in maskers with uncorrelated envelopes in different frequency bands with tone-detection thresholds in maskers with correlated envelopes in different frequency bands. Here, we report neural correlates of CMR in units of the inferior colliculus (IC) of 13 young (5-8 months) and 9 old (greater than 33 months) Mongolian gerbils.
Methods: We recorded from single- and multi-units in the IC of Fentanyl-anaesthetised gerbils using tungsten electrodes that were advanced through a craniotomy dorsally to the IC. We used the "flanking-band paradigm" (Schooneveldt and Moore 1987, doi: 10.1121/1.395639) to test units: A 400-ms or 800ms tone was presented in a continuous noise masker. Tone frequency was either 700 Hz or 4000 Hz. The noise masker with an overall level of 57 dB SPL was composed of two 25-Hz wide bands, one centered on the tone frequency (On-Frequency Band, OFB) and one on a frequency differing by -400, -100, 0, 100, or 400 Hz from the tone frequency (Flanking Band, FB). The two masker bands had either uncorrelated envelopes (UN) or identical (correlated CO) envelopes. Units' rate responses and temporal responses were used to determine tone-detection thresholds.
Results: Regarding the rate thresholds, the units showed little differences between tone-detection thresholds in maskers with uncorrelated and comodulated envelopes indicating little neuronal CMR based on response rate. Also, the frequency difference between OFB and FB had little effect on the rate threshold. Finally, rate thresholds did not show age differences.
In contrast, the response thresholds based on the temporal representation of the envelope showed a significant effect of the frequency difference between OFB and FB. There was a trend that temporal thresholds were higher in uncorrelated maskers than in correlated maskers. Similar to the rate thresholds, there were no significant age differences.

Conclusions: Since Mongolian gerbils show a similar CMR as human subjects in the flanking band paradigm (Tolnai et al. 2022, Abstracts ARO Midwinter Meeting), they constitute a suitable animal model for investigating the mechanisms underlying CMR. The rate thresholds in the IC do not mimic the pattern of the behavioral thresholds. The neurons' temporal thresholds depend on the frequency difference between OFB and FB similar to the pattern observed in behavior. Neither neuronal threshold measure depended on age that corresponds to the small age effect observed in the behavioral study.
Funded by the DFG, EXC 2177

SU127. Effects of Age on Within-Channel and Across-Channel Temporal Processing and Relationship to Speech Perception in Noise

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Category: Aging

Background: Individuals with normal audiometric sensitivity have variable speech perception in noise (SPIN) capabilities, which can worsen with age. Temporal processing is pivotal to speech perception, especially in adverse listening conditions. Auditory decline due to aging manifests both as peripheral pathology and central auditory system changes, leading to altered temporal processing. To disentangle the relative contributions of these changes to speech perception in noise, we measure within-(frequency)-channel and cross-channel temporal processing in normal-hearing individuals across a wide age range. Robust perception of within-channel temporal cues necessitates precise coding at both peripheral and central levels of the auditory pathways, whereas cross-channel processing relies on central mechanisms. We hypothesize that central auditory changes contribute more to difficulties in SPIN.

Methods: Our data collection encompassing comprehensive behavioral and electrophysiological measures of temporal processing is ongoing in human subjects spanning a wide age range (18-71 years) with near-normal hearing sensitivity (pulsed-tone average of thresholds for the frequency range 500 - 6000 Hz less than 25 dBHL). Within-channel assays consist of behavioral gap detection thresholds (GDT) and electroencephalographic (EEG) responses elicited by gaps embedded in 4000 Hz tones with octave-band noise. Across-channel assays probe temporal-coherence processing through (1) a behavioral measure of comodulation masking release (CMR) for detecting 4 kHz tones embedded in modulated narrowband-noise masker and flanked by two well-separated bands of noise that were modulated coherently or incoherently with the on-frequency masker; (2) an EEG-based measure using a novel twenty-tone stimulus where the co-modulation statistics are parametrically varied while keeping within-channel statistics constant. Finally, SPIN performance was measured using two tasks selected to differentially emphasize peripheral masking (word identification in a multi-talker babble), stream segregation, and cognitive load (matrix sentence test with staggered presentation of words from different streams).

Results: Our findings indicate a significant increase (worse) in behavioral thresholds on within- and across-channel tasks with age. Cortical responses elicited by gaps and temporal-coherence changes were less robust with increasing age. SPIN performance also declines with age. Age effects on cross-channel temporal-coherence processing appear to be larger than within-channel alterations, notably in the EEG measures. Our metrics of cross-channel behavioral and EEG temporal-coherence processing are stronger predictors of speech-in-noise performance, especially when tasks emphasize streaming and selective attention. Whereas the behavioral within-channel metric is a significant predictor of SPIN outcome for tasks designed to emphasize peripheral masking.

Conclusions: Our results underscore the importance of central auditory changes in aging as a key contributor to age-related perceptual deficits.

SU128. Using Interaural Time Difference Thresholds to Create Individualized "Mixed-Rate" Processing Strategies for Sound Localization Among Bilateral Cochlear Implant Listeners

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Category: Auditory Prostheses

Background: Bilateral cochlear implants (BiCIs) do not restore sound localization abilities to the full extent exhibited by typical hearing listeners. This is partly because commercial processors are not synchronized across the ears and cannot provide coordinated stimulation. Another issue is that most clinical strategies adopt high-rate stimulation across all electrodes. While high-rate stimulation is important for speech intelligibility, low-rate stimulation is needed for ITD sensitivity. Prior work has shown that mixed-rate strategies, with low- and high-rates of stimulation presented to different electrode pairs, could improve ITD sensitivity while maintaining speech understanding. ITD sensitivity can vary along the electrode array such that the locations with "best" (lowest) thresholds could be different across individuals. Recent work has demonstrated that

directing low-rate stimulation to an electrode pair with the best ITD sensitivity resulted in similar sound lateralization performance as when five electrode pairs received low-rate stimulation. Furthermore, sensitivity to ITD predicted the benefit from mixed-rate strategy for lateralization. The present study extends the investigation of individualized mixed-rate strategies to localization in the free field. We hypothesized that, similar to lateralization, ITD sensitivity is a prerequisite for improved localization when using the mixed-rate strategy. By using a research processor to deliver synchronized ITDs at low rates, we test the prediction that listeners with lower ITD thresholds at the "best" electrode pair will have lower localization error when using the individualized mixed-rate strategy as compared to clinical-like strategies, since the latter do not deliver synchronized ITDs at low rates.

Methods: BiCI listeners (Cochlear Ltd. devices) were recruited for testing. Experimental processing strategies running in real-time were implemented and tested using the CCi-MOBILE, a bilaterally-synchronized mobile research processor. Listeners were tested with four strategies: 1) their everyday clinical strategy on unsynchronized processors, 2) an "all-high" ten-channel strategy with every channel stimulated at 1000 pulses per second (pps) on CCi-MOBILE, 3) an "interleaved" ten-channel strategy with every other channel stimulated at 125 pps while the remaining channels were stimulated at 1000 pps, and 4) a "best" strategy where low-rate (i.e., 125 pps) stimulation was only provided to the cochlear place with the lowest ITD threshold. ITD thresholds were measured with direct stimulation and sound localization was tested in a sound booth. Localization performance was quantified with the root mean square error of the response angles.

Results: Preliminary results (n=7) show that individuals with lower overall ITD discrimination thresholds had lower localization error. Interestingly, the "interleaved" mixed-rate strategy resulted in greater localization error than other strategies, suggesting a trade-off between providing ITD cues with low-rate stimulation vs. maintaining a maximal number of high rate channels.

Conclusions: Individualizing mixed-rate strategies by providing low-rate stimulation to only the "best" pair of electrodes may allow improved sound localization without sacrificing high-rate stimulation.

SU129. Combined Optogenetic and Electrical Stimulation for Spatially and Temporally Precise Stimulation of Cochlear Neurons

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Category: Auditory Prostheses

Background: The cochlear implant uses electrical stimulation to efficiently activate auditory neurons. However, the spread of electrical current from each stimulating electrode in the linear array lowers the spatial resolution of activation and can cause interactions incase electrodes are simultaneously stimulated. Higher spatial resolution of auditory neuron activation has been reported for optogenetic stimulation, but even the fastest optogenetic ion channels struggle to meet the high stimulation rates used in cochlear implants. We have reported that combining electrical and optogenetic stimulation improved response fidelity at high stimulation rates. Since near-threshold light is used to raise excitability of the neuronal population and lower the electrical threshold of activation, we hypothesized that it would also maintain a low spread of activation and low channel interactions. Hence, the spread of activation and channel interactions during combined stimulation were compared against electrical-only, optogenetic-only, and acoustic stimulation.

Methods: The mouse hybrid cochlear implant consisted of 5 platinum rings (0.21-0.29 mm ø) and 5 micro-LEDs (453 nm; 0.27×0.22 mm). The arrays were implanted into the cochlea of acutely deafened transgenic mice (modified with the ChR2-H134R opsin). The micro-LEDs and electrodes were controlled by an in-house custom-built LED driver and a benchtop electrical stimulator, respectively. In this study, the combined stimulus consisted of 1-ms-long light pulse plus a 25 μs/phase electrical pulse delivered 1.5-ms after the onset of the optical stimulus. Neural activity was recorded from the inferior colliculus of the auditory midbrain via a multichannel recording array. The spread of activation was assessed from response images of spike rate across the recording array and compared using the activation width at D-prime of 1 and 2 above the threshold. Interactions between channels were compared by measuring the threshold shift on one channel during simultaneous co-stimulation of another channel at different set intensities relative to threshold.

Results: Our studies confirmed that combined (optogenetic and electrical) stimulation resulted in a significantly narrower spread of activation compared to electrical-only stimulation in the cochlea (p less than

0.01, Two-way ANOVA, n=6). The average activation width at D-prime of 1 and 2 above threshold during electrical-only stimulation was 0.47 ± 0.049 mm and 0.72 ± 0.065 mm, respectively, while activation width during combined stimulation was 0.25 ± 0.039 mm and 0.53 ± 0.070 mm, respectively, the latter being similar to the activation width during acoustic or optogenetic-only stimulation. In one animal recorded to date, the interaction between simultaneously stimulated adjacent channels during hybrid stimulation was approximately 4-times lower compared to electrical-only stimulation.
Conclusions: The narrow spread of activation, reduced channel interactions, and high fidelity achieved via combined electrical and optical stimulation could increase the number of independent channels and allow for simultaneous stimulation of multiple channels, which are vital for improving the speech understanding in recipients of cochlear implants.

SU130. What is the Basis of the Binaural Benefit for Music Sound Quality in Single-Sided Deaf Cochlear Implant Users?

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Category: Auditory Prostheses
Background: Cochlear implants (CIs) allow for good speech perception under ideal listening conditions, but do not provide pitch and timbre information needed for good music perception. Despite poor sound quality with CIs, single-sided deaf (SSD) CI patients [normal hearing (NH) in one ear and CI in the other], often prefer listening to music with binaurally combined acoustic and electric hearing. The source of this binaural benefit is unclear. Is the binaural benefit due to gross binaural restoration or synchronous temporal envelope information across ears? Does the detail of the spectral and/or temporal content in the CI ear matter? We investigated sources of binaural benefit for music quality in SSD CI users by manipulating the input to the CI ear. We expected that the highest binaural ratings would be obtained when unprocessed music was presented in the CI ear, as this was the everyday listening condition.
Methods: The Multiple Stimulus with Hidden Reference and Anchor (MUSHRA) protocol was used to rate sound quality in SSD CI and NH listeners. Music excerpts from multiple genres were used as stimuli. Participants were tested with each ear alone or with both ears. For SSD CI users, sound was delivered to the NH ear via earphone and to the CI ear via direct audio input (DAI); for NH listeners, sound was delivered to each ear via earphone. Multi-channel sine-wave vocoders were used to explicitly control spectral and temporal content delivered to the CI ear; carrier frequencies were matched to the center frequencies of the analysis bands used in each SSD CI user's clinical map. Signal processing conditions included: broadband noise (binaural restoration), noise modulated by the temporal envelope of the music excerpt (temporal envelope benefit without spectral cues), multi-channel vocoding with 20-Hz (spectral envelope benefit) or 1000-Hz envelope filter (spectral envelope benefit with temporal cues), or unprocessed music (the same spectro-temporal information input to NH and CI ears).
Results: Initial SSD CI data suggest that binaural restoration was not the basis of the binaural benefit. For SSD CI users, binaural performance was better than NH-only performance only when unprocessed music or multi-channel vocoders were presented to the CI ear. Interestingly, highest ratings were observed for the multi-channel vocoder with the 20-Hz envelope filter, suggesting that high-frequency temporal cues in the CI ear may be less desirable for binaural music listening. This was most evident for classical music. For NH controls, binaural ratings were higher than monaural ratings only when the unprocessed signal was delivered to both ears.
Conclusions: Preliminary data suggest that spectral information in the CI ear contributes to binaural benefit in SSD CI users, with limited benefit from high-frequency temporal cues. Simplifying signals provided to the CI ear may improve SSD CI users' binaural music sound quality.

SU131. Progress on an Implantable Microphone for Cochlear Implants

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Category: Auditory Prostheses
Background: We present the fabrication and testing of a piezoelectric microphone for use with cochlear implants. The bimorph microphone is made from titanium and thin film polyvinylidene fluoride (PVDF), is shaped like a triangular cantilever, and has a differential output. It is inserted through the facial recess and contacts the manubrium's umbo. We target the umbo because its uni-directional motion produces a well-represented sound signal. As the umbo moves, it displaces our piezoelectric sensor, resulting in a charge accumulation that we amplify with a custom differential charge amplifier.
Methods: We use photolithography and thin film deposition to fabricate our sensors in a nano-microfabrication facility at the Massachusetts Institute of Technology. We have tested eight sensors at Mass Eye and Ear via bench testing and human cadaveric experimentation. Additionally, we have tested our sensors in sheep temporal bones at Columbia University.
Results: The eight sensors behave comparably during bench testing with a piezostack pusher, showing a flat frequency response from 100 Hz to over 20 kHz. When tested in five distinct human temporal bones, they again behaved similarly, with a flat frequency response up to 2 kHz and a robust response to at least 10 kHz. Our microphones show high sensitivity, good linearity, and an equivalent input noise comparable to commercial hearing aid microphones.
Conclusions: We have devised a working implanted microphone, which could be a component of a totally implantable cochlear implant. By using titanium as our conductive material, we are moving towards biocompatibility. We plan to continue investigating biocompatibility while also establishing robustness for years of wear, evaluating the microphone's sound signal by cochlear implant users, and testing the sensors via in vivo trials in sheep.

SU132. An Open Platform for Cochlear Implant Research

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Category: Auditory Prostheses
Background: Advancements in cochlear implant (CI) technology are driven by the efforts of clinicians, industry, and academia, but the impact is limited due to industry concerns of liability and intellectual property concerns. To date, advances in software and encoding strategies have improved CI performance. However, the lack of hardware solutions addressing fundamental challenges like high manufacturing cost and poor spatial resolution have limited widespread adoption and metrics like word recognition. A community-developed implantable platform would allow the implementation of CI innovations reducing cost and improving performance.
Methods: We are developing an open platform for hearing research and CI device development (OpenCI) that includes hardware, software, and documentation. The openly-developed hardware consists of an application specific integrated circuit (ASIC), an intracochlear electrode array, and hermetic biocompatible packaging. The software includes an open data communication protocol that serves as a standard for CIs to maintain compatibility across sound processors while also enabling new features like higher channel count expandability. The documentation comprises of design, manufacturing, and testing data. The platform will integrate with various open-source initiatives such as the CCI Mobile audio processing system and the OpenEar model library. The open-source platform will be broadly accessible to meet the needs of diverse interdisciplinary research groups like basic scientists, hearing researchers, medical/audiology clinicians, and hardware/software developers.
Results: The hardware in the OpenCI platform has been designed with several improvements over commercial state of the art specifications. Among other features, the ASIC can support fully configurable multipolar stimulation, the OpenCI communication standard, and high-channel count scalability. The intracochlear electrode array has 32 multisegmented ring electrodes (configurable to 128 channels), enabling novel methods to focus stimulation. An array prototype was microfabricated in the Biomedical Foundry at Lawrence Livermore National Laboratory under a Quality Management System using a translatable and mass manufacturable process to reduce costly manual assembly in existing devices. We expect the platform and the resources developed here to be made freely available and openly published. We anticipate that they may be used to support novel in vivo animal experiments, first in human studies, validation of in silico models, and even other neurostimulation targets such as vestibular, pain, and peripheral nerve applications.

Conclusions: The presented in-development OpenCI platform for CI advancement and hearing research aims to facilitate and accelerate the adoption of scalable, compatible, and cost effective technology. Additionally, we expect collaboration within the broader community of researchers will be expanded to develop novel experimental modalities and address unmet clinical needs.
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SU133. Cortical Haemodynamic Responses to Auditory-Speech and Visual-Speech Stimuli Predicts Future Speech Understanding Outcomes for Cochlear Implant Recipients.

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Category: Auditory Prostheses
Background: Activation of the auditory cortex in response to both auditory-speech and visual-speech, or lip-reading, has been widely observed in cochlear implant (CI) users. A consensus is yet to be reached on how such activation of the auditory cortex correlates with speech understanding outcomes. In this study, we use brain imaging data collected within one-month after switch-on to predict recipient-specific speech understanding outcomes at one-year post switch-on. To do so, we evaluated cortical responses to auditory-speech and visual-speech stimuli using functional near infrared spectroscopy (fNIRS). We hypothesise: 1) larger activation of the auditory cortex during visual-speech at switch-on is predictive of poorer speech understanding outcomes at one-year, and 2) larger activation of the auditory cortex during auditory-speech at switch-on is predictive of better speech understanding outcomes at one-year.
Methods: 21 post-lingually deaf adult implant recipients were recruited for this study. All recipients were new implant users fitted with Cochlear Nucleus devices. fNIRS recordings were made during a naturalistic audio-or-visual story task within one-month of implant switch-on. The story was presented using 18 blocks of audio-only (sound-only, fixation cross on screen), 18 blocks visual-only (a video of a female speaker, no sound), and 10 blocks of control (fixation cross only, no audio) trials. The order of the trials was pseudorandomised for condition, but the story remained contextually continuous throughout. General linear modelling and waveform averaging were performed for fNIRS channels within the a-priori bilateral auditory cortex region of interest to quantify cortical activation. At one-year after implant switch-on, speech understanding outcomes were assessed using Bamford-Kowal-Bench (BKB) sentences in 8-talker babble noise.
Results: As hypothesised, data from our group of new recipients suggest that poorer speech understanding outcomes is associated with larger visual-speech stimulus activation of the auditory cortex at switch-on (p less than 0.001). Contrary to our second hypothesis, data suggested that poorer speech understanding outcomes were associated with larger auditory-speech stimulus activation of the auditory cortex at switch-on (p less than 0.01).
Conclusions: An assessment of cortical activation within one-month after implant switch-on offers a promising new method for predicting recipient speech understanding outcomes in the long term. This insight can be used to inform appropriate recipient-specific rehabilitation strategies to improve speech understanding outcomes.

SU134. Hearing Through a Bone Conduction Headset

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Category: Auditory Prostheses
Background: In recent times, bone conduction (BC) headsets for communication have gained popularity. Most of these devices place transducers in proximity to the ear canal opening. However, it remains unclear whether the perceived sound from these devices is a result of classic BC theory, transmitted to the inner ear through the bone, or primarily comprises airborne sound emitted directly from the transducers.

Methods: We conducted a study on twenty-one participants with normal hearing, measuring hearing thresholds and ear canal sound pressures with BC transducers positioned either at the mastoid or in front of the ear canal. Additionally, a skull-bone vibration analysis was performed using computational model (LiUHead), with stimulation provided at both positions.
Results: The hearing thresholds, in terms of stimulation force levels, were significantly lower (better) by 20 to 40 dB when stimulation occurred in front of the ear compared to the mastoid. The inter-aural separation was approximately 30 dB higher with frontal stimulation than with mastoid stimulation. It was also found that the sound pressure in the ear canal dominated hearing when stimulation was applied in the frontal position, and this ear canal component exceeded other components contributing to BC hearing by 25 to 30 dB.
Conclusions: When BC sound is applied near the ear canal, as seen in numerous BC headsets for communication, a significant portion of sound transmission occurs through radiated sound entering the ear canal. Consequently, this type of sound transmission closely resembles the process of air conduction stimulation. consequently, these devices are not suitable for audiological measurements, as they can be influenced by middle ear conditions and do not effectively distinguish between conductive and sensorineural hearing losses. However, bone vibration in this scenario is only 10 to 20 dB lower than that at the mastoid, making these devices potentially viable for individuals with conductive hearing impairments.

SU135. Improved Parameterization of a Hybrid Phenomenological-Biophysical Model of Cochlear Implant Stimulation of the Auditory Nerve

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Category: Auditory Prostheses
Background: Computational models of electrical stimulation of auditory nerve fibers (ANFs) extend from simple phenomenological models describing ANF spiking statistics through to detailed biophysical models describing the membrane potential and the activity of voltage-gated ion channels along the length of ANFs. Phenomenological models have the advantage of computational speed and a smaller set of parameters, but they may lack accuracy. The hybrid model of Joshi et al. (JARO 2017) aims to incorporate the best of both phenomenological and biophysical models. This model can accurately describe many response characteristics observed in cat ANF data, such as the increase in the dynamic range of an ANF's response with increasing pulse phase duration (PPD) of a biphasic stimulating pulse. However, preliminary simulations indicate that, with its default parameterization, the model's spontaneous firing rate is much higher than that observed in deafened cat ears, which could influence the dynamic range versus PPD behavior.
Methods: Simulations were run with single biphasic current pulses at a range of current levels to map out the model's discharge probability versus level function. From each probability versus level function, the relative spread (RS) was estimated as a measure of the model ANF's dynamic range. The onset time of the pulse and the PPD were systematically varied to determine how these affected the estimated RS. For each set of onset times and PPDs, the model parameters p_RS and c_RS were scaled by a range of factors to establish how these parameter values map onto the estimated RS values.
Results: The simulation results indicate that the model only produces RS values matching the cat ANF data, including the RS vs PPD effect, if i) the membrane potential is always initialized to the same resting potential and ii) the pulse onset time is close to zero, which are the defaults in the public release of the code. To obtain estimated RS values similar to cat ANFs for short PPDs, the parameters p_RS and c_RS had to be reduced by a factor of 3. This parameter scaling also reduced the spontaneous rate to almost zero, more consistent with the physiological data. However, the RS vs PPD effect is then absent, demonstrating that this effect was indeed due to the abnormally high spontaneous rate.
Conclusions: This study showed that the default parameterization of the Joshi et al. model does not generate appropriate dynamic ranges or rates of spontaneous activity in general. A new parameterization fixes these shortcomings, but further modifications will need to be made to the model to produce an appropriate increase in the dynamic range with increasing pulse phase duration. The code should also be modified so that the initial membrane potential is randomized with an appropriate probability distribution.

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SU136. Bilocated Intracochlear Electrocochleography During Cochlear Implantation to Provide Surgical Feedback

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Category: Auditory Prostheses

Background: Intracochlear electrocochleography (ECochG) during cochlear implant (CI) surgery is a promising method to monitor cochlear trauma. However, variations in the distribution of response generators throughout the cochlea complicate the interpretation of signal amplitude drops. This study aimed to investigate whether simultaneous recordings from two locations within the cochlea could solve this problem. This was done by 1) comparing recordings acquired simultaneously from an apical and a more basal electrode contact during atraumatic electrode array insertions, and 2) simulating the influence of responses generated at different cochlear locations on the recordings. We hypothesized that the response of a more basal contact should follow the response pattern of an apical contact with some delay if no changes of inner ear function occur during insertion of the array.

Methods: In eight standard CI recipients, ECochG recordings were conducted during stepwise insertion of a short temporary electrode array. Simultaneous recordings were acquired from two contacts separated by 4.2 mm, in response to 500 Hz tone bursts with alternating polarity. The difference and sum curves were derived from the recordings. After completion of the recordings, the temporary electrode array was removed and a standard CI inserted. ECochG measurements were compared with simulated hair cell responses generated at distinct locations along the basilar membrane.

Results: Difference curve amplitude and phase changes recorded by the apical electrode are closely resembled in recordings from the more basal contact. This holds for responses generated at the stimulation frequency as well as higher harmonics. A systematic bias between the recordings from the two electrode contacts was observed in one case, reflecting differences in electrode properties or impedance. The simulations showed that localized and widespread hair cell saturation are reflected in higher harmonics recorded at different intracochlear locations.

Conclusions: The proposed approach could facilitate the detection of ECochG response changes relevant for predicting hearing preservation in the future. In addition, higher harmonics analysis may also be relevant for determining local cochlear health based on ECochG recordings.

SU137. First-In-Human Neurophysiological and Electrode Impedance Results for a Novel Penetrating Auditory Nerve Implant During Acute Intraoperative Experiments

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Category: Auditory Prostheses

Background: Cochlear implants (CIs) have played a pivotal role in restoring hearing for individuals within the deaf community. These devices function by stimulating the auditory nerve through an electrode array implanted within the cochlea. Despite more than four decades of research, while CIs have significantly improved speech intelligibility, challenges remain in achieving music perception and reliable speech perception in noisy environments. These limitations are largely attributed to issues related to spatial activation specificity, primarily driven by the need for electrical signals to traverse the cochlear liquid and the bony wall of the cochlea before reaching the auditory nerve, thereby affecting focus and spatial resolution.

To address these persistent challenges, an innovative approach is proposed: direct stimulation of the human auditory nerve using a penetrating electrode array. This approach aims to eliminate the distance between the electrode and the nerve and bypass the current spread caused by cochlear liquid and the bony wall of the

cochlea. While promising results have been observed in experiments with animal models using various electrode technologies and with traditional wire electrodes in humans, stimulating the human auditory nerve with a penetrating microelectrode array is still largely unexplored.

Methods: In a series of intraoperative experiments performed in four patients undergoing surgeries for a tumor removal procedure, an auditory nerve implant (ANI) system developed by Blackrock Neurotech (Salt Lake City, UT, USA) was acutely implanted into the auditory nerve after tumor resection surgery in which the auditory nerve was already exposed. Electric stimulation pulses were generated by a Cerestim R96 stimulator and delivered to the ANI penetrating microelectrode array through a customized silicone molded lead. Before insertion of the ANI system, auditory nerve functionality was verified via electrically-induced auditory brainstem responses (eABRs) produced in response to stimulation within the cochlea and on the surface of the exposed auditory nerve.
Results: In all four patients, the array was successfully inserted into the nerve via a translabyrinthine approach, in which proper bone grooves ensured stability of the cable and array positioning. Impedances of the electrode sites after implantation were stable within the expected functional range of less than 100kOhms. Electrical stimulation achieved low activation thresholds (less than 30μA), which are significantly lower than what is typically observed for CIs.
Conclusions: In summary, we have made substantial progress towards the development of an ANI, including technology development, surgical implantation procedures, and stimulation protocols, which have led to demonstration of this ANI system in the human auditory nerve for the first time. By directly targeting the auditory nerve, these implants offer the potential for significant improvements in hearing outcomes, initially for those not receiving sufficient benefit from CIs and potentially as a future alternative to CIs through additional developments.

SU138. Contribution of Macrophages to Neural Survival and Intracochlear Tissue Remodeling Responses Following Cochlear Implantation

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Category: Auditory Prostheses
Background: Cochlear implants (CIs) restore hearing to deafened patients. The foreign body response (FBR) following cochlear implantation (post-CI) comprises an infiltration of macrophages, other immune and non-immune cells, and fibrosis into the scala tympani; a space that is normally devoid of cells. This FBR is associated with negative effects on CI outcomes including increased electrode impedances and loss of residual acoustic hearing. This study investigates the extent to which macrophage depletion by an orally administered CSF-1R specific kinase (c-FMS) inhibitor, PLX- 5622, modulates the tissue response to CI and neural health.
Methods: 10-12-week-old CX3CR1+/GFP Thy1+/YFP mice on C57BL/6J/B6 background were fed chow containing 1200 mg/kg PLX5622 or control chow for the duration of the study. 7-days after starting the diet, 3-channel cochlear implants were implanted in the ear via the round window. Serial impedance and neural response telemetry (NRT) measurements were acquired throughout the study. Electric stimulation began 7 days post-CI until 28- days post-CI for 5 hrs/day, 5 days/week, with programming guided by NRT and behavioral responses. Cochleae harvested at 10-, 28- or 56 days post-CI were cryosectioned and labeled with an antibody against α-smooth muscle actin(α-SMA) to identify myofibroblasts and quantify the fibrotic response. Using IMARIS image analysis software, the outlines of scala tympani, Rosenthal canal, modiolus, and lateral wall for each turn were traced manually to measure region volume. The density of nuclei, CX3CR1+ macrophages, Thy1+ spiral ganglion neuron (SGN) numbers, and the ratio of the α-SMA+ volume/ scala tympani volume were calculated.
Results: Cochlear implantation in control diet subjects caused infiltration of cells, including macrophages, into the cochlea. Fibrosis was evident in the scala tympani adjacent to the electrode array. Mice fed PLX5622 chow showed reduced macrophage infiltration throughout the implanted cochleae across all time points. However, scala tympani fibrosis was not reduced relative to control diet subjects. Further, mice treated with

PLX5622 showed increased electrode impedances compared to controls. Finally, treatment with PLX5622 decreased SGN survival in implanted and contralateral cochleae.
Conclusions: The data suggest that macrophages play an important role in modulating the intracochlear tissue response following CI and neural survival.

SU139. Pulsed Infrared Light Stimulates the Cochlea by Generating Pressure Waves that Are Detected by Hair Cells

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Category: Auditory Prostheses
Background: Cochlear stimulation with infrared (IR) light has been proposed as an alternative to electrical stimulation because it could provide better frequency resolution in next-generation cochlear implants. However, the mechanism and target of infrared cochlear stimulation remain controversial. Some studies suggest that spiral ganglion neurons are directly stimulated by IR irradiation, making it useful for cochlear implants, but others suggest that hair cells are required, limiting its value in cochlear implantation. Here, we sought to identify the locus and mechanism of IR cochlear stimulation in a live mouse preparation.
Methods: We recorded auditory brainstem responses and cochlear action potentials in response to both sound and infrared pulses. Our surgical approach preserved hearing while permitting infrared irradiation of the cochlea with an optical fiber. Sound- and laser-evoked vibrations in the cochlear apex were recorded using a custom-built optical coherence tomography (OCT) system. Experiments were done in Pou4f3DTR/DTR, VGLUT3-/-, TectaC1509G/C1509G, and TMC1-/- mice. Pharmacological reduction of the endocochlear potential was performed using intravenous furosemide administration.
Results: We show that IR light does not generate an auditory brainstem response (ABR) or cochlear action potential (CAP) when the inner hair cell (IHC) synapse is silenced by knocking out the IHC vesicular glutamate transporter (VGLUT3). Similarly, selective ablation of cochlear hair cells in transgenic (Pou4f3DTR/DTR) mice prevents the generation of an ABR or CAP in response to infrared light pulses, suggesting that hair cells are necessary for the response. Using OCT, we show that infrared pulses generate motion within the cochlea, and that this motion is amplified by outer hair cells (OHCs). In mice with a tectorial membrane that is detached from OHCs (TectaC1509G/C1509G), the infrared-evoked motion is not amplified by OHCs and a CAP is not generated, suggesting a critical role for the hair bundle in amplification and detection of the light pulse. Consistent with this, we find that the endocochlear potential is required to elicit an infrared-evoked cochlear response. Additionally, TMC1-/- mice, in which cochlear hair bundles are non-functional, do not respond to IR stimulation.
Conclusions: Our findings suggest that infrared light generates a mechanical response that is amplified by cochlear OHCs and detected by IHCs, and that spiral ganglion neurons are not directly stimulated by IR light. Our data provides a unifying explanation for several previously conducted studies on infrared cochlear stimulation. We conclude that infrared irradiation is unlikely to be a successful energy delivery method for future cochlear implants.

SU140. Post-Exposure Treatment of Noise-Induced Hearing Loss by Bk Channel Blockers

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Category: Clinical Otolaryngology and Pathology
Background: Noise-induced hearing loss (NIHL) is a common hearing loss. However, so far, there is no any pharmacological treatment, particularly no post-exposure (i.e., therapeutic) treatment available in the clinic. Also, some potential drugs require local cochlear injection since they cannot pass through the blood-labyrinth barrier (i.e., blood-brain barrier), which further limits their clinical application. Our previous study showed that administration of K channel blockers can attenuate NIHL and ribbon synapse degeneration. The BK channel is a predominant K channel in the cochlea. In this study, we tested if systematic administration of K channel blockers after noise exposure can attenuate NIHL and improve behavior performance in NIHL.

Methods: Adult CBA/CaJ mice were exposed to 95-98 dB SPL white noise for 2 hr, one time. K channel blockers were administrated by intraperitoneal injection (i.p.) after noise exposure. Hearing function was assessed by ABR, DPOAE, and other hearing function tests. Mouse behavior was also assessed by acoustic startle response (ASR). Finally, hair cell and ribbon synapse degeneration were examined by confocal microscopy with immunofluorescent staining.
Results: As shown in vitro study, administration of K channel blockers after noise exposure could significantly rescue hearing measured by ABR threshold and ribbon synapse regeneration. We found that systematic administration of a BK channel blocker GAL-20 after noise exposure significantly attenuated noise-induced hearing loss and cochlear synaptopathy and rescued hearing. We also found that administration of GAL-20 could significantly improve behavioral changes in noise-exposed mice. In comparison with non-treated noise-exposed mice, the ASR in the GAL-20 treated mice were completely restored and comparable to the normal level at the control no-exposed mice.
Conclusions: These data indicated that BK channel blockers have a great potential to treat noise-induced hearing loss and cochlear synaptopathy after noise exposure. These data also further support our previously proposed concept that noise-induced K+ excitotoxicity is a major contributor for the noise-induced cochlear synaptopathy.

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SU141. Facilitating Molecular Studies on Archival Human Temporal Bone and Brain Sections Using Poly(ethylene Glycol)-Mediated Celloidin Removal

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Category: Clinical Otolaryngology and Pathology
Background: Archival pathology repositories around the globe house vast collections of human temporal bone (TB) and brain sections that have been embedded and processed in celloidin. These repositories serve as invaluable resources for investigating diseases affecting the auditory and vestibular sense organs, as well as the human central nervous system. However, the utilization of celloidin sections for contemporary molecular-pathological research, such as immunohistochemistry, presents a challenge, which primarily stems from the lack of efficient methods to efficiently remove the aged celloidin matrix from tissue sections without damaging the specimens.
Methods: We developed a protocol employing poly(ethylene glycol) (PEG) to efficiently and rapidly remove celloidin from human TB and brain sections. First, we examined the PEG-celloidin chemistry, evaluating the dissolution kinetics of celloidin in PEGs with varying molecular weights, PEGs terminated with methoxy instead of hydroxyl groups, and at different temperature conditions. Additionally, we investigated the dissolution products of celloidin-PEG 200 using nuclear magnetic resonance (NMR) spectroscopy. Furthermore, we explored the dissolution properties of celloidin in sections with or without adhesive slide coating using albumin. The effectiveness and suitability of the new PEG protocol was assessed using immunohistochemical labeling on human temporal bone and brain celloidin sections.
Results: We identified low molecular weight PEGs, particularly PEG 200, as the most efficient solvent for celloidin. NMR spectroscopy analysis of celloidin-PEG 200 dissolution products indicated no chemical alterations in either the solute or solvent, suggesting a pure solvation process without any chemical modification. A notable finding was that proteins used as adhesive slide coatings hindered celloidin's solvation in PEG. Building upon these findings, we established a protocol for celloidin removal from archival tissue sections, omitting the need for adhesive slide coatings with albumin. This protocol enables rapid and complete celloidin dissolution through immersion in PEG 200 for 30–60 minutes. The new protocol proved to be highly effective in producing specific and strong immunolabeling results.
Conclusions: We present an innovative approach utilizing the sustainable and environmentally friendly solvent PEG 200 in combination with an enhanced section mounting technique, effectively dissolving

celloidin from archived human temporal bone and brain sections, some of which date back up to 40 years. Our novel protocol overcomes significant methodological challenges, rendering decades-old archival celloidin sections readily accessible for advanced molecular biological techniques, such as immunohistochemistry. At the same time, our method fosters a safer, gentler, and more efficient workflow, marking a stride toward sustainable and cutting-edge research in otopathology.

SU142. Quantitative 2-Dimensional Assessment of Endolymphatic Hydrops in the Aging Human Temporal Bone

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Category: Clinical Otolaryngology and Pathology

Background: Endolymphatic hydrops (ELH) is a histologic finding of the inner ear in which the endolymphatic space is enlarged, which can lead to bulging of Reissner's membrane into the scala vestibuli. ELH is most closely associated with Meniere's Disease (MD), which clinically presents as attacks of vertigo, intermittent hearing loss, tinnitus, and aural pressure. Other disease states have also been associated with ELH, including presbycusis or age-related hearing loss (ARHL). ELH is hypothesized to mechanically disrupt the organ of Corti, leading to loss of auditory function that may correlated with ARHL. Here, we quantitively assessed the presence and severity of ELH in aging temporal bone specimens to determine its association with the clinical presentation of ARHL.

Methods: Histological sections from 71 ears (ages 0-95) from the temporal bone collection at Johns Hopkins University School of Medicine were assessed. The specimens included teporal bones categorized as normal, ARHL, MD, and otosclerosis. Mid-modiolar sections from both ears of each specimen we selected, and every turn of the cochlea (top and bottom basal turns, top and bottom middle turns, and apical turn) were photographed at 2x magnification using Nikon Eclipse 80i and CaptaVision+. The software ImageJ was used to collect the following measurements: scala vestibuli area (mm2), scala media area (mm2), scala tympani area (mm2), perilymph area (mm2), length of Reissner's membrane (mm), the angle between Reissner's membrane and basilar membrane (deg), and the angle between Reissner's membrane and spiral limbus (deg). Anatomical measurements were compared to audiometric data to assess correlations between ARHL and ELH severity.

Results: Anatomical measurements varied across normal and ARHL ears (ages 0-95), but no apparent effect of age was observed across specimens. MD and otosclerosis cases showed clear qualitative and quantitative evidence of ELH, including a bulging Reissner's membrane, enlarged scala media, and abnormally large angular measurements.

Conclusions: These findings demonstrate that ELH of the cochlea does not positively correlate with aging in human temporal bone histology. As predicted, there was a clear association between ELH and cases of MD or otosclerosis, which has been supported in previous literature. These results indicate that 2D measurements can be further explored to analyze different inner ear pathologies quantitatively.

SU143. National Perspectives on the Management of Hearing Loss in Patients with Limited English Proficiency

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Category: Clinical Otolaryngology and Pathology

Background: Limited English proficiency (LEP) individuals represent a growing proportion of the US population, and English-speaking medical professionals continue to face challenges in the evaluation, diagnosis, and counseling of LEP patients with hearing loss. Hearing loss introduces additional vulnerabilities,

compounding on healthcare disparities otherwise attributable to language barrier. To this end, this study seeks to evaluate current practices among providers caring for adult LEP hearing loss patients.
Methods: This is a prospective narrative study of hearing loss providers from the twelve US cities encompassing the top ten highest immigrant populations and top ten non-English languages spoken in households, as determined by US Census data. Structured interviews were conducted regarding practice setting, experiences, and perspectives in managing hearing loss in LEP patients, with a subset focus on cochlear implantation (CI). Two study members analyzed data from audio transcriptions using modified grounded theory approach (a four-phase, iterative axial coding scheme for data distillation in qualitative research), with a third reviewer adjudicating.
Results: Of 43 providers contacted, 29 participated (16 otologists, 13 audiologists). On average, LEP patients comprised 34% of a respondent's practice (5-90%;n=28). The most common non-English language reported was Spanish, followed by Chinese languages. Four thematic domains were derived: CI candidacy evaluation, counseling, barriers to care, and ideal resources. Among providers providing a quantitative estimate, 63% (25-97%;n=14) of CI-eligible LEP patients proceeded to surgery, compared to 79% (30-90%;n=9) of English-proficient patients[p=0.23]. Nearly all providers noted patient desire as a major barrier to treatment (97%;n=28), predominantly influenced by cultural perceptions of healthcare (41%;n=12) and health literacy (34%;n=10). Twenty-one respondents (72%) cited a lack of validated non-English/Spanish tests as an additional barrier to treatment. Seventeen respondents (59%) reported improvising on validated speech perception testing, with live translation of English battery (38%;n=11), testing in English (24%; N=7), and use of pediatric word lists (7%;n=2). Fifteen providers (52%) reported use of non-validated evaluations, including pure tone audiometry alone (38%;n=11) and family reports of hearing difficulty (24%;n=7). Nearly one-quarter of respondents forgo speech testing entirely in non-English/Spanish-speaking patients (24%;n=7). In-person interpreters were most frequently proposed as an ideal resource to improve management (62%;n=18). About one-third of respondents expressed desire for a validated testing battery in all languages (31%;n=9).
Conclusions: There is no clear standard for resource access and utilization, nor is there a consistent approach to management of language barrier, in the management of LEP patients with hearing loss. Without regular access to language-specific testing materials, patients with LEP may be systematically delayed in receiving treatments that could improve their hearing and quality of life.

SU144. Coupled Influence of Tympanic Membrane Perforation Parameters on Hearing Loss and Surgical Outcome

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Category: Clinical Otolaryngology and Pathology
Background: Sound wavers in air are converted into mechanical vibration of the tympanic membrane (TM) and are transmitted to the cochlea via the middle-ear ossicular chain. The TM can be perforated by middle-ear infection or trauma, leading to conductive hearing loss and possibly recurrent infections. Myringoplasty is a surgical procedure to close the perforated TM, and the surgical outcomes of the myringoplasty are affected by parameters in pathological conditions and surgical procedures. While the influence of the parameters on surgical outcomes has been explored, most of the investigation focuses on uncoupled effects of each parameter rather than combined effects of the parameters.
This study aims to investigate the coupled effects of parameters in TM perforation and myringoplasty on hearing loss and surgical outcomes.

Methods: Data of ~2500 patients with a TM perforation, approximately 20% of whom underwent a type-I tympanoplasty, were collected and examined retrospectively. Pathological and surgical parameters were analyzed in correlation with hearing loss and surgical outcomes. The investigated parameters include size, location, duration, and etiology of the perforation, gender and age of the patient, status of contralateral ear, presence of active infection or discharge, and mastoidectomy during the myringoplasty surgery. The assessed outcomes were initial hearing loss measured with audiograms, spontaneous closure rate, and anatomical (closure rate), functional success (assessed by postoperative ABG), and complication rate of the myringoplasty

surgery. Multiple regression with statistical analysis was performed to find combined effects of the parameters on the outcomes.
Results: Effects of each parameter on hearing loss and surgical outcomes were similar to the results shown in previous studies. Coupled effects of several parameters were observed suggesting presence of multicollinearity between the parameters.
Conclusions: This study provides new additional data from one of Switzerland's largest patient cohorts in how the parameters in the TM perforation affect spontaneous closure and surgical success, enabling pre-identification of possible risks for postoperative complications or low surgical success.
The coupled effects of the parameters revealed in this study are expected to be evaluated through experimental investigations or numerical simulation in the future.

SU145. Histopathological Study of the Distribution of Schwann Cells and Microglia in the Cochlea of Human Temporal Bones with Neurofibromatosis Type 2

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Category: Clinical Otolaryngology and Pathology
Background: Sensorineural hearing loss may be associated with loss of auditory neurons. In Neurofibromatosis Type 2 (NF2) with bilateral vestibular schwannomas, neural loss has been attributed to local pro-inflammatory cytokine and chemokine release, furthering the sensorineural hearing loss and tumorigenic effect. However, there is indication that aberrant Schwann cell function and activation of local microglia may contribute to sensorineural hearing loss. Here, we aim to identify and characterize Schwann cells and microglia within the inner ear of human temporal bones with NF2 and age matched normal controls
Methods: Immunohistochemical analysis was performed in celloidin embedded archival temporal bone samples of NF2 patients with vestibular Schwannomas along with normal age-matched controls using antibodies for SOX10, IBA1 and neuronal markers. Fluorescent quantification of Schwann cells and microglia was compared to traditional Hematoxylin and Eosin (H and E) stainings in the National Temporal Bone Database (NIDCD).
Results: Fluorescent staining allowed for quantification and characterization of both cells types in control and NF2 positive cochleas. Preliminary results suggest a predominant glial cell population of SOX10 positive Schwann cells within the human cochleas, in addition to a smaller subset of IBA1 positive microglial cells.
As disease progresses, SOX10 positive Schwann cells and neurons are depleted, whereas microglial cell numbers did not change significantly over time and between controls and NF2. Interestingly, we observed a significant change in phenotype from ramified, quiescent morphology in controls to activated, ameboid phenotype in NF2 cochleas.

Conclusions: Here, we show an immunohistochemical characterization of Schwann cells and microglial cells in the cochleas of human temporal bones with Neurofibromatosis Type 2 compared to normal controls.

SU146. Short- And Long-Term Effects of Hearing Aids on Speech Recognition in Older Adults With Age-Related Hearing Loss – Preliminary Findings

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Category: Hearing Loss: Consequences and Adaptation
Background: Hearing aids are the most common intervention for age-related hearing loss, but the benefits they offer in daily listening conditions vary substantially between users. Some of this variability may stem from differences in amplification-induced plasticity. Such plasticity was found in studies that showed that the contribution of amplification to speech in noise increases over time. In this ongoing longitudinal study, we examine plastic changes in the recognition of fast speech, speech in babble noise and dichotic speech in three

timepoints over a six-months period. We assess the contribution of hearing aid use over time compared to individuals with no hearing aids.
Methods: 28 first-time hearing aid users (age 65-88, M = 77) have completed the first two phases so far. The first phase was conducted shortly prior to hearing aid fitting, and the second phase was conducted two months post-fitting. Aided and unaided performance was assessed. We examined the relative contribution of phase, condition (aided and unaided), and their interaction to the perception of fast speech and speech in babble noise.
Results: Speech recognition accuracy was modeled as a function of phase, condition and their interaction. For fast speech there was no significant main effect of phase (OR = 1.00), but overall, perception was more accurate when assessed with hearing aids (OR = 2.03). A significant interaction between phase and condition was also observed, with a larger amplification effect after two months of hearing aid use compared to the first assessment (OR = 2.06). For speech in babble noise, there was a significant main effect of condition, with an increase in aided speech perception (OR = 1.81). The effects of phase (OR = 0.93), and an interaction between phase and condition interaction were not significant (OR = 1.13).
Conclusions: While the contribution of amplification to the recognition of fast speech increased following a period of hearing aid use, the contribution of amplification to speech in noise was present at the time of fitting and remained constant over time. It seems that hearing aid users learn to take advantage of the improved signal for better recognition of fast speech over time. In contrast, the advanced algorithms dedicated to improve the identification of speech in noise provide an immediate and a direct benefit, reducing the need to adapt overtime.

SU147. Neurophysiological, Structural and Molecular Alterations in the Prefrontal and Auditory Cortices Following Noise-Induced Hearing Loss

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Category: Hearing Loss: Consequences and Adaptation
Background: It is well established that hearing loss can lead to widespread plasticity within the central auditory pathway, which is thought to contribute to the pathophysiology of audiological conditions such as tinnitus and hyperacusis. Emerging evidence suggests that hearing loss can also result in plasticity within brain regions involved in higher-level cognitive functioning like the prefrontal cortex; findings which may underlie the association between hearing loss and cognitive impairment documented in epidemiological studies. Using the 40-Hz auditory steady state response to assess sound-evoked gamma oscillations, we previously showed that noise-induced hearing loss results in impaired gamma phase coherence within the prefrontal but not the auditory cortex. Here, we asked whether region-specific structural and/or molecular changes accompany this differential plasticity following hearing loss.
Methods: In the present study, Sprague Dawley rats were chronically implanted with electrodes over the prefrontal and auditory cortices prior to sham or noise exposure (115 dB SPL, broadband noise, 2hrs). The 40-Hz auditory steady state response (ASSR; 150 trials of a 0.5 second 40 Hz acoustic stimulus) was used to (1) determine the magnitude of sound-evoked responses (i.e., event-related potentials, ERPs) and (2) assess gamma phase coherence (i.e., inter-trial coherence, ITC) in distinct cortical regions at 7- and 14-days post sham/noise exposure. To determine whether region-specific structural and/or molecular changes accompany the electrophysiological profiles observed in the auditory and prefrontal cortices, we used Golgi-Cox staining to assess dendritic organization and synaptic density, as well as western blotting to measure changes in synaptic signaling proteins (GAD65, NR2B, PSD95, synaptophysin) in these cortical regions.
Results: We show that following noise exposure, impaired gamma phase coherence within the prefrontal cortex is accompanied by alterations in pyramidal cell dendritic morphology and decreased expression of proteins involved in GABAergic (GAD65) and glutamatergic (NR2B) neurotransmission; findings that were not observed in the auditory cortex, where gamma phase coherence remained unchanged post-noise exposure. In contrast to the noise-induced effects we observed in the prefrontal cortex, plasticity in the auditory cortex was characterized by an increase in NR2B suggesting increased excitability, as well as increases in the synaptic proteins PSD95 and synaptophysin.
Conclusions: Overall, our results highlight the differential effect of noise-induced hearing loss on auditory versus higher-level brain regions as well as potential structural and molecular mechanisms by which hearing

loss may contribute to impaired cognitive and sensory functions mediated by the prefrontal and auditory cortices.

SU148. Comparison of Hearing Aid Fitting Effectiveness With Audiograms From Either User-Operated or Traditional Audiometry in a Clinical Setting

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Category: Hearing Loss: Consequences and Adaptation

Background: The constrained accessibility of hearing tests for clinical decision-making is a significant challenge within the hearing rehabilitation system. With the ongoing global aging of the population, the challenge will most likely become more significant in the future, as a larger portion of the population will profit from hearing aids (HA). Consequently, there is a global need to expand the capacity of audiometry. One promising avenue for expanding this capacity is the integration of user-operated audiometry (UAud). Numerous studies have consistently demonstrated that user-operated audiometry exhibits reliability and accuracy comparable to traditional audiometry. However, its clinical adoption and acceptance remain limited, partly due to its value for clinical use as a base for HA fitting remains unclear. In this study, we sought to address this gap by comparing the effectiveness of HA fitting based on the UAud system to that based on traditional audiometry in a clinical setting.

Methods: The design was a blinded non-inferiority randomized controlled trial. 215 adults referred for hearing aid treatment were included in the analysis. Participants was tested using both traditional audiometry as well as the UAud system (which is based on the AMTAS method) at baseline. Participants were randomized to receive HAs fitted based on either UAud (UAud group) or traditional audiometry (control group). Outcome measures were assessed at two time points: baseline and a follow-up conducted a minimum of 12 weeks after hearing aid fitting. The primary outcome was changes in self-assessed hearing, assessed by the Speech, Spatial and Qualities of Hearing Scale (SSQ12) overall score from baseline to follow-up. Secondary outcomes include aided speech-in-noise performance assessed by speech reception thresholds in noise (SRTN) using the Hearing In Noise Test (HINT) at follow-up, as well as changes in the SSQ12 subscales scores between baseline and follow-up.

Results: Both groups exhibited a significant improvement in SSQ12 scores. A constrained linear mixed model analysis demonstrated that the UAuds group's before-after changes in self-assessed hearing was non-inferior to that of the control group, as the differences in SSQ12 scores improvements were not statistically significant, and the upper bound of the 95% confidence interval for the difference between group means fell below the predefined non-inferiority margin. The UAud group also demonstrated non-inferiority to the control group across all secondary outcome measures.

Conclusions: As the outcome of HA fitting based on UAud was non inferior to that based on traditional audiometry, it's suitable for clinical use as a base for HA fitting in the general clinical practice. The use of UAud could play an important role for clinicians in need of effectively treatment of the growing number of HA users in the future.

SU149. Open Board

SU150. The Relationship Between Tinnitus Annoyance and Work-Related Noise Exposure History in Older Adults With Sensorineural Hearing Loss

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Category: Hearing Loss: Consequences and Adaptation

Background: Exposure to noise at work is one of the major risk factors contributing to the onset of tinnitus. Rates of tinnitus has been shown to range from 35 to 77% among adults with noise-induced hearing loss. Not all who suffers from tinnitus are significantly bothered by it, but for those who experience a severe degree of tinnitus it can lead to depression, anxiety, and poorer quality of life. However, no single cure exists for the condition, and evidence is still lacking on the causes of the onset of tinnitus. Knowledge of factors associated with tinnitus annoyance are important for prevention and rehabilitation in audiological clinics. Thus, the aim of the current study was to investigate if tinnitus is more prevalent among older adults with a noise exposure history and whether older adults with previous work-related noise exposure suffer from a more severe tinnitus than those without an occupational noise exposure history.

Methods: The study was designed as a prospective observational cohort study and included 1176 older adults (≥ 60 years) with bilateral sensorineural hearing loss referred for hearing aid rehabilitation. A non-standardized health-related questionnaire that contained questions on demographic details a long with questions on previous occupational noise exposure to estimate their life-time noise exposure were distributed. A noise emission index was used to calculate their total equivalent noise exposure levels. The tinnitus handicap inventory (THI) questionnaire was used to assess tinnitus annoyance and sent to those answering yes to having experienced a ringing in their ears. All patients underwent a standard pure-tone and speech audiometry (n=1171 complete audiograms).

Results: Out of the 1176 included adults (mean age ± SD: 71.6 ± 6.8 years) 43% answered yes to suffer from tinnitus. Tinnitus was significantly more prevalent among occupationally noise-exposed older adults (X2(2, N=1175) = 43.7, p less than 0.001) with 54% of the noise-exposed reporting tinnitus compared to 35% in the non-exposed group. Regression analysis revealed that the reported THI score was not statistically significant higher among noise-exposed adults com-pared to the non-exposed adults with tinnitus when adjusting for age, sex, better-ear hearing thresholds and hearing aid experience (β: 3.1 [95%CI: -0.5; 6.7], p=0.09).

Conclusions: An occupational noise exposure history was associated with a higher prevalence of tinnitus in older adults with sensorineural hearing loss. However, the severity of tinnitus assessed with the THI questionnaire was not significantly related to noise exposure status. Other tinnitus questionnaires might be more sensitive to measure differences in tinnitus annoyance. More clinical attention should be given to older adults with a noise exposure history in hearing rehabilitation.

SU151. Refinement of Inner Hair Cell (IHC) Dysfunction in a Phenomenological Auditory Nerve (AN) Model Using Physiological and Single-Unit Recordings Following Selective IHC Dysfunction

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Category: Hearing Loss: Consequences and Adaptation

Background: A precise computational auditory model capable of simulating neural responses in healthy auditory systems and those with varying degrees and types of hearing loss, in response to any desired stimuli, can serve as a powerful tool for exploring hypotheses in hearing science. The phenomenological model proposed by Bruce et al. (2018) simulates neural responses of auditory-nerve (AN) fibers in normal and hearing-loss conditions. However, a recent study (Patra et al., 2023) highlighted the need for improvement in the model for predictions of rate level functions, modulation coding, and evoked potentials for selective inner-hair-cell (IHC) dysfunction. Bruce et al.'s 2018 hearing loss model uses AN-fiber spiking rates from animals with noise-induced hearing loss (OHC/IHC dysfunction combined), making it complex to validate the model for individual hearing loss mechanisms. Insights gained from studying single-unit auditory nerve responses in animal models with specific hearing loss inductions contribute to a deeper understanding of hearing-loss mechanisms, enhancing more precise computational hearing-loss models.

Methods: We compared AN single-unit spike rates between animal models (chinchillas) with normal hearing (NH) and carboplatin-induced selective IHC dysfunction (CA-induced). Our project focuses on comparing average and instantaneous rate-level functions (RLFs) between the CA and NH models. Results show that

with CA induction, the saturation points in the RLF shift to higher sound levels, accompanied by a decrease in saturation and spontaneous rates compared to NH animals. These alterations cannot be accounted for in the model by simply reducing the weight of a single IHC dysfunction parameter, CIHC. We proposed refinements to the model for IHC dysfunction, involving not only adjusting CIHC but also introducing a negative DC shift to the model's IHC nonlinearity. This adjustment better replicates the lower saturation rates and spontaneous rates observed in our physiological dataset. We further optimized parameters related to the IHC dysfunction model to minimize differences between rate-level functions, dynamic range, and saturation rates in physiological data and model responses. For this optimization, we adjusted the AN model's properties such as characteristic frequency (CF) and spontaneous rate based on individual cell properties in the dataset. Lastly, we assessed the refined IHC-dysfunction model's performance in simulating evoked potential responses to various amplitude-modulated stimuli.
Results: Our findings suggest that the current IHC-dysfunction model does not fully capture all aspects of the mechanisms observed in selective IHC-dysfunction physiological studies. Preliminary results indicate that the proposed refinement to the IHC-dysfunction model in this study provides a more precise prediction of neural responses of animal models with selectively isolated IHC damage.
Conclusions: This study suggests that leveraging insights from selective, isolated forms of hearing loss can be valuable in the development of more accurate computational models for hearing loss.

SU152. Cortical Extracellular Matrix Glycoprotein Density Increases After Acoustic Noise Trauma in Tinnitus Animals Only

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Category: Tinnitus
Background: Most scientist agree that tinnitus, the perception of a sound without external physical source, is the result of an interaction of a damage to the peripheral auditory system and central neuroplastic adaptations to the new, changed auditory input. Here we investigate tinnitus related adaptations in the auditory cortex (AC) 13 d after its induction by quantifying the density of the extracellular matrix (ECM) in the primary fields of the AC of Mongolian gerbils (Meriones unguiculatus). The ECM density has been shown to be relevant for neuroplastic processes within the cortex and can be seen as a marker for the stability of synapses within a certain area.
Methods: We used a mild monaural acoustic noise trauma (2 kHz, 115 dB SPL, 75 min) in 9 gerbils to induce tinnitus, or sham monaural control sound exposure (2 kHz, 65 dB SPL, 75 min in 3 gerbils. A possible tinnitus percept was assessed by the gap prepulse inhibition of the acoustic startle (GPIAS) response paradigm; 4 of the trauma animals did show tinnitus related changes in the GPIAS paradigm 13 d after trauma (T group), the remaining 5 did not show tinnitus related behavioral response changes (NT group). None of the 3 control animals showed signs of a tinnitus percept (C group). The ECM density 13 d after trauma was quantified using immunofluorescence luminance of Wisteria floribunda lectin-fluoresceine-5-isothiocyanate (WFA-FITC) on histological slices of the primary AC, relative to the brainstem as reference area.
Results: We found that the WFA-FITC luminance of the AC of NT animals was not significantly different from that of control animals, independent on the side of the cortex (ipsi- / contralateral to trauma). On the other hand, we found a significant increase of luminance in T animals' ACs ($p=0.001$) compared to NT or control animals cortices. This effect was exclusively found on the AC side contralateral to the trauma ear ($p=0,002$) while no significant differences were found on the ipsilateral side ($p=0.24$).
Conclusions: These results point to a process of stabilization of synaptic connections in primary AC, which may be involved in the chronic manifestation of tinnitus.

SU153. Open Board

SU154. Open Board

SU155. Characterizing Functional Changes of the Central Auditory System in Tinnitus

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Category: Tinnitus

Background: Tinnitus is widely thought to be triggered by overcompensation (gain) in the central auditory system in response to hearing loss, leading to phantom percepts and abnormal loudness recruitment. While compensatory central changes are common in hearing loss, recent animal studies suggest that changes to specific non-lemniscal circuits in the dorsal cochlear nucleus (DCN) are involved in tinnitus.

Methods: To examine the role of DCN changes in tinnitus, we will leverage a large battery of psychoacoustic measurements and electroencephalography (EEG). We will assess whether individuals with tinnitus exhibit deficits in perceptual tasks thought to be specifically mediated by DCN function, as opposed to functions that are thought to be supported by other structures along the auditory pathway. To probe DCN function, we will measure sensitivity to frequency notches in broadband noise sounds. In addition, we will examine central gain at multiple levels along the lemniscal pathway using EEG.

Results: Analyses will compare psychoacoustic performance and EEG neurometrics between individuals with persistent tinnitus, and a control group with matched audiograms but no (or occasional) reported tinnitus. Preliminary results suggest that individuals with tinnitus do not exhibit greater gain at different levels of the ascending lemniscal pathway compared to controls with similar degrees of peripheral integrity.

Conclusions: The behavioral and EEG data from this ongoing work will provide insight on the physiological characteristics of the central auditory system in tinnitus.

SU156. Effect of Photobiomodulation on Tinnitus Induced by Acoustic Overexposure

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Category: Tinnitus

Background: Tinnitus, an abnormal perception of sound within the ear despite external auditory stimuli, is believed to result from aberrant nerve activity along the central auditory pathway, including the cochlear nucleus (CN). Furthermore, the causes of tinnitus are diverse, and tinnitus induced by noise exposure is on the rise. Despite its high prevalence, effective treatments remain unclear. Photobiomodulation (PBM) impacts cellular activity by delivering low-intensity light energy (less than 500 mW) that does not generate heat. In the field of audiology, PBM has shown beneficial effects on hearing loss caused by various damaging factors and has demonstrated protective effects on central neurons. Moreover, positive outcomes of PBM have been observed in animal models of salicylate-induced tinnitus and clinical studies involving patients. Therefore, this study aims to investigate the changes that occur after noise-induced (NI) tinnitus and evaluate the effect of PBM on NI tinnitus in the auditory nervous system.

Methods: Five-week-old C57BL/c6 mice were used. To induce NI tinnitus, the right ear of each mouse was occluded, exposing only the left ear to a unilateral 110 dB SPL at 16 kHz narrowband noise for 5 hours. Auditory Brainstem Response (ABR) and Gap-Prepulse Inhibition of Acoustic Startle (GPIAS) were measured before and after noise exposure, at 1 day, 3 days, 1 week, 2 weeks, and 4 weeks post-exposure. Photobiomodulation (PBM) using an 808nm diode laser (at 165 mW/cm2, 1 hour per day for a total of 5 consecutive days) was performed 4 weeks after noise exposure. ABRs were measured before and 2 weeks after PBM. GPIAS was assessed before noise exposure, during the 5 days of PBM irradiation, and 2 weeks after PBM. Changes in TRPV1, VGLUT1, and VGLUT2 were investigated.

Results: Following noise exposure, ABR thresholds increased at frequencies of 8, 16, and 32 kHz, while GPIAS decreased compared to the control group. At 2 weeks after PBM irradiation, ABR thresholds increased at all tested frequencies, and GPIAS significantly increased. The NI tinnitus ears exhibited reduced spiral ganglion cells in Rosenthal's canal of the cochlea compared to the control group. In the cochlear nucleus (CN), TRPV1 and VGLUT2 expression significantly increased after noise exposure, while VGLUT1 expression decreased compared to the control group. After PBM application, contrary to the NI tinnitus results, TRPV1 and VGLUT2 decreased, while VGLUT1 increased.

Conclusions: Noise exposure resulted in increased ABR thresholds and reduced GPIAS values, indirectly indicating the presence of NI tinnitus. Furthermore, the NI tinnitus models showed increased TRPV1 and

VGLUT2 expression and decreased VGLUT1 expression in the CN. However, when PBM was applied, the results were opposite to those observed after tinnitus induction, suggesting that PBM could alleviate tinnitus symptoms.

SU157. Exploring the Mechanisms of Stress-Induced Tinnitus: Transcriptomic Analysis of the Prefrontal Cortex and Hippocampus in a Rat Model

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Category: Tinnitus

Background: Although the role of stress in the development and persistence of tinnitus is well-established, the specific molecular mechanisms involved remain poorly understood. This study aims to investigate the changes in the brain occurring in a chronic stress-induced tinnitus animal model by conducting a transcriptome analysis of the brain using RNA-sequencing (RNA-seq).

Methods: Twenty Sprague Dawley rats were exposed to 10 consecutive days of chronic restraint stress lasting for 2 hours each day. After assessing tinnitus development using the gap pre-pulse inhibition of the acoustic startle reflex (GPIAS) test, brain tissue from the prefrontal cortex and hippocampus was collected from 15 rats: 5 with evident tinnitus (Stress-induced Tinnitus, ST), 5 with noticeable absence of tinnitus (Stress-induced Non-Tinnitus, SNT), and 5 without stress (control group, CG). Comparative RNA-seq analysis was conducted to investigate gene expression profiles among these groups.

Results: Compared to the control group, the ST group exhibited 971 and 463 differentially expressed genes (DEGs) in the prefrontal cortex and hippocampus, respectively (FDR less than 0.05). The enrichment analysis of the prefrontal cortex revealed the downregulation of gene sets associated with neurotransmitters and synapses in the ST group. In contrast, gene sets related to the cell cycle and immune pathways were significantly upregulated in the prefrontal cortex of the ST group. Additionally, the steroid biosynthesis gene set was downregulated, while the ECM and immune-related gene sets showed upregulation in the hippocampus of the ST group.

Conclusions: Genes associated with neurotransmission and synapse were downregulated in the prefrontal cortex, while immune-related genes were upregulated in both prefrontal cortex and hippocampus in the rats with ST. Our understanding of the molecular pathways governing gene expression in the brain during ST will provide essential insights and data for the development of precise tinnitus management strategies in the future.

SU158. Efficacy and Safety of Intratympanic Botulinum Toxin Injection on Middle Ear Myoclonic Tinnitus: Clinical Outcomes in a Large Case Series

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Category: Tinnitus

Background: This study aims to investigate the efficacy and safety of intratympanic Botulinum toxin (IT-BTX) injection as a novel clinical treatment option for middle ear myoclonic tinnitus (MEMT)

Methods: Medical records and tinnitus questionnaires of the patients with MEMT who underwent IT-BTX at Seoul St. Mary's Hospital from 2019 to 2022, followed up more than 6 months were retrospectively reviewed. The dosage of BTX was 5U~10U depending on the size of middle ear cavity. Efficacy of IT-BTX on MEMT as well as its safety were evaluated by audiology tests and tinnitus questionnaires.

Results: Out of a total of 75 patients who underwent IT-BTX, 57 patients who completed the questionnaires at pre- and post- IT-BTX and followed-up more than 6 months were enrolled. Among these patients, 26 (45.6%) were male and 31 (54.4%) were female, with a mean age of 37.6 years (9-73). Fifteen patients (26.3%) received IT-BTX more than twice, while 42 patients (73.6%) received a single injection. Ten patients (17.5%) underwent middle ear tendon resection (METR) after IT-BTX. Tinnitus handicap inventory (THI) scores

decreased significantly from pre- IT-BTX (44.31±26.8) to 31.42±23.6 (p less than 0.001), 32.92±24.9 (p=0.004), and 25.71±22.9 (p less than 0.001) at 1-, 3-, and 6-months post-IT-BTX, respectively. Additionally, visual analogue scales (VAS) of loudness (LD), awareness (AW), annoyance (AN) and effect on life (EF) significantly decreased post-IT-BTX (p less than 0.05): LD from 4.19±2.4 to 2.47±2.0, 2.89±2.2, and 2.38±1.9 at 1-, 3-, and 6-months; AW from 41.75±29.5 to 24.38±23.3, 26.94±23.3, and 22.10±21.7; AN from 5.00±2.8 to 2.82±2.1, 3.47±2.4, and 2.78±2.2; EF from 4.82±2.8 to 2.89±2.2, 3.28±2.4, and 2.73±2.2 at 1-, 3-, and 6-months post-IT-BTX . Notably, 40.3% of the IT-BTX patients experienced a complete cure of their MEMT symptoms, while 50.8% demonstrated partial resolution, and only 8.7% showed no response after IT-BTX. Comparing delta values between single injection group and multiple injection group revealed significant differences in VAS LD, AW, and EF between 1-month and 6-month intervals, indicating potential needs for additional injections due to the remain symptom after 1 month or symptom recurrence after 6 months. Importantly, no patient showed any complications such as hearing loss, dizziness, facial palsy or swallowing difficulties during the mean follow-up period of 22.2 months after IT-BTX.

Conclusions: IT-BTX seems to be an effective and safe modality for treating MEMT, providing substantial symptom improvement without complications and presenting a novel compelling therapeutic option prior to considering METR.

SU159. Tinnitus Affects the Response to Sound in the Inferior Colliculus

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Category: Tinnitus

Background: Tinnitus is the perception of sound in the absence of an external sound source. It is estimated that worldwide over 740 million people are affected, with 10-20% experiencing bothersome tinnitus. Currently, there is no cure available. One reason is the lack of an objective non-invasive test. Another problem is that the underlying mechanisms are poorly understood. A subset of neurons in the inferior colliculus (IC) shows a phenomenon in which they have an increased spontaneous firing rate and increased firing rates in response to sound stimuli following a long-duration sound (LDS). As tinnitus has been associated with increased spontaneous activity, we tested if this phenomenon is altered in mice with or without behavioral signs of tinnitus.

Methods: We recorded multi-unit activity from IC of unilaterally sound exposed mice with and without behavioral signs of tinnitus. Sound exposure was a 1 h presentation of a 2 kHz wide narrow-band noise (centered at 16 kHz). One ear was blocked from sound and animals were awake during sound exposure. Behavioral tinnitus assessment was performed via active avoidance paradigm tests. Only animals with normal hearing in the unexposed ear were included in this study. All electrophysiological recordings were performed on anaesthetized animals. Using a double-shank 32-channel low-impedance probe we recorded from the IC both ispi- and contralateral to the sound exposed ear. Using our 'Novel Stimulus Paradigm' we recorded the responses to tone pips before (PRE) and after (POST) the presentation of an LDS (narrow-band noise (1/3 octave) centered at the tone pip frequency).

Results: In response to 3 ms tone pips we found that response durations exceeded the stimulus duration in both IC. However, sound exposed animals (tinnitus and non-tinnitus) showed a significantly shorter response duration than non-sound exposed control animals. Contrastingly, sound exposed animals showed a higher firing rate in response to PRE tone pips than the controls. A tinnitus effect was present in recordings from the IC contralateral to the sound exposed ear with tinnitus mice showing the highest PRE firing rates in response to the stimulus presentation.

When comparing the responses PRE to POST we found that while the control animals show both channels with an increased firing rate POST as well as channels with a decrease, sound exposed animals show almost exclusively a decrease. Recordings from tinnitus animals, however, show less of a suppression compared to non-tinnitus animals in both the ipsi- and the contralateral IC.

Conclusions: Recordings from the IC in sound-exposed mice show a difference in firing rate in response to tone pips depending on the tinnitus status. This might reflect the reported increased spontaneous activity in tinnitus mice. Since the effect is reflected in recordings in response to sound, it might be used in non-invasive testing.

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SU160. Influence of Auditory Experience With Jittered Input on Spatial Hearing of Cochlear Implanted Rats

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Category: Binaural Hearing and Sound Localization

Background: Sound localization in patients with bilateral cochlear implants (biCI) is a commonly described problem. A major cause is the difficulty patients have in perceiving interaural time differences (ITD), especially if they suffer from prelingual deafness. This led to the hypothesis that a lack of binaural hearing experience during an early critical period may be a reason for the poor ITD sensitivity of these patients. However, recent work by our research groups has shown that neonatally deafened (ND) rats, bilaterally supplied with cochlear implants (CIs) in young adulthood, can be trained to use ITDs to lateralize sounds with remarkably low ITD thresholds (~50 µs) if the biCIs are precisely synchronized from the beginning resulting in the presentation of informative pulse timing (pt) ITDs. Here we investigate whether the inexperienced auditory system can develop good ITD sensitivity when the ptITDs are jittered from onset.

Methods: Neonatal rats were deafened using kanamycin and bilaterally implanted with CIs in young adulthood. Subsequently, rats learned to lateralize electrical biphasic current pulse trains in a 2-alternative-forced-choice task. For six weeks, rats were trained with jittered ptITD and interaural level difference (ILD) information. Using a pulse rate of 250 pps and a pulse duration of 0.2 seconds, ITD values were drawn independently from a set of ±{0, 20, 40, 60, 80, 100, 120} µs where negative values denote left-ear leading. ILD values were drawn independently from a set of ±{0, 1, 2, 3, 4, 5} dB. Jitter values changed per pulse and were randomly drawn from a distribution, ranging from -61.44 µs to +61.44 µs in 20.48 µs steps. After training, rats were tested on ITD and ILD sensitivity with and without jittered pt information. ITD and ILD sensitivities were determined using a psychometric curve fit model.

Results: Under jittered stimulation conditions, all biCI rats showed very good ITD and ILD sensitivities over the normal physiological range. After removal of the jitter, all rats still showed good ITD and ILD perception albeit with a trend toward reduced sensitivity, indicated by a reduced slope of the psychometric functions at 0 µs ITD and 0 dB ILD.

Conclusions: In summary, the behavior results demonstrate that ITD and ILD sensitivities of the early deafened auditory system are not reduced when exposed to jittered pulse timing information during initial training. Surprisingly, ITD sensitivity appeared to be comparable or even slightly improved under jittered versus unjittered stimulation conditions. This suggests that the development of good ITD sensitivity in biCI patients does not necessarily require microsecond precise ptITD presentation, but that jitter of up to ±60 µs can be tolerated or even improve perception.

SU161. A Fast and Accurate Algorithm of Sound Localization for Smart Hearing Aids

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Category: Binaural Hearing and Sound Localization

Background: The human auditory system can localize multiple sound sources using time, intensity, and frequency cues contained in the sound received by the two ears. Being able to spatially segregate the sources helps detection and perception in a challenging condition when multiple sounds coexist and compete.

Methods: We performed model simulations and constructed a real-time system to explore an algorithm for localizing multiple sources in azimuth with binaural (i.e., two) microphones. The algorithm relies on the "sparseness" property of a variety of daily signals in the time-frequency domain, and sound coming from different locations carrying unique spatial features will form clusters. Based on a previously developed interaural normalization procedure, the model generated spiral patterns for sound sources in the frontal

hemifield. Next, a dummy head with in-ear binaural microphones was constructed to record sound coming from various horizontal locations in a noisy environment.
Results: Our algorithm performed better than the Generalized Cross-Correlation Phase Transform approach for speech and complex sound. In anechoic conditions, low-frequency models yield better results than high frequencies. When implemented in the dummy-head system, we achieved better results at higher frequencies due to the existence of a significant low-frequency humming noise in the environment.
Conclusions: The results served as a proof of concept that the algorithm is practical to be incorporated in the next-generation hearing aids.

SU162. Accounting for Individual Variability in the Recovery of Sound Localization Performance Following Acute Spatial Cue Distortion

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Category: Binaural Hearing and Sound Localization
Background: Human pinna morphology is known to impact sound source localization. The unique shape of the pinna acts to filter incoming sound in a direction-dependent and highly individualized manner. Acute changes in effective ear shape degrade localization performance, but it has been demonstrated that with sufficient passive exposure and/or explicit training, negative impacts of modified ear shape on sound localization can be largely ameliorated. However, large individual variability has been noted in almost every step of the localization recovery process. Here, we aimed to examine sources of such variability. Individual behavioral performance on a localization training task following an acute change in effective pinna shape was evaluated in terms of individual pinna filter characteristics and extended high-frequency hearing (EHF).
Methods: 9 normal-hearing subjects (4 female; pure-tone thresholds ≤20dB HL bilaterally; EHF thresholds 9-16 kHz also measured) completed ~45 minutes of daily sound localization training with audiovisual feedback for two weeks wearing low-attenuation earplugs bilaterally. Training was completed in a darkened hemi-anechoic chamber using a set of 12 loudspeakers located at 15° intervals from -30° to 45° along the mid-sagittal plane in the front and rear hemifields. Subjects also completed pre- and post- testing with open and plugged ears on a more extensive set of 24 speakers spanning 360° azimuth and 60° elevation. Separately, 3D scans were taken of each subject's head/pinnae, and pinna dimensions were quantified. Digitized pinnae (right ears only) were then merged onto a standard base designed to couple to an acoustic test fixture (G.R.A.S. 45-CB). Merged pinnae were printed in soft (Shore 30A) polymer and coupled to the test fixture to facilitate measurement of head-related transfer functions (HRTFs) with open and plugged ears.
Results: Immediately following insertion of the hearing device, subjects demonstrated significant increases in localization errors along the vertical plane and in the confusion of front and back sources. After two weeks of training, significant improvement was seen in both dimensions. Post-testing at untrained source locations demonstrated greater generalization in azimuth than elevation. Correlational analyses quantified relations between behavioral outcomes and individual pinna, HRTFs, and audiometric features.
Conclusions: Following acute disruption of sound localization via insertion of low-attenuation earplugs, near-normal localization ability was recovered after two weeks of training with feedback. Although all subjects improved, significant individual variability was noted. Further evaluation will work to specify the extent to which such variability is accounted for by individual variability in audible HRTF features with open and plugged ears.

SU163. Open Board

SU164. Performance on a Novel Combined Sound Localization and Speech-In-Noise Task in Bilateral Cochlear Implant Recipients

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Category: Binaural Hearing and Sound Localization

Background: Binaural hearing is essential for localizing sound and understanding speech in noise. Due to their potential for restoration of binaural hearing, bilateral cochlear implants are a desirable solution for bilateral hearing loss. While it is well established that bilateral cochlear implantation is superior to unilateral implantation for restoring sound localization and speech comprehension in noise for patients with severe-to-profound hearing loss, it remains the case that bilateral cochlear implantation is inferior to normal hearing when it comes to accuracy of localization and speech-in-noise understanding, without a clear source for the majority of performance outcome variability. To our knowledge, there has not been a granular analysis of the compensatory mechanisms for optimizing sound localization and speech-in-noise performance in patients with bilateral cochlear implants. To gain insight into these adaptive behaviors, we used a novel testing paradigm that simultaneously tests speech-in-noise perception and localization while tracking head movements in a 360o-configuration and real-time ear-level acoustic cues to monitor ear-level signal-to-noise optimization. The objective of this study was to quantitatively and objectively study the listening behavior of individuals in this population to gain a deeper understanding of the adaptive behaviors used to improve performance on tasks of binaural hearing.

Methods: Subjects with normal hearing or bilateral cochlear implants were tested in a dark, semi-anechoic chamber equipped with a 24-speaker array equally-spaced in a 360-degree configuration. On each trial, an orienting stimulus followed by a Harvard IEEE sentence was presented from one of 12 randomly selected target speakers in a background of pink noise at seven different signal-to-noise ratio levels from -10 dB to +10 dB. Participants were instructed to behave naturally and encouraged to move their heads freely to localize sound and their task was to repeat the sentences and report sound source location. Head movement was continuously tracked using an electromagnetic head-p 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 (movement delay in ms, absolute displacement in degrees, and response time in ms), localization accuracy (in root-mean-square error and linear best-fit characteristics across targets), and speech-in-noise performance (percent correct as a function of signal-to-noise ratio) between subject groups. Furthermore, we will quantify ear-specific acoustic input, including ear-level signal-to-noise ratios.

Conclusions: Our study provides a detailed account of sound localization and speech-in-noise perception in bilateral cochlear implant recipients, including unique compensatory listening behaviors and acoustic cue adjustments. This study uncovers some of the subtle strategies and challenges faced by bilateral cochlear implant users in localizing sound and comprehending speech in noise. Data presented here will inform the ultimate goal of computing metrics of binaural hearing to better clinically characterize binaural listening impairment and more accurately assess outcome benefit for bilateral cochlear implantation.

SU165. Comparison of Vowel Fusion Percepts With Headphones and Loudspeakers

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Category: Binaural Hearing and Sound Localization

Background: The ability of a listener to understand speech in noisy listening environments can be impaired by abnormal binaural fusion—the integration of two or more sounds presented to both ears into a single perceived object. Listeners with hearing loss experience abnormally broad binaural fusion of sounds, spanning 1-4 octaves in pitch, which is associated with difficulties understanding speech in noise (Reiss et al., 2017; Oh et al., 2019). To better understand the processes underlying binaural fusion, this project seeks to develop a method of measuring binaural fusion in an animal model. One method we can utilize is a dichotic vowel perception task, shown to provide a measure of vowel fusion via the detection of vowel percept averaging (Reiss and Molis, 2021). Previous studies of vowel fusion used headphones, and the objective of this study is to determine whether the vowel fusion percept is altered under free-field playback, i.e., via loudspeakers. Headphones can dampen environmental sounds and help improve auditory perception, however, they produce less natural acoustic environments and are difficult to adapt to animal studies. Validation of the Reiss and

Molis findings with loudspeakers in humans will allow application of a similar vowel perception task for an animal model. We hypothesize that there is no difference in vowel perception between playback through headphones and loudspeakers.
Methods: Vowel fusion perception was measured in four normal hearing (NH) listeners. Synthetic vowels with different fundamental frequencies (F0) were used, and each set contained seven vowels at each F0. The stimuli were presented through two headphones, or two loudspeakers at ±60°. Subjects were trained using diotic single-vowel presentations of the seven vowels at different F0s. During the main task, subjects were presented with dichotic single vowels—double vowels interleaved with diotic vowels. . During testing, only four vowels were presented, but all seven trained vowels were available as response options. The subjects were asked to indicate which one or two vowel(s) were heard. A confusion matrix was constructed to contrast the stimuli presented versus the responses made by the subjects, which could include single as well as double-vowel responses.
Results: Preliminary findings suggest that there is little within-subject variability between the confusion matrices for the single vowel fusion percepts measured with the two systems. However, there was a fair amount of inter-subject variability that was often greater than the within-subject variability between playback systems.
Conclusions: Comparison of vowel fusion between headphones and free-field playback suggests that subjects experience similar vowel fusion regardless of the apparatus used. This consistent effect could mean that similar vowel fusions can be expected for animal models presented with vowel stimuli via loudspeakers. Work was funded by NIH/NIDCD R01 DC013307.

SU166. Patient-Derived Organoids Modelling Human TMPRSS3-Related Hearing Loss

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Category: Development: Human Subjects
Background: The Transmembrane serine protease 3 (TMPRSS3) gene is in the top 5 of inherited mutations associated with hearing loss which involves the degeneration and death of hair cells in the inner ear. Organoids serve as invaluable tools in modeling genetic hearing loss by replicating the complexity and cellular interactions of specific parts of the inner ear affected by the mutations. Through the use of induced pluripotent stem cells (iPSCs) derived from patients with genetic disorders, organoids can be generated to mimic the patient's unique genetic background. Researchers can then induce the differentiation of these iPSCs into the relevant cell types of the inner ear organoid, allowing for the study of disease-specific cellular and molecular mechanisms.
Methods: A patient diagnosed with TMPRSS3 autosomal recessive variant mutation was admitted for cochlear implant (CI). During surgery for the CI, fibroblasts isolated from a patient were collected and reprogramed to become iPSCs. The generated iPSC colonies were isolated, passaged and checked for pluripotency markers expression before proceeding. Organoids from the commercial human embryonic stem cell line H9 were also generated as a control group. Differentiation into otic organoids was initiated by timely supplementation of Wnt agonist followed by SHH agonist and Wnt antagonist and kept for maturation until day 200. The organoids were observed using light microscopy and analyzed after being fixed, sectioned and stained for hair cell markers as well as apoptotic and ion channels markers using immunohistochemistry.
Results: The fibroblasts isolated from the patient were successfully reprogrammed into iPSCs and confirmed by various pluripotency markers. Spheroids were also formed using the mutant iPSCs with similar efficiency to the control. Immunostaining of mutant organoids at different time points shows the normal formation of hair cells with functional mechanotransduction channels. Reduced expression of BK channels was observed in the maturing mutant organoids compared to the control.
Conclusions: This study enables investigations into how genetic mutations contribute to the progression of hearing loss and possibly provides a model of response to potential treatments, ultimately facilitating the development of targeted therapies and personalized medicine approaches for patients with TMPRSS3-related hearing loss.

SU167. Changes in Subjective Verticality Under Optokinetic Flows and Related Pupil and Cortical Responses: Focusing on the Impact of Cognitive Impairment

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Category: Multisensory Processing/Interactions

Background: As the world's population increases in age, the chance of visual, vestibular, or cognitive functional impairment increases. As a result, the elderly people should endeavour to integrate such defective multiple sensory inputs by consuming more cognitive resources for increased communication among related cortical and subcortical areas. The pupil response has been introduced to vestibular research as a surrogate marker for required cognitive demand in such multisensory signal processing. So, there are demands to see the differences in cognitive demand and cortical response for cognitively impaired patients in integrating conflicting visual-vestibular inputs.

This study aimed to evaluate differences in the accuracy of internal estimates and pupil responses between normal and cognitively impaired group in integrating conflicting optokinetic visual inputs into the otolith signals in perceiving subjective verticality and to find correlated cortical responses

Methods: The pupil changes and cortical responses from electroencephalogram were taken from normal and cognitively impaired participants in performing subjective visual vertical (SVV) under rotational optokinetic flows in roll plane. The visual stimuli and SVV test procedure were implemented in the virtual reality interface. The pupil changes and error angles of SVV were compared between groups and cortical responses were also analyzed. Various correlations were calculated among error angles, pupil changes, and quantified cognitive function.

Results: Error angles were significantly increased under rotational optic flows for both groups and the cognitively impaired group showed significantly larger error angles than normal group ($p=0.014$*). Pupil size was increased in all trials by 0.48 to 0.80 mm compared with baselines. The cognitively impaired group showed significantly larger pupil changes than normal group ($p=0.009$*). Significant correlations between the error angles and the pupil changes for both groups ($p=0.021$*). A significant correlation was also found between pupil changes and K-MMSE in cognitively impaired group ($p=0.040$*). Significantly higher cortical activations were found in occipital lobe and frontal lobes during the tasks and significant correlations were found with pupil changes especially for beta and gamma band responses on occipital lobe.

Conclusions: The cognitively impaired group showed larger error angles and pupil changes, which suggests that they require higher cognitive demands to integrate conflicting optokinetic flows into the otolith signal yielding erroneous internal estimates of verticality. Higher cortical activity was found in occipital lobe suggesting activation of visual cortex with reciprocal inhibition of candidate vestibular cortices.

SU168. Unveiling the Neural Circuitry of Salience: Rough Sounds Processing in Mice

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Category: Multisensory Processing/Interactions

Background: Baby cries, city alarms and screams share a common property that confer sounds a rough sonic texture inducing unpleasant sensations and increasing attention in humans. Recently, it has been shown that human scream exploits a privileged acoustic niche called "roughness" characterized by amplitude modulations of the temporal envelope of sounds between 30 Hertz (Hz) and 150 Hz. Behavioral studies have demonstrated that sound aversiveness follows a non-linear profile and, in humans, is maximal in the roughness range. Subjective aversiveness is not only explained by responses in the classical auditory system but may target non-primary auditory regions situated in deep temporal and limbic structures involved in emotional responses that may belong to the salience network. However, the recruitment of non-canonical auditory pathways in the processing of rough sounds remains still unknown. As distress vocalizations play a major role in

sociobiological communication across many species, we aim at investigating how such sounds are processed in mice.
Methods: To answer this, we studied mice's behavior in presence of rough sounds. We also probed the circuits activated by amplitude-modulated sounds using c-fos, an immediate early-gene (marker of neural activity).
Results: We studied mice's behavior in presence of rough sounds, and we showed that mice are sensitive to amplitude-modulated sounds and present the same non-linearity in the roughness range as humans. Probing the circuits activated by amplitude-modulated sounds using c-fos, an immediate early-gene (marker of neural activity), we showed that rough sounds specifically activate cortical, subcortical, and limbic areas.
Conclusions: Our results are compatible with the hypothesis of the recruitment of a salience network involved in aversion processing and exogenous attention. This is particularly encouraging as it supports the idea that these circuits are ancient and conserved and reinforces the validity of the mouse model to further explore these circuits in comparison to humans.

SU169. Musical Groove Listening Does Not Enhance Primary Motor Cortex Activation

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Category: Multisensory Processing/Interactions
Background: Groove is the pleasurable urge to move to the beat of music, a universally experienced human phenomenon. Motor networks have been shown to be engaged during active music listening. This auditory-motor linking is important in motor preparation and planning. It is yet unknown, however, if musical groove causes engagement of the primary motor cortex during music listening. The predominant role of the primary motor cortex is to execute movement. Because listeners are often inspired to move to music with groove, we hypothesized greater engagement of the primary motor cortex during musical groove listening. The present study represents a novel approach to studying groove using electroencephalography (EEG) to see how high-groove music may enhance sensorimotor processing. Specifically, we asked whether high-groove music, as defined by Janata et al. (2012), would promote greater motor system activity than low-groove music, noise, or silence, as shown by faster latency and/or higher amplitude of the lateralized readiness potential (LRP), an event-related potential measure of neural response preparation and execution in the primary motor cortex.
Methods: In Experiment 1, 21 participants were presented with one of two symbols (@ and #) on a computer monitor and pressed a button with their left or right hand, depending on which symbol was presented, while listening to high-groove music, low-groove music, noise, or silence. Throughout the duration of the experiment, EEG was recorded at the scalp and we calculated stimulus-locked and response-locked event-related potentials (ERP) by averaging across correctly responded, non-artifact trials. The LRP was calculated for each participant as the difference ERP between electrodes C3 and C4. Latencies and amplitudes of the LRPs were calculated and entered into four one-way Bayesian ANOVAs with sound condition (high-groove music, low-groove music, noise, or silence) as the lone factor. In Experiment 2, 32 participants were tested with the same stimuli, procedure, and analysis, with the following exception: instead of responding to visual stimuli, participants pressed a button with their left or right hand, depending on whether they heard an occasional increase or decrease in volume in the music or noise.
Results: None of the analyses in either experiment showed differences in LRP latency or amplitude due to differences between the high-groove or low-groove conditions, as measured by BF10 values in favor of the alternative hypothesis. Several of the ANOVAs yielded BF01 values greater than 3 in favor of the null hypothesis.
Conclusions: We found no convincing evidence that high-groove music enhances activity in the primary motor cortex. Future studies should use other brain activity measurement and manipulation techniques to conceptually replicate our findings, including techniques that can separately measure different motor and auditory cortical areas.

SU170. Cross-Modal Stimulation in Changing Hearing Sensitivity and Tinnitus in Humans

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Category: Multisensory Processing/Interactions

Background: Cross-modal stimulation can drive neural plasticity across the auditory pathway that can be leveraged for rehabilitative purposes. One example is the application of paired sound and somatosensory or autonomic stimulation in alleviating tinnitus. Multiple studies in animals and humans have demonstrated that electrical stimulation of certain body regions or nerves (e.g., the surface of the tongue or neck, or trigeminal or vagus nerve) paired with sound stimulation can reduce tinnitus symptoms. Yet, there is still a scarcity of human data on the ability to alter hearing sensitivity or tinnitus perception through paired stimulation involving electrical stimulation of the ear. Here, we developed a cross-modal stimulation paradigm that delivered paired sound and electrical stimulation on the cymba concha and investigated the effects of this paradigm on hearing sensitivity and tinnitus perception.

Methods: Participants with various hearing profiles were recruited into the study, with and without tinnitus. Five participants have completed the study (three with tinnitus). All participants were given 30 minutes of paired stimulation (PS) on five separate visits. Each PS trial consisted of a 25-Hz burst of 6 electrical pulses delivered to the left cymba concha and a target 250-ms bilateral sound stimulus (a 6 kHz tone, narrowband noise centered at 6 kHz, or notched white noise, approximately 25 dB SL above thresholds of relevant frequencies). A total of 600 PS trials (⅓ burst/s) were delivered during a PS session. A calming background sound of string instruments was consistently presented during the PS sessions. Individual perceptual thresholds of electrical stimulation were obtained before every PS session. The electrical pulses were delivered at 110% of the perceptual threshold. To assess possible behavioral and electrophysiological changes, a test battery of audiograms, questionnaires, auditory brainstem responses, and the cortical auditory evoked potentials was used before and after the 5 PS sessions. For tinnitus participants, tinnitus loudness and frequency were evaluated with custom tests to help determine the location of the tinnitus notch in the white noise stimulus.

Results: No significant changes in the post-stimulation audiograms have been observed in the preliminary data. All tinnitus participants reported a temporary reduction or even complete suppression in tinnitus after the last session, which was confirmed by a reduction in the matched tinnitus loudness level. Ongoing data collection and analysis will fully assess the pilot observations and reveal any possible changes in the electrophysiological responses.

Conclusions: Preliminary results suggest that the paired sound and electrical stimulation on the ears have the potential to reduce tinnitus. Improvements and customization in the current paradigm per participant are needed to induce sufficient plasticity that can be reflected in fundamental hearing capabilities. An effective stimulation paradigm can lead to new techniques for improving hearing health.

SU171. Otoacoustic Test of Middle Ear Transmission Changes Associated With Increased Intracranial Pressure

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Category: Otoacoustic Emissions

Background: Otoacoustic emissions (OAEs) have been proposed in order to get noninvasive estimates of the intracranial pressure (ICP). The transition from orthostatic to clinostatic body posture is associated to an increase of the ICP, and this is reflected by the increase of the OAE phase. An ICP increase is also expected to occur in microgravity. The extent and timescale of the ICP recovery to normal values during long-term exposure to microgravity are not known. In a few astronauts, some vision symptoms were observed, defined as the spaceflight associated neuro-ocular syndrome (SANS).

It was demonstrated by Avan and coworkers that the physical quantity straightforwardly affected by ICP variation is the OAE phase difference. They hypothesized that the increase of the ICP would cause an increase of the middle ear stiffness. Therefore, the imaginary part of the load impedance measured in the ear canal would be shifted downward. As a more indirect consequence, the middle ear energy reflectance would also

increase in the low-frequency range. Reactance and reflectance are related to the OAE phase change through relations involving ratios of complex numbers, each step adding statistical uncertainty and introducing systematic errors.

In this study, we report and discuss measurements of OAE phase variation associated with postural changes and with exposure to microgravity conditions on the International Spatial Station (ISS). As well as showing the phase variation of the DPOAE short latency component, we also show how the ICP increase affects the middle ear impedance.

Methods: The experimental chain sensitivity to ICP changes induced by postural changes was assessed by means of on ground experimental session on healthy volunteers changing the position from vertical to horizontal. High resolution DPOAE spectra of five astronauts involved in a long-term spatial mission on the ISS were also acquired, before, during and after the flight, using an ER10B+ probe. A time frequency wavelet transform based algorithm was applied in order to select the zero latency component phase. The ICP variation was estimates from the phase difference. The preliminary Thevenin calibration of the ER2 allowed us to measure the complex load impedance, essentially representing the middle ear impedance.
Results: A statistically significant increase of the ICP was estimated both in the clinostatic condition and during the exposure to microgravity. Large inter-subject variability was observed among subjects, and poor reproducibility in some subjects due to unsatisfactory fitting of the probe in the ear canal. The study of the load impedance confirms that the DPOAE phase change is due to increased middle ear stiffness.
Conclusions: The DPOAE phase variation offers a reliable indirect estimate of the ICP variation. The hypothesis that the OAE phase variation should be mainly due to the middle ear stiffness increase is confirmed.

SU172. Monitoring the Course of Endolymphatic Hydrops With a Joint Reflection-Distortion OAE Profile

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Category: Otoacoustic Emissions
Background: Endolymphatic hydrops (EH) is a pathology involving an increased volume of endolymph in the cochlear duct. The ensuing alterations in cochlear morphology can cause disruptions in cochlear mechanics, including the generation of otoacoustic emissions (OAEs). Hence, OAEs may be an effective tool for diagnosing and monitoring the course of EH in humans. Here, in a handful of individuals with EH, we rapidly and jointly measure the two distinct classes of emissions—OAEs arising from nonlinear distortion and those arising from linear reflection—over the course of one year to better understand their utility and to define the reliability of both emission types in this group.
Methods: A joint OAE profile was generated from the near-simultaneous measurement and relational analysis of distortion-product (DP) OAEs and stimulus-frequency (SF) OAEs. OAEs were recorded longitudinally (approximately every other month for one year) in ears with EH and in disease-free ears with normal hearing. Both OAEs were evoked with tones swept across five octaves and 10-12 stimulus levels at one octave per second. Stimulus level was calibrated in forward-pressure level and OAE level was corrected to emitted-pressure level. In this preliminary report: (1) we examine whether previously documented effects of EH on DPOAEs and SFOAEs persist across repeated test sessions (with distortion emissions reduced more than reflection emissions), and (2) we define the test-retest reliability of DPOAEs and SFOAEs in individuals with EH compared to normal hearers.
Results: Preliminary results from this longitudinal study show reduced or non-measurable DPOAEs in the region of EH involvement, combined with SFOAE levels that were less reduced or near-normal, most notably at high stimulus levels. Most subjects with EH showed this OAE pattern, but not all. Although SFOAE levels are generally higher than DPOAE levels in normal hearers, most notably at low-to-mid frequencies and high stimulus levels (and this control group was no exception), individuals with EH showed exaggerated differences between the two emission types, most of which can be attributed to selectively decreased DPOAE levels. A preliminary assessment of test-retest reliability showed that subjects with EH had more changes in OAE level from test to test compared to disease-free individuals.
Conclusions: Consistent with a previous report from our group (Stiepan et al., 2023), the results suggest that OAE generation processes are disrupted by the pathophysiology of EH, which may include a stiffening of the

cochlear partition and damage to the outer hair cell bundle. The intracochlear generation and/or transmission of nonlinear-distortion emissions may be more disrupted than that of coherent-reflection emissions. Additionally, OAE measurements are less repeatable in ears with EH compared to normal hearers. Data collection is ongoing.

SU173. Accuracy of the Joint Reflection-Distortion OAE Profile in Detecting Mild Hearing Loss: Preliminary Findings

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Category: Otoacoustic Emissions

Background: Measuring and analyzing both distortion- and reflection-type otoacoustic emissions (OAE) in the same ear yields what we term a "joint-OAE profile". This profile provides a glimpse into cochlear function and dysfunction by accessing two distinct OAE generation mechanisms. We hypothesize that this joint approach will provide improved detection of hearing loss.

Methods: The unmixed distortion-component of the 2f1-f2 DPOAE (a distortion emission) and the stimulus-frequency OAE or SFOAE (a reflection emission) were recorded at 10-12 stimulus levels to create Input/Output (I/O) functions across five octaves with tones swept at 1 octave/second in 300 adult ears: 117 normal hearers and 183 ears with mild hearing loss (HL). Only OAEs corresponding to elevated audiometric thresholds were analyzed. Stimulus level was calibrated in forward pressure level (dB FPL) and OAE levels were converted to emitted pressure level (dB EPL).

Results: A universal OAE profile for mild HL emerged. In ears with mild-moderate HL: (a) OAE levels and "source strength" (i.e., OAE level re: stimulus level at steepest slope) are reduced, more so for distortion vs reflection emissions; (b) the compression "knee" on the I/O function is elevated for distortion OAEs only; and (c) reflection emissions show a steepened slope of growth. These trends occurred for mild-moderately impaired ears regardless of the etiology of hearing loss. It is noteworthy that the two OAEs were differently affected by cochlear damage, suggesting that measuring both together may improve diagnostics.

Using clinical decision theory, Receiver Operating Characteristic (ROC) curves were created for various DPOAE and SFOAE variables and multivariate clusters to assess their performance in classifying ears as normal-hearing or hearing-impaired. The hit rates achieved showed at least equivalent but most often improved performance compared to results reported over the last three decades using discrete-tone OAEs and conventional calibration. Hit rates greater than 0.90 (allowing for 10% false positives) were achieved from 0.75 to 12 kHz. Improvement (re: past work) was most evident in the low-to-mid-frequencies where the addition of the SFOAE was impactful. OAE amplitude at low stimulus levels, source strength, compression knee, and maximum slope derived from OAE I/O functions were the best predictors (and most powerful when combined). The methodological factors contributing to these improvements were explored.

Conclusions: The joint-OAE profile showed improved performance in the detection of mild hearing loss. It is particularly noteworthy that this cohort with hearing loss included the most difficult-to-detect (and most-often missed) impairments, i.e., slight-to-mild hearing loss. Mean dB HL for this group ranged from 29 to 38 dB HL across frequency. Overall, findings indicate that the swept-tone joint-OAE profile, including nearly simultaneous measurement of distortion- and reflection-type emissions, combined with advanced calibration provides significant improvements in the detection of slight-to-moderate hearing loss.

SU174. Enhanced Otoacoustic Emissions in College Musicians: Role Training Recency and Noise Exposure

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Category: Otoacoustic Emissions

Background: Otoacoustic emissions (OAEs) are a non-invasive method of measuring cochlear function. Studies of OAEs in musicians have yielded varying results, ranging from evidence of diminished OAEs in musicians, suggesting noise-induced cochlear hearing loss, to no difference when compared to non-musicians or even a trend for stronger OAEs in musicians. The goal of this study was to use a large sample of college students with normal hearing to compare OAEs in musicians and non-musicians and examine the potential effects of noise exposure and training recency on OAEs.
Methods: All participants (n=160) completed a survey about recent noise exposure (previous 12 months), and a subset of participants (71 musicians and 15 non-musicians) wore a personal noise dosimeter for one week to obtain a more nuanced and objective measure of exposure. The purpose of these noise measurements was to assess how different exposure levels may affect OAEs before the emergence of a clinically significant hearing loss. OAEs were tested using both transient-evoked OAEs (TEOAEs) and distortion-product OAEs (DPOAEs).
Results: As predicted from the literature, musicians experienced significantly higher noise levels than non-musicians based on both subjective and objective measures. Yet, despite higher noise levels, we observed stronger TEOAEs and DPOAEs in musicians compared to non-musicians in the 1-5 kHz range. Among musicians, active musicians and inactive musicians had similar OAE amplitudes and SNRs.
Conclusions: Our findings suggest an enhancement of cochlear function in young adult musicians that may not require active, ongoing musical practice and is not strongly undermined by recent noise exposure. Further studies are needed to determine if musicians are protected against damage caused by noise exposure.

SU175. Contributions to Otoacoustic Emission Amplitudes Beyond Outer Hair Cells: Effects of Sedation and Inner Hair Cell Dysfunction

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Category: Otoacoustic Emissions
Background: Otoacoustic emissions (OAEs) are commonly used in clinical and research settings to screen for hearing loss and to monitor for changes in hearing status. OAEs are often interpreted as a measure of outer hair cell (OHC) function, but other peripheral factors also influence OAE amplitudes. Small but consistent amplitude increases in distortion product otoacoustic emissions (DPOAEs) and stimulus frequency otoacoustic emissions (SFOAEs) in chinchillas with selective inner hair cell (IHC) damage induced by carboplatin have been seen in our lab and others. We hypothesize that increased OAE amplitudes secondary to carboplatin primarily result from decreased afferent input to the medial olivocochlear (MOC) efferent fibers that control OHC gain. In humans, these non-OHC contributions may explain the significant variability in OAE amplitudes among adults with normal hearing thresholds.
Methods: When using traditional OAE calibration techniques to determine stimulus levels, OAE amplitudes are highly susceptible to variation in probe placement and individual differences in ear canal acoustics (Charaziak and Shera, 2017, JASA). Since we aimed to investigate small changes in OAE amplitudes beyond OHC involvement, we utilized improved calibration methods that enhance the reliability of the measures--forward pressure level (FPL) and emitted pressure level (EPL). Swept DPOAEs and SFOAEs were recorded from 0.5-16 kHz in young, unexposed chinchillas with normal hearing sensitivity in two conditions: awake and under xylazine/ketamine sedation. The same measurements were made in chinchillas before and after exposure to carboplatin.
Results: Preliminary results suggest that sedation and carboplatin exposure both increase DPOAE and SFOAE amplitudes. Changes to amplitudes are frequency-dependent. These results are consistent with a reduction in the strength of the MOC reflex mediated by (1) anesthesia and (2) reduced primary afferent input following selective IHC and IHC stereocilia damage. In both cases, reduced activity of the MOCR effectively disinhibits the OHC response. Future analyses will explore the frequency dependence and compare changes to distortion and reflection components of the OAEs.
Conclusions: Though OAEs are classically viewed as being dominated by OHC function, the potential effects of changes to both afferent and efferent pathways are observable when using suitable calibration. Thus, a broader perspective on the factors influencing OAE amplitudes is warranted to refine the interpretation and enhance the diagnostic utility of OAEs when applied to studies of sensorineural hearing loss.

SU176. Components Identified in SFOAEs Derived From a Nonlinear Cochlear Model Composed of Fluid-Coupled Oscillators

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Category: Otoacoustic Emissions

Background: Stimulus-frequency otoacoustic emissions (SFOAEs), i.e., OAEs evoked with a single pure tone, are assumed to be generated due to linear reflection of forward-traveling waves from mechanical irregularities in the cochlea. Theoretical studies indicated that in addition to mechanical irregularities, additional tones used to extract SFOAEs during their measurement may perturb the impedance of the basilar membrane (BM) and generate a new component of SFOAEs. In addition to this, by using an analytical solution for SFOAEs in a nonlinear cochlear model, we showed that backscattered wavelets may also perturb the BM impedance and create an additional component with long latency.

Methods: The analytical solution for SFOAEs in the cochlear model is derived for either "true" SFOAEs obtained by vector subtraction of the response from the model with irregularities and from the smooth model, or for SFOAEs simulated with the method of suppression. To minimize the interaction between backscattered wavelets, a single irregularity with a Gaussian shape is added to the feedback force. To find the analytical solution, we subtracted the linear part of the feedback force in the model and isolated the effect of nonlinearity in the model into a single term which we call the nonlinear force.

Results: In the true SFOAEs, we can identify two main components of SFOAEs: a component due to linear reflection and a component due to backscattered wavelets which perturb the nonlinear force. The component due to linear reflection grows approximately linearly with tone intensity at the lowest intensities (less than about 25 dB), but the component due to perturbation of the nonlinear force has a cubic growth at these intensities, and the opposite phase than the component due to reflection. A Taylor expansion of the nonlinearity explained the reason for the cubic growth and its change when the operating point in the nonlinear function was altered. A suppressor presented simultaneously with the probe tone perturbed the nonlinear force, which generated a new SFOAE component. For the suppressor 2.1 octaves above the probe tone, the suppressor component dominated the entire SFOAEs and grew approximately linearly with the probe tone level.

Conclusions: The analytical solution of the model allowed for the identification of SFOAE components due to nonlinearities. One component is due to the perturbation of the nonlinear force by backscattered wavelets. This component has an opposite phase to the reflection component and a long latency. The other component is due to perturbation of the nonlinear force by a suppressor. This component is dominant if the suppressor has a much higher frequency than the probe tone (e.g. 2.1 oct. above the probe tone), grows linearly with the probe tone intensity, and has a short latency.

SU177. Perception of USVs by the BTBR Mouse Model of Autism

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Category: Psychoacoustics

Background: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder that is characterized by social communication deficits and stereotyped, repetitive behaviors. Several researchers over the years have indicated hearing abnormalities in children with ASD, but it is not clear if these abnormalities contribute to communication deficits. Researchers have utilized several mouse models of autism to study communication deficits as these mouse models produce abnormal ultrasonic vocalizations (USVs). However, very few studies have investigated the hearing abilities of these mouse models, and none have looked at the perception of USVs, which are thought to be used for communication, in these mice.

Methods: We trained BTBR and control C57BL/6 mice using an operant conditioning paradigm with positive reinforcement on either a detection task or a discrimination task. Each mouse only participated in one experiment, but within that experiment, each mouse completed every condition. We collected absolute thresholds for four USVs (chevron, upsweep, downsweep, and complex) in quiet or in a noisy background. Absolute thresholds for each USV were compared across strain and sex. We also tested whether the mice

could discriminate between different categories of USVs and between USVs produced by different strains of mice.
Results: USV thresholds from BTBR mice are elevated compared to those from other strains of mice. However, they tend to show similarities to other strains for sex and background condition. Female BTBR mice appear to have slightly lower (better) thresholds compared to male BTBR mice. BTBR mice show some elevation in USV in noise thresholds compared to USV in quiet thresholds. Additionally, BTBR mice are able to discriminate between different USV categories, similar to previously published work in CBA/CaJ mice (Screven and Dent, 2019). The BTBR mice are also capable of discriminating between the same category of USV produced by different mouse strains, but not between USVs produced by two mice of the same strain.
Conclusions: These are the first experiments conducted in awake and behaving BTBR mice to look at the perception of natural USVs. Individuals with ASD tend to struggle with speech perception and especially speech in noise detection compared to typically developing individuals. Currently, it appears that the BTBR mouse model of autism shows similar behavior as humans with ASD. However, more work needs to be done to understand the potential underlying mechanisms of these perceptual behaviors.

SU178. Adaptation to Sentences and Melodies When Making Judgments Along a Voice/Non-Voice Continuum

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Category: Psychoacoustics
Background: Perceptual contrastive adaptation effects can be used to identify important perceptual features or categories. Our previous work revealed contrastive adaptation effects between voice and non-voice categories, where ambiguous sounds following repetitive presentation of musical instrument tones tend to be perceived as voice, and vice versa. The current study investigated whether the effect generalizes to adaptors with higher ecological validity, namely sentences and melodies.
Methods: Forty participants (age range: 18-40 yrs, mean = 21.8 yrs) completed the study online. In each trial of the experiment, participants listened to an adaptor, followed by an ambiguous test stimulus. Participants categorized the test stimulus as either "voice" or "instrument" by pressing a key. To create the test stimuli, 10-step continua between a "voice" (female /a/, /o/, or /u/ vowels) and an "instrument" (bassoon, horn, or viola) were generated for each possible pair. The adaptor stimuli were sentences spoken by female and male voices, and melodies with matching pitch range and duration played on bassoon and French horn. Adaptor type (voice vs. instrument) was tested as a within-subject variable, and adaptor voice gender (female vs. male) was tested as a between-subject variable. To quantify each participant's perception of the test stimuli, the point of subjective equality (PSE) between voice and instrument was calculated by fitting the percentage of "voice" responses as a function of continuum step with a logistic function.
Results: A mixed-effects ANOVA was performed to examine the effects of adaptor type and voice gender on PSE. A significant main effect of adaptor type was observed, showing that the test stimulus was more likely to be identified as a voice following a musical melody and vice versa. No significant main effect of voice gender or interaction was observed, suggesting the effect was comparable regardless of whether the adaptors and test stimuli share the same speaker gender or F0 range.
Conclusions: Contrastive adaptation to voice and non-voice stimuli is a robust effect that does not rely on the repetition of simple adaptors. The effect can generalize across speaker gender and fundamental frequency range, suggesting broad applicability and potential relevance to everyday perception. [Work supported by NIH grant R01DC005216.]

SU179. Impact of Reduced Spectral Resolution on Temporal-Coherence-Based Source Segregation

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Category: Psychoacoustics

Background: Hearing-impaired listeners struggle to understand speech in background noise, even when using state-of-the-art cochlear implants (CIs) or hearing aids. Successful listening in such environments depends on the brain's ability to organize a mixture of sound sources into distinct perceptual streams (i.e., source segregation). In normal-hearing listeners, temporal coherence of sound fluctuations across frequency channels supports this process by promoting the perceptual grouping of sound elements belonging to a single acoustic source. We hypothesized that reduced spectral resolution in CI/electric hearing (from current spread) and in acoustic hearing with sensorineural hearing loss (from broadened tuning) degrades segregation based on temporal coherence, in turn impacting speech understanding in noise. This is because the likelihood that a single sound source dominates the activity driving any specific frequency channel decreases as the frequency resolution of that cochlear channel decreases; concomitantly, the correlation in activity across different channels increases.
Methods: To test our hypothesis, we used a combination of online behavioral experiments and physiologically plausible computational modeling of across-channel temporal-coherence-based segregation (Viswanathan et al., JNeurosci, 2022). We compared model predictions of comodulation masking release (CMR; a correlate of across-channel temporal-coherence processing) and speech intelligibility in noise as a function of CI current spread to behavioral measurements with simulated CI listening (N=20 for CMR; N=48 for speech in noise). We also obtained predictions for CMR as a function of degree of simulated outer-hair-cell (OHC) dysfunction.
Results: Consistent with our hypothesis, model predictions suggest that CI current spread reduces CMR and speech-in-noise outcomes. Furthermore, these model predictions were shown to be consistent with our behavioral data with simulated CI listening. Finally, our model also predicts that reduced spectral resolution due to OHC dysfunction degrades important cues for source segregation.
Conclusions: Together, these results suggest that reduced spectral resolution relative to normal hearing impairs temporal-coherence-based source segregation. This impaired source segregation may in turn contribute to speech-in-noise deficits in hearing-impaired populations.

SU180. The Impact of Inharmonicity on Voice Denumerability and Discrimination in Polyphonic Music

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Category: Psychoacoustics
Background: Although the perception of speech in the presence of other talkers has received considerable attention in the auditory research literature, the perception of polyphonic music has received much less. Source segregation is often thought to be facilitated by pitch and harmonicity. We tested this hypothesis in the context of music by studying the perception of sung polyphonic music while manipulating the harmonicity of the constituent voices. We predicted that harmonicity within individual voices would be more important for music than speech.
Methods: Vocal passages were generated from four-second MIDI excerpts of music scores found on MuseScore, using female vocal recordings. Inharmonic sounds were created by introducing a unique random deviation ('jitter') to the first 30 harmonics of each tone between 10% and 30% of the fundamental frequency (F0).
Two experiments were run. Experiment 1 involved presenting listeners with short passages of between 1 and 5 simultaneous voices. The listeners' task was to estimate the number of voices in each passage. Experiment 2 required participants to actively follow one of the voices within the same polyphonic passages and to report whether a probe tone presented after the passage corresponded to the pitch of the last note in the attended voice. In both experiments, the degree of inharmonicity of the voices was varied parametrically.
Results: Experiment 1 revealed a significant effect of both inharmonicity level and the number of voices on performance, indicating that the denumerability of voices becomes more difficult as the number of voices increases and the degree of inharmonicity increases. Experiment 2 also showed a dependence on performance on both the number of voices and the degree of inharmonicity, as well as an interaction indicating that the impact of inharmonicity on performance varied depending on the number of voices present. In both experiments, average performance remained above chance in the inharmonic conditions.
Conclusions: Introducing inharmonicity to the individual voices within polyphonic music resulted in poorer performance in both voice denumerability and the ability to hear out individual voices within the mixture.

However, despite the negative effect of inharmonicity, as in comparable work on speech perception, inharmonicity did not make either task impossible. Thus, it seems that even in music, where pitch is thought to be a much more critical cue than in speech, the introduction of inharmonicity does not lead to a catastrophic degradation in our ability to hear out competing simultaneous voices.
[Supported by NIH grant R01DC005216.]

SU181. Behavioural and Electrophysiological Measures of Hearing in the Abnormally Wobbly Gait (AWG) Mutant Mouse Strain

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Category: Psychoacoustics
Background: A spontaneous, recessive mutation recently discovered in C57BL/6J laboratory mice (Mus musculus) resulted in offspring with an "Abnormally Wobbly Gait" (AWG) phenotype. The AWG phenotype—observable by postnatal day (PND) 15—affects males and females equally and results in hyperactivity, continuous circling, and head-tossing behaviour. Despite abnormal locomotion, AWG mice are able to self-regulate: they can feed, drink and mate with other mice. Based on their phenotypes, AWG mice were suspected to have problems with the limbic system and possible hearing loss.
Methods: We measured and compared hearing thresholds and acoustically-evoked behaviour using auditory brainstem response (ABR) and acoustic startle response (ASR) recordings for AWG (AWG; n = 15) wild-type littermate control (WT; n = 9), heterozygous littermate control (HET; n = 11) and C57BL/6J control mice from a different source (C57; n = 12). We measured ASRs by placing mice in a foam-lined arena and presenting a loud burst of white noise (107 dB SPL at 10 cm, 200 ms duration) three times for each subject. Several raters (n = 19) watched video recordings of ASR trials and rated their confidence of startle events on a Likert scale (1– 4). Intraclass correlations (ICCs) were used to compare ASR scores between raters. We measured ABR hearing thresholds by placing subdermal electrodes near the mouse's auditory bullae. Characteristic ABR electrophysiological waveforms were recorded and averaged over repeated (n = 512) acoustic stimulation with tone bursts (frequencies: 8, 16, and 32 kHz; 5 ms duration) and clicks (0.1 ms). The maximum playback amplitude for tones and clicks was 85 dB SPL. The ABR hearing thresholds were standardized using the Suthakar and Liberman (2019) algorithm.
Results: We found that C57 and WT mice exhibited strong ASR scores (C57 mean = 3.01; WT mean = 3.04) whereas AWG mice often showed no response to the white-noise stimulus (AWG mean = 1.16). Interestingly, HET mice had a weaker ASR score than both WT and C57 mice (HET mean = 2.69). The ASR scores were very consistent across raters (ICC = 0.732; F(119,1042) = 58.6, p less than 0.01). The ABR recordings for C57, WT, and HET mice all showed characteristic waveforms with peaks and troughs for tone bursts and click stimuli. The ABR thresholds for C57 mice (mean +/- standard error: 46 +/- 0.8 dB SPL) were significantly lower than WT (50 +/- 1.5 dB SPL) and HET mice (55 +/- 2.4 dB SPL) in response to click stimulation. Most AWG mice did not exhibit a clear ABR waveform with a detectable threshold at 85 dB SPL.
Conclusions: Overall, the results suggest that AWG mice do not show an ASR because they have a profound hearing loss (i.e. with no ABR evoked) compared to WT, HET and C57 control mice.

SU182. Individual Differences in Vowel Spectral Contrast and Auditory Enhancement Effects

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Category: Psychoacoustics
Background: The perception of tones and speech can be influenced by neighboring sounds as broadly captured by auditory context effects. An example of this effect is auditory enhancement, observed when an individual target component within a masking complex tone becomes perceptually more prominent when the masker-target combination is preceded by a copy of the masker, leading to lower detection thresholds of the target. Context effects can also influence the perception of speech sounds. For instance, the identity of a vowel

can be influenced by the spectral content of the preceding sentence. Both auditory enhancement and vowel spectral contrast effects suggest the existence of a perceptual process that favors new acoustic events over ongoing energy, yet it remains unclear whether the same neural mechanisms are responsible for both. This study tested the hypothesis that both effects reflect the same underlying mechanisms by determining whether individual differences in performance are correlated between the two tasks.
Methods: Thirty normal-hearing native speakers of American English completed an auditory enhancement task and a vowel categorical boundary task in counterbalanced order. Vowel contrast effects were measured by the perceptual shift in the categorical boundary of a vowel following a precursor sentence that was filtered to spectrally enhance the envelope of either vowel. Auditory enhancement was measured as the difference between detection thresholds of a target paired with a masker when preceded by either no precursor, or with the masker alone.
Results: Consistent with existing findings, significant context effects were observed both in speech and non-speech. The average auditory enhancement effect was over 17dB. For the vowel identification task, the spectrally contrastive precursor sentence generated an average perceptual shift in the categorical boundary of nearly 3 steps along a 10-step vowel continuum. Although substantial inter-individual variability in performance was found in both tasks, no significant correlation was found between the size of the context effects in the two tasks.
Conclusions: Although robust enhancement and vowel spectral contrast effects were observed, the lack of correlation between the two effects does not provide support for the hypothesis that the two effects reflect the same underlying mechanisms. However, it remains possible that more similar speech and non-speech stimuli may be processed in more similar ways. Currently ongoing studies will test vowel discrimination within a paradigm more similar to classic auditory enhancement. The experiment assessed the relationship between context effects by examining perceptual shifts in vowel boundaries either with or without a spectrally enhanced precursor sentence, to the detection threshold difference between a target that was or was not preceded by a masker. The significant effects observed were highly variable, but not correlated with one another, contrary to the prediction that both effects rely on the same underlying mechanisms.

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SU183. Incidental Learning Enhances Auditory Sensitivity in Real-World Soundscapes

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Category: Psychoacoustics
Background: Can memory for a context, such as a familiar auditory environment, enhance sensitivity to a sound that is present in that environment? Here we asked that question by first exposing listeners to soundscapes, half of which had a brief pure tone embedded within them. They were then tested on their ability to detect faint tones in these soundscapes to determine whether sensitivity was higher for tones presented in soundscapes experienced previously as having embedded tones, compared to those experienced without tones.
Methods: In each of four learning blocks, participants (N = 123; 85 EEG) heard 40 memory-cue (a particular soundscape with an embedded lateralized pure tone) and 40 neutral-cue (soundscape alone) trials. The soundscape-tone pairing in the memory trials were identical, allowing participants to form associations between the soundscape and the location of the tone (left/right). Soundscape assignment to each condition was counterbalanced across participants. To ensure that the associations were formed incidentally, no reference was made to the lateralization of the tone and its relation to the soundscape. At test, every soundscape contained a faint lateralized tone (accuracy = 94%). For memory-cue trials, the tone appeared on the same side as it did during learning. For neutral-cue trials, the tone appeared on a pseudo-randomized side. Participants localized the tones as quickly as possible by pressing a key and indicated whether they had an explicit memory for each soundscape. To index learning-related changes, we measured the acoustic change complex (ACC), using high-density electroencephalography. Elicited when changes to an ongoing stimulus occur, larger ACC amplitudes at tone onset suggest greater neural detectability of that tone within the soundscape at the level of the auditory cortex. Specifically, we tested the hypothesis that learning of tone-soundscape pairs, indexed by ACC amplitudes, is associated with greater auditory sensitivity.

Results: Reaction times for localizing the tone at test were faster for memory-cue trials than for neutral-cue ones, suggesting that the soundscape cued the tone ($p = .039$). For memory-cue trials, greater ACC amplitudes during learning were associated with greater lateralization accuracy at test ($p = .017$), suggesting that the neural salience of the tone embedded in the soundscape at learning is important for auditory sensitivity at test. Further, accuracy was better on trials in which the soundscape was explicitly remembered ($p = .00002$). Together, larger ACC amplitudes may relate to associative memory formation, while explicit memory for the soundscape may support processing efficiency during tone localization.
Conclusions: These results illustrate how auditory experience can enhance auditory sensitivity. Incidental exposure to complex everyday sounds can lead to associative representations that are robust enough to improve auditory perception. This knowledge may contribute to the development of technologies and prostheses to aid people with hearing impairment.

SU184. Syllable-Rate-Adjusted-Modulation (SRAM) Predicts Clear and Conversational Speech Intelligibility

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Category: Other, Speech Intelligibility Prediction
Background: Objectively predicting speech intelligibility is an important area in hearing research. The classic method has relied on signal-to-noise ratios (SNR) to successfully predict speech intelligibility. One exception is clear speech, in which a talker intentionally articulates as though speaking to someone who has hearing loss or is from a different language background. At the same SNR, clear speech produced higher intelligibility than conversational speech. Despite numerous efforts, no objective metric can predict the clear speech benefit at the sentence level.
Methods: We proposed a novel objective intelligibility metric that used Speech Rate Adjusted Modulation (SRAM) power to predict the speech intelligibility of both clear and conversational speech under multiple SNR levels. SRAM estimated the modulation power spectrum of the speech envelope with a time window of 1 s. And then removed spectrum components below the syllable rate for intelligibility prediction.
We compared SRAM with three established objective speech intelligibility metrics: envelope-regression-based speech transmission index (ER-STI), Hearing-Aid Speech Perception Index version 2 (HASPI-v2) and short-time objective intelligibility (STOI); and five popular modern automatic speech recognition systems (ASR): Amazon Transcribe, Microsoft Azure Speech-To-Text, Google Speech-To-Text, Facebook Wav2vec2, and OpenAI Whisper.

Results: SRAM can correctly predict the intelligibility of both clear and conversational speech as well as the clear speech benefit. It obtained a low normalized root-mean-square error (NRMSE) of 4.4% between predicted intelligibility and the human performance.
Among all the reference metrics, ER-STI produced the best results with NRMSE of 7.2%. HASPI-v2 and STOI only predicted the increase of intelligibility as SNR increases, not the clear speech benefit. Out of five ASR systems, Google obtained a best NRMSE value of 6.7%, and Whisper came at second with NRMSE of 6.9%. The rest ASR systems all struggled to predict the clear speech intelligibility correctly.

Conclusions: In this study, we presented SRAM, an objective speech intelligibility metric based on modulation power above the syllable rate. We also presented an automatic syllable rate estimation algorithm in cases where speech script was not available. The SRAM outperformed not only the modulation power model without the syllable rate adjustment but also three acoustic-based objective metrics and five ASR systems in terms of predicting both the speech reception thresholds and the clear speech benefits. Importantly, with a one-second window, the SRAM can predict sentence-level intelligibility. The importance of syllable rate on predicting speech intelligibility suggested that human may not rely on the information at the modulation frequencies below the syllable rate to understand speech.

SU185. No Learning Effect and Small Variations in Ripple Test Outcomes With Different Acoustic Conditions

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Category: Psychoacoustics

Background: Speech perception outcomes vary greatly among cochlear implant (CI) recipients since it is affected by among others cochlear health and cognitive factors. Non-linguistic tests like the spectral-temporally modulated ripple test (SMRT; Aronoff and Landsberger, JASA, 2013) measure spectral resolution without a major role for cognitive factors. A study in normal-hearing subjects was performed to examine the effect of type of transducer (loudspeaker versus headphones), sound level and learning in the context of a clinical study in CI users which includes the SMRT and electrophysiological assessments of cochlear health.

Methods: Twenty normal-hearing subjects performed SMRT (version 1.1.3) 12 times. The temporal modulation was fixed at 5 Hz while the spectral modulation was varied in 1-up/1-down fashion using a three-alternative forced-choice paradigm with 20 cycles/octave as reference stimulus. The threshold was determined by averaging the last 6 reversals. Both headphone and speaker in free field were used, the stimuli were presented at three levels (55, 65, and 75 dB SPL), and each of these six acoustic conditions was presented twice. The subjects also performed a consonant-vowel-consonant (CVC) test in noise at -5 and -10 dB speech-in-noise ratios.

Results: A higher sound level (75 dB SPL) was associated with slightly lower SMRT scores (repeated measures ANOVA, p=0.001), while free-field conditions resulted in higher, i.e., better SMRT scores (p=0.01). No significant effect in order of measurement (first or second) was observed (p=0.79). Interactions among the three factors were not significant (p greater than 0.3). While average SMRT scores varied within a narrow range between 8 and 12 cycles/octave, a positive correlation between speech perception and SMRT scores was observed (R = 0.44, p = 0.05, for -5 dB speech-in-noise ratio).

Conclusions: Conducting the SMRT at sound levels between 55 and 65 dB SPL in free field conditions appears optimal. The slight decrease in performance with level can be explained by broadening of the auditory filter. The better performance using free field presentations can be ascribed to diffraction around the head and resonance in the auditory canal, leading to frequency dependent amplification and attenuation. Since significant learning effects were not observed, only a short procedural test round seems to be sufficient for testing in patients.

SU186. Long-Term Experience with Amplification May Contribute to Broad Binaural Fusion

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Category: Psychoacoustics

Background: Binaural fusion occurs when stimuli presented to the two ears are perceived as one sound. In normal-hearing listeners, this fusion typically only occurs for tones with small frequency differences across ears less than 0.1-0.2 octaves. However, hearing-impaired (HI) listeners often experience broad fusion, fusing across large frequency differences of as much as 3-4 octaves (Reiss et al., 2017). This broad fusion was found to be correlated with several subject factors, including younger age at testing, early age of onset of hearing loss and long duration of hearing aid use. The present study further investigated these demographic variables in a larger population of HI listeners to determine which of these factors have the strongest associations with broad binaural fusion.

Methods: Binaural fusion was measured in 27 adult (14 male, 13 female) and 8 pediatric (7 male, 1 female) subjects with hearing loss. Stimuli were dichotic pure tones presented simultaneously to the reference and contralateral ears. The reference tone in the one ear was held constant at 1600 Hz, while the comparison tone was varied in frequency around the reference at each trial. Fusion range, a measure of the breadth of fusion, was calculated as the frequency range over which fusion occurred at least 50% of the time. Demographic data collected included age at testing, age of hearing loss onset, hearing loss duration, and duration of hearing aid use in years.

Results: Preliminary findings in adult subjects showed a significant positive correlation between fusion range and duration of hearing aid use (r = 0.38, p =0.03), but not duration of hearing loss, age at testing, or age of onset of hearing loss. No significant correlations were seen with any factors in pediatric subjects; however, the number of subjects to date is small.

Conclusions: The findings suggest that long-term experience with amplification may influence the development of broad fusion in hearing aid users. Funded by NIH/NIDCD R01DC013307.

SU187. The Interactions of Spatial and Pitch Cues in Auditory Scene Analysis

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Category: Psychoacoustics

Background: The human auditory system can decompose complex sound mixtures into distinct perceptual auditory objects that are associated with physical sound sources in particular locations. This fundamental perceptual ability ('Auditory Scene Analysis' or 'ASA') relies on various perceptual and physical sound properties, including periodicity, spatial, and temporal cues.

Both pitch and spatial cues are known to influence sequential grouping (Plack, 2018). The impact of pitch in ASA has been well characterized using Van Noorden's (1975) ABA paradigm, in which two streams of tones, 'A' and 'B' are interleaved, and the factors contributing to perceptual segregation of the two streams are explored. It remains unclear how spatial and pitch cues trade off in perceptual organization. We introduce a spatial dimension to the ABA paradigm through the displacement of the A and B sequence sources.

Methods: We utilized a 91-element geodesic dome (radius 1m) loudspeaker array, capable of 9th-order ambisonic rendering (Sonible, Austria), located in a sound-attenuating room ("Audiodome"). Participants were seated in complete darkness with their heads at the center of the dome and ears aligned at 0° elevation, fixating on an LED in front of them. Our group has previously demonstrated that the minimal audible angle achievable with the configuration used here is 1.03° off the midline (Zargarnezhad et al., this meeting).

Each trial consisted of 32 repetitions of an ABA triplet (A: 125ms pure tone, f=400 Hz; B:125ms pure tone, f= or greater than 400 Hz; 125ms inter-triplet interval; 16-sec overall). The A and B streams were presented from two distinct symmetrically located positions on the horizontal plane. Listeners were instructed to report their perception (segregated/integrated) throughout the trial via keypresses (Cusack et al., 2004).

We first estimated the minimum required Df and Ds values at which the ABA sequence is perceived as segregated 90% of the time (i.e., Df and Ds 90% thresholds), employing an adaptive procedure for each dimension. Then the range of Df and Ds between 0 (no A-B difference) and each threshold were each divided into 7 equally spaced values (inclusive) and these values were combined to form a two-dimensional, 49-coordinate, psychophysical space. Perception at each of the 49 coordinates was then tested 10 times each. We measured the proportion of time in which the 2 streams were perceived as segregated.

Results: Very preliminary results suggest that small spatial separations lead to robust segregation even with very small Dfs.

Conclusions: This study aims to elucidate the interactions between spatial and pitch cues in organizing perception during a sequential streaming task by introducing a spatial dimension to a well-established paradigm used to probe factors that contribute to ASA. While data collection is still in progress, we anticipate elucidating the relative weight of pitch and spatial cues in ASA, for this paradigm.

SU188. Training a Deep Neural Network to Analyze Mouse Facial Grimace as a Measure of Painful Perception of Sound

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Category: Psychoacoustics

Background: Painful hyperacusis is a condition characterized by the perception of pain to normally-tolerated sound (Tyler et al., 2014). The Mouse Grimace Scale (MGS) was developed to assay facial changes correlated to painful stimuli (Langford et al., 2010). In her dissertation research, Lorraine Horowitz applied the MGS to a noise-induced model of hyperacusis showing an increase in grimacing to sound in a subset of noise-exposed mice. The MGS requires isolating clear frames of a video and having a blinded observer score facial

parameters on a 3-point scale of not present to severe. This approach has the potential to introduce bias at multiple stages. Le Moëne and Larsson adapted the MGS to identify changes to facial grimace in profile using markers manually placed on clear video frames of the mouse face. We have applied a similar approach but have reduced bias and analysis time by training a deep neural network to placed markers on the mouse face.
Methods: Six-week-old C57BL6/J mice of both sexes were placed into individual polycarbonate containers within an acoustic chamber fitted with overhead speakers. Mice were awake and freely moving. Mice received an acclimation period of 30 minutes to their containers the day before the sound exposure protocol. The sound exposure protocol began with ten minutes without sound played from the speakers (No Sound), then 70 dB of broadband sound was played for two minutes; the sound increased 10 dB every two minutes until reaching 120 dB. Then, the mice were returned to the No Sound condition for two minutes. Each video was approximately 25 minutes in length and was filmed in 4K resolution at 30 frames per second and was cropped to analyze one of two mice at a time. The deep neural network DeepLabCut was trained to place twelve (12) markers on the face of in each frame of video where the mouse was in profile (Mathis et al., 2018, Le Moëne and Larsson, 2023). Lines between markers (skeletons) were created. Angles between certain skeletons and ratios of certain skeletons were used to generate measurements of facial parameters.
Results: Six facial parameters were measured: Eye Ratio, Ear Ratio, Ear Angle, Ear Position, Snout Position, and Mouth Position. All measurements were significantly different across sound levels. The facial changes measured at 120 dB were consistent with perceived pain. Compared to manual analysis, the number of frames analyzed per video increased when machine learning was applied.
Conclusions: Analysis of facial grimace can be used to assay perceived pain to sound in mice. Mice exposed to sound at the human discomfort threshold of 120 dB exhibit facial changes associated with pain (Plutchik, 1963). The use of DeepLabCut machine learning has presented new opportunities to collect unbiased data in a more efficient manner.

SU189. The Circularity of Audition: Perceptual Similarity Underlies Memory Performance for Simple Tones

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Category: Psychoacoustics
Background: Memory is characterized by both remarkable precision and a disheartening fragility. What determines whether information is remembered well or poorly? Recent work in the visual domain has demonstrated that, when the perceptual similarity of a visual stimulus space is carefully mapped, memory errors are straightforwardly predicted by this similarity (Schurgin et al., 2020). 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 in general, irrespective of modality.
Methods: 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, we performed a rating experiment where participants listened to two tones and rated their perceived similarity on a 1-7 scale. The pure tone space consisted of two octaves separated by ¼ semitone intervals (96 tones; n = 127) while the circular Shepard tone-space was made up of 360 tones (n = 124). For both spaces, each tone (300ms) was separated by a 500ms interval.
Next, a new set of participants were randomly assigned to a memory experiment with either pure (n = 30) or Shepard tones (n = 30). Participants maintained one or three randomly sampled tones in working memory (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.

Results: 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. 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.

Conclusions: 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.

SU190. Neural Correlation of Speech Envelope Tracking for Background Noise in Normal Hearing

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Category: Speech Perception

Background: Everyday speech communication often occurs in environments with background noise, and the impact of noise on speech recognition can vary depending on factors such as noise type, noise intensity, and the listener's hearing ability. However, the extent to which neural mechanisms in speech understanding are influenced by different types and levels of noise remains unknown. This study aims to investigate whether individuals exhibit distinct neural responses and attention strategies depending on noise conditions.

Methods: We recorded electroencephalography (EEG) data from 20 participants with normal hearing (13 males) and evaluated both neural tracking of speech envelopes and behavioral performance in speech understanding in the presence of varying types of background noise. Participants engaged in an EEG experiment consisting of two separate sessions. The first session involved listening to a 12-minute story presented binaurally without any background noise. In the second session, speech understanding scores were measured using matrix sentences presented under speech-shaped noise (SSN) and Story noise background noise conditions at noise levels corresponding to sentence recognitions score (SRS).

Results: We observed differences in neural envelope correlation depending on noise type but not on its level. Interestingly, the impact of noise type on the variation in envelope tracking was more significant among participants with higher speech perception scores, while those with lower scores exhibited similarities in envelope correlation regardless of the noise condition

Conclusions: The findings suggest that even individuals with normal hearing could adopt different strategies to understand speech in challenging listening environments, depending on the type of noise.

SU191. Analysis of Word Type Specific ERPs Obtained From Continuous Speech EEG

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Category: Speech Perception

Background: Recently, in cognitive neuroscience, artificial stimuli such as single sentences have been replaced by more natural stimuli such as continuous speech. Since the localisation of language processing in the brain is already known, the next critical step is to study the functionality of these neural circuits and processes. Thus, it is necessary to apply experimental procedures with a high temporal resolution such as electroencephalography (EEG) in order to capture and identify these mechanisms. However, EEG is highly prone to measurement errors as the surface electrodes collect all kinds of electromagnetic noise from physiological and non-physiological sources. We want to present a procedure to remove those artifacts and provide evidence that it is possible to extract event related potentials (ERPs) from EEG data recorded during listening of an audio book.

Methods: We developed an evaluation pipeline, tested on EEG data of 36 participants. The pipeline consists of two major steps: spectral filtering (bandpass: 1 Hz-20 Hz) and a custom, highly reproducible, version of independent component analysis (ICA) filtering. Thus, we defined one channel (Fp1) as our electro-oculogram channel (EOG) and tested which independent components significantly correlate with this channel. All independent components that had a correlation above a fixed threshold were removed. To validate our results, we examined the grand average ERPs across all 36 subjects for different word types – nouns, verbs, adjectives, adverbs and numbers. Thereby, we contrasted word type ERPs with random time points and also compared

different word types (verbs vs. adjectives) by using a permutation test computing the root-mean-square amplitudes of 10,000 different random splits over five consecutive time windows from -200 ms before until 800 ms after stimulus onset.
Results: Comparison of the average word type ERPs with random time points confirmed that they were unique signals and not pseudo-ERPs caused by artifacts. Permutation test, shows that there is a significant difference between the ERPs evoked by verbs compared to adjectives in the interval from 200 ms to 400 ms ($p = 0.01$) and from 600 ms to 800 ms ($p = 0.001$) after word onset.
Conclusions: Our findings strongly suggest random time point controls to verify data quality when using continuous EEG for speech analysis. Furthermore, we want to emphasize the importance of employing adaptive artifact control procedures that yield in subject-specific results that can be replicated across studies.

SU192. Sex-Based Differences in TEOAE Results and Reproducibility Rates in Newborns

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Category: Otoacoustic Emissions
Background: Females have better auditory performance throughout their lifespan than males, including overall performance in newborn screening TEOAE. However, it is not well proven whether factors such as test timing and result reproducibility influence this observed difference.
Methods: This retrospective cohort study utilized data from May 31, 2019, to August 14, 2023, obtained from the University of Texas Medical Branch newborn hearing screening TEOAE results. The data included age and sex of the subjects, TEOAE outcomes, Signal-to-Noise Ratio (SNR) values (dB) at frequencies 1K-, 1.5K-, 2K-, 3K-, and 4KHz, and overall reproducibility percentages. A total of 240 subjects, 120 males and 120 females each, were subdivided into age at test, 0-1, 2, and 3+days old. A comparative analysis was performed on the left and right ear mean SNR values across age groups, and then subsequently compared between males and females. All testers were female.
Results: At 4kHz, for females, the right ear consistently exhibited the highest SNR value except for group 3+days. For males, the left ear exhibited the highest SNR value except for group 3+days. On Day1, female vs male left ears demonstrated similar SNR values at 10dB, whereas in the right ear, females had larger SNR values at 10.76dB, and males had 8.98dB. On Day2, females outperformed males at 4kHz, 11.74dB left ear and 12.53dB right ear vs. 10.62dB left ear and 10.19dB right ear, respectively. However, females showed a significant discrepancy in reproducibility rates between ears: the left ear was 83%, while the right ear was 49%, (p less than .001). On or after Day3, females exhibited a higher SNR in the left ear, 12.36dB, compared to males, 9.72dB. However, comparable right ear: 10.46dB(females) and 10dB(males). All groups maintained appropriate reproducibility rates ranging from 70-86%, with the sole exception being the 49% Day2 female right ear. The linear uptrend observed in the day2 group suggests that this might be a more optimal and dependable day for newborn TEOAE testing. However, the difference in reproducibility serves as a potential mediating factor. Nonetheless, these findings highlight gender and day-specific differences in TEOAE tests, emphasizing the potential for tailored clinical approaches in newborn auditory evaluations.

Conclusions: The observed variations in TEOAE reproducibility rates between male and female emphasize an important subtlety that could impact early diagnosis, interventions, and the length of newborn hospital stays. The patterns observed in this study highlight the value of testing two days post-partum and gender-focused analysis in neonatal auditory assessments. The disparities in results emphasize the necessity of multiple-day testing for accurate assessment and the potential for gender as a consideration in evaluation criteria. Upcoming research will expand the sample size and investigate the potential impact of tester's gender on the differential results in male and female newborn TEOAE results and reproducibility.

SU193. Speech Sound Discrimination by Mongolian Gerbils Before and After Noise Trauma

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Category: Speech Perception

Background: Exposure to loud noise can lead to difficulties in speech perception. Especially in the presence of background sounds, people who suffer from noise-induced hearing loss have difficulties in speech comprehension. However, the underlying mechanisms resulting in compromised speech reception after noise injury are still under debate. This study investigates the discrimination of speech sounds in Mongolian gerbils as a model organism. Gerbils show a good low-frequency hearing with a similar sensitivity as humans for the frequency range of human speech, which makes them a suitable model for investigating discrimination of speech sounds. Here, we report data on the discrimination of logatomes (CVCs - consonant-vowel-consonant combinations, VCVs - vowel-consonant-vowel combinations) by young gerbils before and after undergoing an acoustic trauma to reveal possible noise-related changes in the perception of speech sounds.

Methods: Nine young Mongolian gerbils were trained to perform an oddball target detection paradigm in which they discriminated a deviant CVC or VCV in a sequence of CVC or VCV standards (for details see Jüchter et al., 2022). The experiments were performed with an ICRA-1-noise-masker with speech-like spectral properties. After the gerbils completed the whole set of behavioral conditions, they underwent an acoustic trauma (anesthetized, 2 - 4 kHz, 115 dB SPL, 2 h) and afterwards collected data for the complete set of conditions again. Response latencies served to generate perceptual maps employing multidimensional scaling reflecting the gerbils' internal representations of the sounds before and after noise trauma. To evaluate which features of vowels and consonants are most important for discrimination before and after trauma, the dimensions of the perceptual maps were correlated with different features of the speech sounds. For evaluating peripheral auditory function, auditory brainstem responses and envelope-following responses were measured before and at three different time points after acoustic trauma. Further, peristimulus time responses (Jeffers et al., 2021) were measured at the round window after the gerbils finished behavioral data collection to investigate the functional state of the auditory nerve fibers.

Results: The perceptual representations of vowels in multidimensional scaling were very similar before and after trauma and perceptual distances were highly correlated. The gerbils discriminated vowels predominantly based on differences between spectral features of formants. Although showing similar patterns in the perceptual maps, the perceptual distances of consonants before and after trauma were only weakly correlated. The discrimination of consonants mostly depended on combinations of their articulatory features. However, the relative importance of the different articulatory features for consonant discrimination changed after noise injury.

Conclusions: In contrast to a very stable discrimination of vowels despite an acoustic trauma, noise injury showed a larger effect on the gerbils' perception of consonants with a change in the relative importance of features used for consonant discrimination.

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SU194. The Effects of Temporal-Spectral Asynchrony on Speech Intelligibility in a Multi-Talker Listening Environment

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Category: Speech Perception

Background: One of the important cues used in auditory scene analysis is thought to be temporal coherence, where sounds that share the same ongoing patterns of onset and offset timing are more likely to be perceived as a single source than when they are not synchronized. Although known as a highly salient grouping cue, the effects of temporal coherence have been studied mostly with simple repeating tone sequences, and its role in segregating more complex sounds, such as speech, has remained less studied. We aimed to test the role of temporal coherence across frequency in our ability to segregate multiple completing talkers by selectively desynchronizing the speech signal in adjacent spectral channels. Our predictions were: 1) disrupting temporal coherence should affect segregation, leading to a selective deficit for speech-in-speech over speech-in-noise tasks; 2) Temporal coherence of both the target and masker speech should be important in segregating competing perceptual streams.

Methods: Participants were instructed to report the words from target sentences, which were embedded in speech-shaped noise or a competing talker. The stimuli were filtered into eight contiguous frequency bands, and the bands were desynchronized with respect to each other. Experiment 1 (n=24) compared speech-in-noise and speech in the presence of a single-talker masker, with both target and masker desynchronized by maximum between-channel delays of between 0 and 120 ms in 40-ms increments. Experiment 2 (n=20) examined the same range of asynchrony, but only with a single-talker masker, and with the asynchrony imposed on either target alone, masker alone, or both.
Results: In Experiment 1, listeners' accuracy declined significantly with increasing between-channel delay. A significant interaction between delay and masker type (noise or single talker) reflected the fact that a small but significant difference between the maskers was found only when the delay was 40 ms and 80 ms. Experiment 2 showed that altering the synchrony of bands in the target produced similar results to those found in Experiment 1; in contrast, asynchronies in the masker speech had little effect on the target's intelligibility.
Conclusions: The experiments were designed to provide a better understanding of the role of temporal coherence in speech segregation. The results showed relatively small differences in the effect of temporal coherence between noise and speech maskers. In addition, only the temporal coherence of the target affected performance. The results suggest that temporal coherence may not play as critical a role for speech as has been found for the non-speech stimuli traditionally used to investigate auditory scene analysis.
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SU195. Feasibility of Continuous Speech-Evoked Electroencephalography to Understand Language Comprehension

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Category: Speech Perception
Background: The difference in neural activation during speech perception between native and non-native speakers is essential to understanding language comprehension. Brain responses to a short token, such as a word or phonemic stimuli, have been widely investigated. However, the previous approaches may not sufficiently provide information for comprehending continuous-speech in the human brain. This study investigated the feasibility of continuous speech-evoked electroencephalography (EEG) data to understand language comprehension in native and non-native speakers.
Methods: Participants included 20 Korean native speakers and 20 English native speakers. Korean and English sentences were presented via loud speakers. The brain activity in response to continuous speech (durations were about 2.5 s) was measured using the 64-channel EEG system. The temporal response function (TRF), which computes the relationship between a stimulus and the evoked EEG, and phoneme-related potential (PRP), which characterizes EEG responses to typical phonemic stimulus, were computed to analyze the difference associated with language proficiency between native and non-native speakers.
Results: The results show that the amplitude of TRFs (a forward model) in native speakers was significantly higher than in non-native speakers. However, the backward model better reconstructed the envelope of continuous speech stimuli in non-native speakers than in natives. The amplitude and peaks of PRP were higher and clearer in native speakers. Both approaches (TRF and PRP) to analyze continuous speech evoked EEG may be feasible to understand language comprehension.
Conclusions: This study showed the distinction in brain activation patterns across native- and non-native speakers when listening to the sentences using the approaches of TRF and PRP. Nonetheless, additional research is needed to understand better how to interpret the outcomes generated by these methods. This work was supported by the National Research Foundation of Korea grant (NRF-2020R1A2C2003319).

SU196. Recruitment of Latent Neural Resources During Speech Recognition in Blast-Exposed Veterans

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Category: Speech Perception
Background: Among the population of Service members with normal or near-normal audiometric hearing, blast-exposed listeners are roughly 2.5x more likely to experience subjective or objective hearing deficits than those without a history of blast exposure. These "functional hearing difficulties" may result from traumatic brain injuries (TBIs) induced by blast forces. The "latent resource hypothesis" suggests that TBI is generally associated with increased recruitment of neural mechanisms involved in auxiliary support of novel or challenging tasks. Here, we test whether blast-exposed listeners exhibit increased recruitment of latent neural resources during a speech recognition task.
Methods: Sixty Veterans (31 blast-exposed) performed a 3-AFC variant of the Coordinate Response Measure during fMRI scanning. The task involved identification of keywords spoken by a female talker in the presence of a competing male talker (+3 dB target-to-masker ratio). The two-talker mixtures were processed using a spectrotemporal modulation filtering technique that allows prediction of fMRI response from idiosyncratic modulation distortion patterns imposed on each trial. The "weights" in this predictive model describe a spectrotemporal receptive field (STRF) in the two-dimensional speech modulation power spectrum. Conveniently, the modulation power spectrum separates acoustic cues related to: (i) the pitch of the male talker, (ii) the pitch of the female talker, and (iii) phonetic content shared across both talkers. Pitch-sensitive brain regions were defined as those whose STRFs consistently included a response (i) or (ii). Non-negative matrix factorization was applied to pitch-sensitive STRFs to separate neural responses to the female talker from those to the male talker, and a whole-brain map of activation to the male talker was obtained for each listener as the inner product of the canonical "male" STRF and the actual STRFs at each brain location in that listener. A second application of non-negative matrix factorization was used to separate these whole-brain maps into discrete cortical networks that best captured variability across listeners. We focused on the male (competing) talker under the assumption that brain networks responding to background speech would be most sensitive to the effects of blast exposure.
Results: A network including the dorsolateral prefrontal cortex and the inferior parietal lobe was significantly more active in listeners with a greater cumulative history of blast exposure and other types of mild TBI, even after controlling for high-frequency audiometric thresholds, age, PTSD symptom severity, and cognitive ability. These brain regions are known to be involved in effortful processing during speech recognition and other challenging cognitive tasks.
Conclusions: This finding suggests that blast exposure leads to increased recruitment of latent neural resources to accommodate for the presence of competing speech. Crucially, overall task performance was held constant (67% correct) for all listeners, suggesting that blast-exposed listeners must allocate more neural resources to obtain the same level of performance.

SU197. Decoding of Imagined Speech and Music

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Category: Speech Perception
Background: Listening to music evokes spectrotemporally detailed neural responses measured with noninvasive EEG and MEG. Recent experiments (Di Liberto et al, 2021, Marion et al, 2021) revealed that imagining music also induces analogous responses, but with roughly the inverse polarity and delayed (~100ms), presumably because imagination is a top-down predictive process in contrast to the bottom-up listening. Here, we report on three follow-up studies demonstrating: predictions of imagined responses to music from their corresponding listening responses; extensions of these findings to the listening and imagination of speech; and generalizations to other recording systems (e.g., MEG and dry EEG systems).
Methods: All IRB-approved measurements of brain responses were conducted in subjects listening to music and speech stimuli while in (wet-BrainVision) 64-channel EEG, a (dry-CGX) 32 channel EEG, or 157 channel MEG. The original listened and imagined music recordings involved 21 subjects (Marion et al, 2021). 15

subjects participated in the MEG recordings of listened and imagined speech and music (Rezaeizadeh, 2022). Three subjects listened and imagined sequences of words in the dry-EEG set-up.
Results: Three key results are summarized here.

1. EEG responses to imagined and listened music exhibited a striking inversion of their response waveforms. Given the data from 21 subjects, a multilayered convolutional encoder-decoder was successfully trained to map (and thus predict) the EEG recordings from the listened to the imagined music, and vice versa. This nonlinear mapping was stable, accurate, and generalized to the recordings from all subjects.

2. MEG recordings of listened and imagined responses were made with the same musical signals. In addition, speech in the form of short poems was listened to and imagined by 15 subjects. We found that within each subject's responses, linear mappings between the listened and imagined responses can be reliably computed. A significant finding here is that mappings computed with music or speech responses were similar and generalized to one another well, indicating that the nature of the measured responses was similar and likely reflected an acoustic-like representation in the early auditory cortical stages.

3. The final experiments involved dry-EEG recordings with a smaller number of channels. Recordings of imagined and listened speech in 3 subjects exhibited polarity inversions similar to those seen in earlier experiments. Furthermore, the imagined responses were sufficiently precise that they could be reliably related to their listened counterparts. Finally, we also tested if the previously computed nonlinear mapping (with music and wet-EEG system) can be adapted and applied directly to mapping the speech listened responses and predicting its imagined counterpart.

Conclusions: Experiments with EEG and MEG recordings reveal the nature of imagined audio, provide reliable mappings to predict them from their listened counterparts, thus laying out a realistic path to decoding imagined speech and music.

SU198. Robust Evaluation of the Cortical Encoding of Word-Level Expectations Using the Temporal Response Function

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Category: Speech Perception
Background: Speech comprehension involves an active neural process mapping sounds into abstract meanings. As part of that process, words are detected and their meaning is interpreted based on the preceding semantic context. Previous work has provided strong evidence in favour of a predictive processing account of lexical processing, where the meaning of a word is actively shaped by lexical predictions based on the preceding semantic context. While recent work demonstrated the possibility of probing that same mechanism in scenarios involving natural speech listening, also leading to work proposing the tantalising possibility of robust relationships between neural processes and artificial large language models, there remains considerable uncertainty on how exactly lexical predictions are built by our brains. One issue is that the metrics used in non-invasive electroencephalography (EEG) and magnetoencephalography (MEG) studies typically exhibit small effect sizes, severely limiting the type of analyses and conclusions that can be drawn from them. This study demonstrates two metrics for robustly assessing lexical surprise from neurophysiology signals, substantially improve the state-of-the-art.
Methods: For probing neural lexical prediction mechanisms, the present study employed univariate and multivariate temporal response function analyses (uTRF and mTRF respectively) to assess the reliability of evaluation metrics previously used in the literature. Afterwards, two novel metrics are introduced using the mTRF methodology and multivariate stimulus features including word onsets and lexical surprise extracted from GPT-2. The first metric relies on prediction correlations, where the correlations are calculated by focussing on time-points where lexical surprise is expected to impact the prediction. The second metric is derived from the TRF weights, by contrasting features that do and do not relate with lexical surprise. These metrics were validated through simulations and then applied to a real publicly available EEG data, which was recorded as participants (N = 19) listened to a classic work of fiction. The effect-size for each metric was calculated by comparing the results with null distributions obtained by randomising the order of lexical surprise values returned by GPT-2.

Results: EEG metrics of word-level expectations based on EEG prediction correlations and TRF weights were compared between the proposed metrics and the vanilla TRF evaluation. Numerical results on both simulated and real EEG data indicated that the proposed metrics produces larger values (t-test, p less than 0.001). TRF results for each metric were compared with a corresponding null distribution (shuffled lexical surprisal), showing that the proposed metrics achieve effect-sizes that are over 80% larger than the vanilla TRF evaluation.
Conclusions: The two evaluation metrics introduced here substantially improve the metrics used in the literature without increasing the complexity and computational requirements. The increased effect size is expected to enable more detailed investigations into the prediction mechanisms generating lexical expectations during natural speech comprehension.

SU199. Individual Variability in Behavioral and Neural Measures of Categorical Perception

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Category: Speech Perception
Background: Categorical perception (CP) of speech sounds is a phenomenon where some sound pairs are perceived more similarly (i.e., within category) as compared to other sound pairs (i.e., across category), despite the acoustic difference between the sound pairs being constant. People vary in "categoricity," the degree of sensitivity to acoustic variability within a sound category, with some being less categorical in their judgments than others. While these individual differences are well established, our understanding of how individual differences in perception relate to individual differences in the neural processing of sound is incomplete. Past studies (e.g., Bidelman et al., 2013) examined CP using behavioral and neural measures; however, most studies used identification tasks that force judgments to be categorical. There has been a push (Kapnoula et al., 2017) to use tasks that allow more gradience in participants' judgment, such as ratings along a visual analog scale (VAS). The current study examined individual differences in CP in healthy young adults using a VAS behavioral task and neural indices of sound processing via Frequency Following Responses (FFR).
Methods: 77 (21M, 54F, 2 NB) monolingual native adult (18-30 years) speakers of American English with no history of speech, language, and hearing disorders participated in this study. To elicit behavioral and neural responses, we used a 7-point /ba-da/ continuum (each stimulus 230 ms). Behavioral responses were collected using VAS. We extracted the response curve and estimated the slope (steeper slope = higher CP) and the consistency of participant ratings. We recorded the FFR, a phase-locked auditory evoked response, to these seven stimuli presented randomly (1500 trials/stimulus). The FFR recordings were pre-processed using a 70-3000 Hz band-pass filter. For each participant, we extracted a neural differentiation index reflecting the degree to which FFRs differed across the stimulus continuum (higher value = greater differentiation). We also extracted a measure of neural consistency, the degree of FFR similarity across trials of the same stimulus. Previous work in children suggests that indices of FFR differentiation and FFR consistency predict phonological awareness, the ability to recognize that words are formed from individual sounds (Hornickel et al., 2009; 2013).
Results: Overall, in this young adult dataset, individual variability is high across both behavioral (slope: mean=0.69, SD=0.41; consistency: mean=-1.90, SD=0.95) and neural measures (FFR consistency (r-value): mean=0.72, SD=0.10; FFR differentiation (r-value change) mean=0.006, SD=0.034). Data collection for this project has recently ended, and exhaustive analysis linking perceptual and FFR data is currently underway. However, a preliminary analysis focusing on the tail ends of the behavioral data suggests that listeners who are most vs. least categorical differ in their level of FFR differentiation.
Conclusions: From the preliminary findings of the current study, there is initial evidence of individual variability in behavioral and neural measures of CP.

SU200. Multi-Session Training in the Evening Schedule Promotes Speech Learning

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Category: Speech Perception

Background: Speech learning can be affected by a variety of subject-internal (e.g., musical experience, cognitive abilities, general perceptual aptitude) and subject-external (e.g., amount of stimulation, stimuli variability) factors. A growing body of scientific literature (e.g., Earle and Myers, 2014, 2015; Qin and Zhang, 2019) has uncovered the role of sleep as a significant subject-internal factor than can affect speech learning. More specifically, it has been found that those who are trained in the evening learn better as compared to those trained in the morning due to consolidation that happens during sleep. Another reason that the morning group lag in performance is due to interference from the sounds of their native language during daytime (Earle and Myers, 2015). However, whether an extended training dosage with training sessions spanning across multiple days overcome the effects of interference from the native language for the group trained in the morning, is currently unknown. In the current study, using a 5-session artificial language training paradigm (Maggu et al., 2018, 2019), we investigated whether the effects of this potential native-language-interference for the group trained in the morning could be overcome by introducing a training-based subject-external factor i.e., daily dosage for five consecutive days.

Methods: In the current study, we compared young adults who were trained in the morning (8-10 am; n=16) vs. those who were trained in the evening (6-8 pm; n=16) on a 5-session dental-retroflex pseudoword-picture association training paradigm. More specifically, we evaluated whether conducting a multi-session training paradigm reinforce learning such that the effects of possible interference for training in the morning session can be overcome. In other words, we predicted that if training consecutively for five days led to overcoming the possible interference in learning during the morning session, we would see no significant difference between learning in the morning vs. evening, both on identification (trained stimuli) and discrimination (generalization) tasks.

Results: We found that even after getting their learning reinforced with a five-session training, the morning group was not able to catch up to the level of learning achieved by the evening group. In addition to the difference in learning performance between the groups, we found that the morning group also performed worse than the evening group on the generalization to novel contrast (not used in the training paradigm). In other words, even though providing daily dosage of training to the morning group helped them learn over time, it was not sufficient to help them match the level of the evening group.

Conclusions: The current findings, even with a multi-session paradigm, are consistent with the previous findings that support enhanced performance by training in the evening. These findings have clinical implications toward scheduling of speech therapy.

SU201. Investigating Contributions of Temporal Processing on Speech-In-Noise Understanding in Middle-Aged Adults

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Category: Speech Perception

Background: Difficulty understanding speech-in-noise in the absence of hearing loss is a longstanding puzzle in auditory research and hearing health care. As standard clinical tests appear insensitive to identifying the issue, auditory processes not engaged in existing clinical batteries must be explored. It is known that the ability to separate speech from competing noise is dependent on the auditory system's coding of temporal cues. While both animal and human research have demonstrated degraded temporal coding in older populations, it has been less studied in middle age—when speech-in-noise difficulty is first reported. This study addresses this by measuring temporal processing in middle-aged adults and investigating the extent to which these measures contribute to speech-in-noise understanding. Here, we measured temporal encoding at the peripheral, central, and perceptual levels of the auditory system using electrophysiology and psychoacoustic tasks. These measures were related to performance on two separate measures of speech-in-noise understanding that differed in cognitive and sensory demands.

Methods: Eighty-two adults aged 30-50 years with puretone thresholds ≤ 25dB across standard audiometric frequencies participated in this study. Extended high frequency thresholds were also collected. Speech-in-noise understanding was measured using two speech tasks: (1) AzBio Sentence Lists with colocated target and 10-talker babble, and (2) Spatial release from two talkers (SR2) using the closed-set Coordinate Response Measure corpus presented in colocated and separated conditions. Peripheral temporal coding was evaluated

by calculating the percent change in the peak-to-trough amplitude of the compound action potential in response to slow and fast click rates. Central temporal coding was measured by recording the frequency following response to a /da/ stimulus and extracting phase-locking strength to the stimulus fundamental frequency. Perceptual temporal processing abilities were measured using a dichotic frequency modulation detection task and, in a subset of participants (n = 32), an amplitude modulation detection task.
Results: Initial correlational analyses revealed only significant correlations between perceptual measures of temporal processing and speech-in-noise performance on the spatialized condition of the SR2 task (SR2-SEP). No relationships were observed between predictor variables and performance in the colocated speech-in-noise tasks. Age nor hearing sensitivity correlated with measures of speech-in-noise understanding. The linear regression model that provided the best prediction of SR2-SEP performance (adjusted R2: 0.192) included both psychoacoustic measures of temporal processing.
Conclusions: This study investigated the role of temporal processing in speech-in-noise understanding while minimizing the confounds of aging and reduced hearing sensitivity associated with older age. While physiologic measures of phase-locking did not contribute to speech-in-noise performance, perceptual measures of sensitivity to temporal fine structure and temporal modulation did. However, these measures only accounted for ~20% of variance in speech-in-noise performance, supporting the idea that additional variables should be considered in determining factors influencing speech-in-noise difficulty.

SU202. The Human Middle Ear in Motion: Direct Visualization and Movement Quantification Using Dynamic Synchrotron-Based X-Ray Phase-Contrast Microtomography

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Category: Middle and External Ear
Background: Characterizing the vibrations of human middle ear ossicles during sound transmission is of great interest in clinical research. However, the small size, location within the temporal bone, and tiny movements of the three auditory ossicles make this measurement extremely challenging.
Methods: Dynamic synchrotron-based X-ray phase-contrast microtomography is used to visualize the 3D motion of the ossicular chain under acoustic stimulation, with a voxel size of 2.75 μm. A post-gating algorithm is used to temporarily resolve fast micromotions at 128 Hz, coupled with a high-throughput pipeline to efficiently process the large tomographic datasets. Seven healthy ex-vivo fresh-frozen human temporal bones were scanned, and quantitative parameters describing the six degrees of freedom of the rigid body motion of each ossicle were computed for two sound pressure levels.
Results: With the extracted vectors for the translation and rotation of the ossicles, we could visualize their periodic movements for one movement cycle at 128 Hz and two sound pressure levels: 110 dB SPL and 120 dB SPL. Moreover, we could show that the main axis of rotation is perpendicular to the incudomalleolar joint and consistent with the lever-like rotation around the joint. Although the intersample variation was high, we could see a decrease in displacement amplitude along the ossicular chain for all samples. Further, when we compare the two sound pressure levels, we found that with an increase of 10 dB SPL, the amplitude increased by a factor of 1.7 at the umbo, 1.4 at the lenticular process, and 1.2 at the stapes footplate.
Conclusions: For the first time, we could directly visualize the micromotion of the human ossicular chain. The pipeline confirms previous reports of an amplitude decrease along the ossicular chain from the umbo to the stapes footplate and with decreasing sound pressure. The obtained knowledge will help us better understand the biomechanics of the human middle ear and may help to improve middle ear prostheses further.

SU203. Mechanical Impedance of the Human Head at Different Stimulation Position, Static Forces, and Interface Areas

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Category: Middle and External Ear
Background: The output force generated by a bone conduction transducer (BCT) depends on the mechanical load to which it is connected. While the mechanical impedance for a 15 mm diameter interface at the mastoid with a static force of 5.4 Newtons has been well characterized, contemporary usage involves BCTs of diverse sizes positioned at various locations on the skin-covered head. These transducers may be affixed using adhesive without exerting any static force or secured with a headband employing different static forces. Consequently, it is imperative to investigate how mechanical impedance varies on the skin-covered head in relation to factors such as position, size, and static force.
Methods: We conducted measurements of mechanical point impedance across a frequency spectrum spanning from 100 Hz to 15 kHz, involving 30 participants. These measurements were taken at three distinct head positions: (1) the mastoid, (2) the forehead, and (3) immediately in front of the ear canal opening. To control the static force applied, we employed a lever system and examined six discrete static force levels ranging from 0.5 to 7 Newtons. In the investigation of the influence of size on impedance, three circular interfaces with diameters of 10 mm, 15 mm, and 25 mm were used.
Results: The mechanical impedance profile exhibited a distinct pattern, characterized by different regions at varying frequencies. Specifically, it displayed:
1. A stiffness-controlled region at lower frequencies.
2. Resonance occurring between 0.8 and 4 kHz.
3. A mass-dominated region at frequencies exceeding the resonance range.

Comparing the mastoid and forehead positions, they demonstrated quite similar impedances, albeit with the forehead position showing slightly greater stiffness. On the other hand, the placement in front of the ear exhibited significantly lower stiffness, leading to a lower resonance frequency compared to the other two positions. When examining the impact of interface size, size had minimal influence on stiffness but had a substantial effect on mass. Larger interface areas were associated with greater mass, and larger masses corresponded to lower resonance frequencies. Additionally, the static force applied had a notable influence on stiffness, with higher static forces yielding increased stiffness.

Conclusions: The mechanical impedance of the skin-covered head was affected by the three parameters studied. The highest mechanical impedance was observed when the BCT was placed on the forehead, employing the largest interface area and the highest static force. Conversely, the lowest mechanical impedance was found when the interface was positioned in front of the ear, using the smallest interface area and the lowest static force. In this configuration, the mechanical impedance levels were approximately 30 dB lower compared to the highest levels observed on the forehead.

SU204. Assessing the Safety of Non-Invasive Electrical Stimulation in the External Auditory Canal

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Category: Other, External Auditory Canal
Background: To cure diseases, medications have been used, and for diseases that are not responsive to medication, surgical interventions have been employed. However, these methods often come with significant side effects. In contrast, electroceuticals has minimal side effects compared to oral medications, Alternative approaches to invasive techniques, such as deep brain stimulation and epidural stimulation, have been explored due to their limitations. Non-invasive techniques, on the other hand, offer advantages such as portability, cost-effectiveness, user-friendliness, and high acceptance among users.
Methods: Electrical stimulation was administered using the YPS-201b device at levels of 1mA, 2mA, 5mA, and 16mA, all set to a 1000Hz pulse frequency. A safe and tolerable stimulation of 2mA was considered, with 5mA being slightly higher. A symmetrical square waveform was used, and gel was applied to enhance conductivity. Each stimulation session lasted 5 minutes with a one-day rest period.
Auditory function was assessed via auditory brainstem responses (ABRs) before and after stimulation in the left ear using tone pips. Subdermal needle electrodes were used, and thresholds were measured from 20 to 80dB at 16kHz.

Tissue damage from electrical stimulation was histologically examined by excising the electrode-contacted portion of the external auditory canal (EAC). The EAC was paraffin-embedded, sliced to 5㎛ thickness, stained with Hematoxylin and eosin, and observed under an optical microscope. Damage severity ranged from no burn to first, second, or third-degree burns.

Results: We visually classified burns from electrical stimulation into zero-degree (no damage), first-degree (redness or small blisters), and second-degree (significant redness and inflammation) as there were no specific standards for the external auditory canal (EAC). Endoscopic images showed EAC stages: no burn, first-degree, and second-degree. Tympanic membrane and EAC showed no damage at 1mA and 2mA stimulation. At 5mA, first-degree burns occurred, and at 16mA, no normal condition was observed, with equal first and second-degree burns. Auditory brainstem responses (ABRs) revealed hearing deterioration starting from 5mA, reaching a maximum of 55dB at 16mA. Post-stimulation EAC sections were examined under an optical microscope, categorizing damage from zero-degree to third-degree burns. Optical microscopy confirmed observations, except for a first-degree burn in the 2mA condition not visible to the naked eye.

Conclusions: This research aimed to investigate how much electrical stimulation a non-invasive electrode can withstand when inserted into the EAC. The research results showed that up to 2mA at 1000Hz, no significant changes were observed in the rat's outer ear. This study provides a reference point for determining the safety limits of stimulation in humans as well. However, further research is necessary to thoroughly examine the effects of non-invasive electrical stimulation on the EAC.

SU205. Deficits in Sensory and Neural Processing of Auditory/Vestibular Cues in Zebrafish Spen Mutants

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Category: Other, Sensory and Neural Processing of Auditory/Vestibular Deficits

Background: Previous studies have shown that genetic forms of human hearing loss mainly affect the peripheral auditory system. In contrast, the genetic basis of central defects in the auditory/vestibular system remains unclear despite the existence of central forms of hearing loss and imbalance in humans. We identified a novel zebrafish spen mutant, which has recessive auditory/vestibular defects within the sensory and neural processing of auditory/vestibular, offering an opportunity to explore peripheral and central deficits in hearing and balance.

Methods: Immunofluorescence, in situ hybridization, EdU labeling, AEBR, VIEM, VSR, and OMR, high-speed calcium imaging

Results: In humans, de novo mutations in split ends (SPEN) are associated with childhood intellectual disabilities, craniofacial defects, and impaired hearing and vision. Here, we identify a nonsense mutation in the zebrafish spen that was isolated in a mutagenesis screen for recessive auditory/vestibular defects. The zebrafish spen mutant phenotype also includes development defects in the jaw and behavioral deficits in the optomotor response, suggesting that the visual system is impaired. Although the balance defects are pronounced in spen mutants, selective deficits in vestibular reflexes are observed. Surprisingly, the vestibuloocular reflex 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 vestibulospinal reflex (VSR) is initially executed, then greatly attenuates over time. These results suggest that the vestibular defects are central in origin in spen mutants. Zebrafish use saccular hair cells for hearing and we see a 55% reduction in sound-evoked calcium transients in this cell type. However, the auditory evoked behavioral response (AEBR) is severely reduced or absent, also suggesting that a central processing defect may contribute to this phenotype. Given the possibility of central deficits and the neurodevelopmental disorder in human patients, we also characterized development and function within the CNS of zebrafish spen mutants. We found that cell proliferation in the brain is dramatically yet transiently delayed in spen mutants between 5 and 7 days postfertilization, likely affecting the maturation and/or function of neural circuits. 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, potentially providing an explanation for defective behavioral reflexes in spen mutants that require activation of tail movements.
Conclusions: Collectively, our data indicate that mutation of spen causes a transient block in cell proliferation in the CNS and suggest that deficits occur in the sensing and/or processing of auditory/vestibular and visual cues. Future efforts will focus on establishing whether there is a link between the sensorimotor defects and aberrant glial cell activity.

SU206. Developing and Validating a Calibration Method to Reduce Wave Peak Amplitude Variability in Auditory Evoked Potentials

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Category: Other, Electrophysiology
Background: Hearing loss due to aging and noise exposure in various animal models can begin with cochlear synaptopathy – a synaptic loss between inner hair cells and spiral ganglion neurons without other permanent cochlear damages. Cochlear synaptopathy can be detected by significant decreases in auditory brain stem (ABR) wave-1 amplitudes in animals under sedation with subdermal electrodes. However, these effects translate poorly for clinical diagnosis possibly due to the clinical use of non-invasive surface recording electrodes which have higher impedances and susceptibility to noise interference. To address these issues, we designed a new electronic circuit that generates an electric calibration pulse (CalPulse) signal time locked to each ABR recording trace. This CalPulse is intended to be used as a calibration reference to adjust the measured amplitudes of AEP wave peaks and reduce amplitude variability.
Methods: Two CalPulse signals were designed using our circuit: square waves and sine waves. Amplitude repeatability of the two CalPulse signals were compared first in vitro with subdermal needle electrodes submerged in .9% sodium chloride. The CalPulse circuit was integrated into a standard one channel recording montage by jumping the positive and negative poles to the recording electrodes. Using this method, 4 different integration configurations were tested (configuration 1-4). Amplitude repeatability of the two CalPulse signals at the four configurations were compared In vivo by testing five CBA/CAJ mice (3M/2F). Based on these verification measures, the most stable and repeatable CalPulse signal type and integration configuration was selected and validated by measuring the correlation between CalPulse amplitude and ABR wave-1 amplitude in 40 adult 8 month old CBA/CAJ mice (20M/20F). Measures were repeated every month for four months (8-11 months).
Results: In vitro saline measures indicate that both signal types were stable across three current levels. In vivo measures indicated that configuration 1 and 2 produced the least amount of amplitude variation for the sine wave signal only. The sine wave CalPulse at configuration 1 was selected for further investigation. ABR wave 1 amplitude correlated significantly with CalPulse amplitude. Using this CalPulse type/configuration, we adjusted the wave-1 amplitudes by calculating the wave-1:CalPulse ratio which significantly reduced the coefficient of variance within the sample. Raw wave-1 measures indicated no significant change in amplitude from 8 to 11 months while the ratio-based calibrated wave-1 measures exhibited a significant and progressive decline in amplitude by month 10.
Conclusions: The CalPulse circuit was well supported by our verification measures, validation measures, and longitudinal data. Future human testing is planned to test possible differences in type and placement of human ABR electrodes compared to animal electrodes. Ultimately, we plan to apply this new device for subtyping of hearing loss in human diagnosis.

SU207. Open Board

SU208. Morphological and Behavioral Alterations in Sprague Dawley Rats Following Single Intraperitoneal 3'3-Iminodipropionitrile Administration

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Category: Vestibular: Basic Research and Clinical
Background: With the multitude of literatures following the mechanisms of vestibular pathologies, we explore the potential onset of behavioral deficiency on its correlation with vestibular synaptopathy. Significant studies follow damage mechanisms with severe hair cell deterioration with ablation of synapses.
Methods: The following study determines the use of a neurotoxic agent, 3,3'-Iminodipropionitrile (IDPN), to provide an animal model which can induce synaptic reduction without hair cell damage. Following damage, we observe several behavioral discrepancies that can be accounted for animal vestibular pathologies from existing literatures. Additionally, we look at the morphological differences of the vestibular system with routinary staining procedures.
Results: Intraperitoneal administration of IDPN showed a dose dependent response in terms of behavioral and structural changes in rats. An increase in behavioral discrepancy with changes in tail lifting was initially observed at IP600 with more aggressive changes at IP1000. IDPN treated animals also showed time-dependent decrease in capability to balance themselves within a rotating barrel starting at IP600. Same incidence was also observed with its curling behavior, which indicates misguided re-orientation of the animal to the ground, starting at IP600 until IP1000. The EthovisionXT behavioral analysis within an open field exploration shows no variability between groups as certain animals tend to maintain its position as contrast to existing literatures. Interestingly, upon staining with Myosin 7a (Myo7a) for hair cell markers, we observed an initial decrease at IP600 with increasing severity at both striola and extrastriolar regions. In contrast, we found an earlier decrease of synapse counts with Calcium-terminal binding protein 2 (Ctbp2) marker starting at IP400 within the utricle.
Conclusions: Our study shows initial findings on the structural changes of utricle upon dose-dependent administration of 3,3'-Iminodiproprionitrile in parallel with its behavioral manifestations within a 7-day observation period. Although there are some behavioral manifestations started at IP400, it is recommended to test with a more sensitive protocol that can detect and quantify minute changes with contrast to severe damage models.

SU209. Vestibular Perceptual Thresholds Correlate With Endolymphatic Volume in Patients With Meniere's Disease

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Category: Vestibular: Basic Research and Clinical
Background: Meniere's disease (MD) is a disabling inner ear syndrome characterized by fluctuating sensorineural hearing loss, episodic vertigo, ear fullness, and tinnitus. Delayed gadolinium (Gd)-enhanced high-resolution magnetic resonance imaging (MRI) of the inner ear has been shown to enable in vivo visualization of cochlear and vestibular endolymphatic hydrops. Clinical audiometric and vestibular tests have not consistently correlated with the severity of endolymphatic hydrops. We hypothesized that vestibular perceptual thresholds, which refer to the smallest appreciable stimulus detected by the participant in translations and rotations of body movement, may be more precise than traditional vestibular function tests. Here, we compared vestibular perceptual thresholds and endolymphatic volume of the hydropic ear in patients with definite MD.
Methods: Twenty (20) adults with definite unilateral or bilateral MD underwent perceptual threshold testing and 3T MRI scans. Five perceptual thresholds were performed using a MOOG 6 degree-of-freedom motion platform: three linear motion translations at 1.0 Hz (inter-aural [y-translation], naso-occipital [x-translation], and earth-vertical [z-translation]), one rotary motion at 1.0 Hz (yaw rotation), and head-centered roll-tilt (HCRT) at 0.2 Hz, upright about a naso-occipital, earth-horizontal axis centered between the ears. Delayed Gd-enhanced MRI scans were performed at 4 hours following administration of intravenous contrast. Subjects also underwent video head impulse testing (vHIT), bithermal caloric irrigation, and cervical vestibular evoked myogenic potentials (cVEMP). Volume measurements of endolymphatic space were calculated using Osirix MD on a positive endolymph image (PEI). The PEI was generated by subtracting a 3D-fluid attenuated inversion recovery (FLAIR) image sequence from a heavily T2-weighted CISS image sequence.
Results: Average vestibular perceptual thresholds were: 0.0325 (0.0184) m/s2, 0.0385 (0.0259) m/s2, 0.1685 (0.1262) m/s2, 1.6277 (1.1771) deg/s, 0.5401 (0.4038) deg/s, for the y-translation, x-translation, z-translation, yaw rotation, and HCRT, respectively. Average endolymphatic volume of the hydropic ear was 18.205 (6.458)

mm3. A positive linear correlation was observed between the endolymphatic volume and perceptual thresholds in the z-translation (r2=0.72, p=0.00003), y-translation (r2=0.32, p=0.019), and HCRT (r2=0.33, p=0.017). No significant correlation was observed between endolymphatic volume and perceptual thresholds from other motions. Clinical vestibular tests (vHIT, calorics, cVEMP) and disease duration did not correlate with endolymphatic volume.
Conclusions: To our knowledge, this study is the first to demonstrate that vestibular perceptual thresholds, a physiologic measurement of vestibular function, may correlate with endolymphatic volume of the vestibule, an morphologic marker of disease. The z-translation threshold is believed to reflect saccular function, the y-translation threshold represents utricular function, while HCRT signifies a combination of vertical and utricular function. Perceptual threshold testing may provide insight into the underlying pathophysiology of MD (e.g. elevated z-translation and y-translation thresholds suggest saccular and utricular dysfunction, respectively, while increased HCRT thresholds suggest vertical canal and/or utricular impairment).

SU210. Modernizing Vestibular Function Testing Through Multi-Frequency Examination of the Vertical Vestibulo-Ocular Reflex

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Category: Vestibular: Basic Research and Clinical
Background: Standard sinusoidal harmonic acceleration (SHA) testing measures the functionality of the horizontal vestibulo-ocular reflex (VOR) by rotating patients at pre-determined frequencies along a fixed earth-vertical axis. In addition to reliably determining site and side of vestibular lesions to differentiate peripheral from central pathology, SHA testing may also be used to monitor compensation and/or vestibular rehabilitation progression. Recently, our group has developed a computerized rotational testing paradigm for assessment of the vertical canals (right/left anterior and right/left posterior semicircular canals), which utilizes SHA testing via off-vertical, head eccentric delivery of whole-body oscillations in the horizontal (yaw) plane. Here, we sought to characterize normative values at several mid-range frequencies (0.08, 0.16, and 0.64 Hz) and determine the viability of vertical SHA testing as a novel clinical test.
Methods: Sixteen healthy males and 23 healthy females (n=39) with a mean age of 25.7 (±5.6) years and no history of vestibular disorders or traumatic brain injury were enrolled following written informed consent (#20190034). Following standard oculomotor testing, horizontal and vertical SHA testing was performed at 0.08, 0.16, and 0.64 Hz in a manually reclined rotary chair (Neuro-Otologic Testing Center [Neurolign USA, Inc.]) with the head turned either 45 degrees to the left (LARP) or right (RALP) positions. Eye position and velocity were measured using a binocular infrared video-oculography system recording at a frame rate of 250 fps (res: less than 0.1 deg) with sensors in the pitch (x), roll (y), and yaw (z) planes. Horizontal and vertical canal gain values were calculated as the ratio between peak slow phase component of horizontal eye velocity to peak yaw gyro velocity and the ratio between peak slow phase component of vertical eye velocity to peak pitch gyro velocity, respectively.
Results: Horizontal and vertical SHA testing was well tolerated by all study participants. At 0.08 Hz, the mean horizontal, LARP, and RALP gain were 0.53 (± 0.21), 0.49 (±0.17), and 0.48 (±0.16), respectively. Similar trends were observed at 0.16 Hz, where the mean horizontal gain was 0.53 (±0.20), LARP gain was 0.47 (±0.15), and RALP gain was 0.48 (±0.15). Lastly, at 0.64 Hz, the mean horizontal gain, LARP, and RALP gain were 0.69 (±0.22), 0.59 (±0.14), and 0.62 (±0.16), respectively. Regardless of plane examined, we observed that gain increased proportionally with increased chair velocity. Further, both average VOR gain and VOR phase observed in the horizontal, LARP, and RALP planes were well-correlated across all frequencies tested (0.08, 0.16, and 0.64 Hz; p less than 0.05), indicating comparable vestibular system responsivity irrespective of canal orientation.
Conclusions: Vertical SHA testing may be a useful tool for assessment of vestibular responsivity over a range of clinically relevant frequencies. Our lab is actively exploring this technique among healthy controls and individuals with defined vestibular pathologies.

SU211. Oculomotor, Vestibular, and Reaction Time (OVRT) Assessment in Parkinson Patients

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Category: Vestibular: Basic Research and Clinical

Background: Our long-term objective is to evaluate patterns of oculomotor, vestibular, reaction time, and cognitive (OVRT-C) functions in Parkinson (PD) patients using portable, virtual reality type eye-tracking technology. This technology allows precise, objective, and non-invasive evaluation of oculomotor and vestibular systems using simple battery of tests. The premise of this research is that several neurological conditions—including PD—are characterized by unique, specific patterns of oculomotor, vestibular, and cognitive behaviors that are measurable and can be used to distinguish between healthy adults and patients and, ultimately, provide diagnostic utility. In this early exploratory study, we seek to identify characteristic OVRT-C metrics that distinguish between (1) controls and (2) patients diagnosed with PD. Secondly, determine if patterns of oculomotor and vestibular results have utility as a biomarker and monitoring tool for PD.

Methods: In this early exploratory study, we assessed twenty-three participants in total (16 patients and 7 controls). Each participant completed a comprehensive battery of 23 OVRT-C tests included horizontal and vertical saccades and smooth pursuits, optokinetic, vergence pursuit and step tests, gaze, light reflex, subjective visual vertical and horizontal, auditory, and visual reaction time, memory guided saccades. and a measure of ocular tremor. Tests were presented consecutively and ranged from 10 to 40 seconds in length, for a total time of approximately 15 minutes. Output variable metrics included oscillation of eye/head for all battery of tests was created. Analysis was completed offline, and raters were blinded to diagnosis.

Results: Early exploratory analyses using univariate Logistic Regression Models identified OVRT-C metrics that discriminated between control subjects and PD patients. For the 23 (64 total to be evaluated) subjects tests thus far, horizontal smooth pursuit correctly classified 71.4% controls and 93.8% patients, antisaccade: correctly classified 100 % controls and 75% of patients, Oculomotor tremor / oscillation of eye/head correctly classified 71.4 % controls and 93.8% of patients. Horizontal smooth pursuit, vertical, predictive, random and antisaccade saccades tests, and oculomotor oscillation of eye/head demonstrated significant promise in being capable of discriminating between patients and controls.

Conclusions: The diagnosis of Parkinson (PD) is challenging due to its clinical overlap with other disorders. Objective findings might improve clinicians' recognition and diagnosis of PD. The preliminary data suggests that OVRT-C testing could be a useful tool in diagnosing PD, including head and eye oscillation, all of which are usually included in standard vestibular testing batteries using video nystagmography. These early exploratory results found that video oculography can distinguish patients with known PD from known controls. A complete analysis is pending more recruitment. To further assess diagnostic value, studies are ongoing to evaluate if OVRT-C can discriminate patients with unknown disease status.

SU212. Vestibular Dysfunction in Individuals With Parkinson Disease With Freezing of Gait

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Category: Vestibular: Basic Research and Clinical

Background: Freezing of gait (FoG) is a disabling motor feature associated with advancing Parkinson disease (PD). FoG involves an abrupt cessation or reduction of leg movement during walking and turning which often results in falling. FoG tends to persist despite dopaminergic medication suggesting a pathomechanism independent of nigrostriatal dopaminergic denervation. Given the vestibular system's role in sensing head rotations and maintaining balance, vestibular dysfunction may be involved in the pathophysiology of FoG. In fact, the vestibular system has been speculated to be involved in PD for decades, but results tend to be inconclusive.

Methods: We propose to assess vestibular dysfunction as it relates to FoG in subjects with PD while taking account the severity of nigrostriatal dopaminergic denervation. The gross recruitment will include 64 subjects from the VAAAHS Movement Disorders Clinic and the University of Michigan (U-M) Movement Disorder Center (MDC). Clinical assessments will evaluate the vestibular system across a broad frequency and will

include the video head impulse test, sinusoidal harmonic acceleration (SHA) range using a rotatory chair, and bithermal water caloric irrigation. Nigrostriatal dopaminergic denervation will be assessed utilizing the [11C]PE2I PET scan. Neuroimaging measures will be utilized to better assess dopamine loss and vestibular dysfunction in the development of FoG.
The presence of FoG will be determined by a combination of assessments including the Snijders et al. freezing of gait provocation protocol and the Movement Disorder Society-revised Unified Parkinson's Disease Rating scale (MDS-UPDRS) items 2.13 and 3.11. The diagnosis of presbyvestibulopathy (PVP) will be based clinically on abnormal performance on the modified Romberg as well as using the Bárány Society vestibular testing criteria. PVP status will be further examined utilizing a principal component analysis of all collected vestibular data to gain better mechanistic insights.

Results: Preliminary analysis validates our methodology by reflecting the physiology of the vestibular system. For example, vestibulo-ocular reflex (VOR) gain increases with rotational frequency while phase tends to decrease. Furthermore, we observed lower VOR gain in freezers (n = 13) when compared to non-freezers (n = 20) at 0.32 Hz. This finding relates vestibular dysfunction to FoG as turns during everyday ambulation are more likely to occur at intermediate frequencies of rotation.
Conclusions: Our summary statistics have provided initial understanding of how vestibular function may differ between PD persons with FoG and PD persons without FoG. We will investigate further whether these differences are maintained independent of nigrostriatal dopaminergic losses. Additionally, evaluation of freezing as a predictor of PVP or vice versa and its association with PVP will be determined via logistic regression and the Chi-square test respectively. Positive findings may augur novel treatment approaches, such as portable thermoneuromodulation or galvanic stimulation in PD persons with postural and gait changes, in particular those with freezing of gait.

SU213. Age-Dependent Changes in Sound-Evoked Vestibular Myogenic Potentials in Preclinical Models of Noise Overexposure

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Category: Vestibular: Basic Research and Clinical
Background: The vestibular system plays a crucial role in providing critical input for balance and posture by encoding changes in head rotation, translation, and gravity. Similar to the cochlea, the vestibular endorgans also respond to loud acoustic stimuli, with the otoliths being particularly susceptible to damage caused by hazardous noise exposures. Previous pre-clinical work has characterized the noise-induced changes in the morphological and functional features of the saccule and utricle combining imaging techniques and the study of vestibular short-latency evoked potentials (VsEPs). However, due to challenges in recording VsEPs in humans, the clinical assessment of vestibular function in noise-exposed subjects rather relies on vestibular evoked myogenic potentials (VEMPs). Although prior work has elucidated the neural basis of VEMPs, their use in preclinical studies to characterize the pathophysiology of vestibular dysfunctions has been limited, primarily due to the low level of reproducibility and high variability of the responses elicited in animal models so far.
Methods: In this study, we employed a standardized preclinical cVEMP setup and test protocol developed by our group that closely mimics clinical methodologies. We provide a detailed characterization of cVEMPs evoked in two groups of rodents exposed to noise at different age (n=6/group). Male Brown Norway rats (14-18 weeks and 54-58 weeks) with normal hearing and vestibular function were screened using a Smart EP evoked potentials system (Intelligent Hearing Systems, USA). The animals were exposed to 4-16 kHz noise at 110 dB SPL for 1 hour. Changes in auditory brainstem responses (ABRs) and cervical vestibular myogenic potentials (cVEMPs) evoked by pure tone bursts at 1 and 8 kHz were monitored up to 28 days post-noise.
Results: ABR measurements revealed that, while the younger cohort is characterized by a permanent threshold shift following noise exposure, the older one presents a temporary threshold shift with complete recovery by D14. cVEMP assessments showed age-related differences in vestibular sensitivity to noise. At 14 weeks of age, transient changes in the threshold of myogenic potentials evoked by 1 and 8 kHz stimuli

occurred. The saccular responses evoked post noise by 90 dB SPL stimuli at both frequencies present temporary latency increase, recovering by Day 14, with a permanent amplitude decrease.
At 54 weeks, high-intensity noise caused permanent threshold shifts only in responses to low-frequency stimuli. The thresholds of cVEMP evoked by 8 kHz stimuli recover by D7. In older animals, cVEMPs latency remain unaffected by noise exposure at any of the frequency tested while transient changes in the amplitudes of the myogenic potentials are still observed.

Conclusions: These results suggest that cVEMPs represent a reliable diagnostic test in a preclinical setting, with significant implications for understanding early and long-term changes and potentially identifying the neural basis of vestibular disorders, including noise-induced vestibular deficits.

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SU214. Natural History of Vestibular Dysfunction in USH1C Mice

Jessica Landry*[1], Zelma Guisela Iriarte[2], Caroline Sit[2], Aaron McCullough[1], Matthew Ward[1], Youguo Xu[2], Tianwen Chen[2], Gwenaelle S.G. Geleoc[3], Hong Zhu[2], Wu Zhou[2], Jennifer Lentz[4]

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Category: Vestibular: Basic Research and Clinical
Background: Usher syndrome (USH) is a rare genetic disorder characterized by the loss of hearing, balance, and vision. Approximately 2.5% of USH is caused by mutations in the USH1C gene, however the frequency is higher in the Acadian populations of Louisiana and Canada due the USH1C c. 216G greater than A founder mutation (216A). The multi-sensory losses are present at different ages in USH patients; however, the natural clinical course is not known. The goal of this study was to define the temporal course of vestibular dysfunction in USH1C mice carrying two copies of the 216A mutation, and to define the long-term effects of antisense treatment on balance behavior.
Methods: USH1C mice were treated via semi-circular canal injection with antisense oligonucleotides (ASOs) targeting the 216A mutation at postnatal day 2 and aged to 1 year. Balance behavior and vestibular function were assessed in wild type (WT), untreated and ASO-treated USH1C mice at various ages over a one-year time course. Fine motor coordination and balance behavior were assessed by rotarod and balance beam analyses. To define the progression of canal and otolith dysfunction, rotational and translation vestibulo-ocular reflex (rVOR and tVOR) tests were performed. The peripheral vestibular function was further assessed by measuring single vestibular afferent activities in response to head rotation and translation.
Results: On the rotarod, untreated USH1C mice were found to have significantly reduced latency to fall compared to WT mice at all ages tested. Whereas, at 12 months-of-age, ASO-treated USH1C mice showed longer latency to fall than untreated USH1C mice and were not significantly different from WT mice. Untreated USH1C mice were also not able to complete the balance beam test at any age. Whereas, at 12-months-of-age, ASO-29 treated USH1C mice were not significantly different from WT mice. USH1C mice displayed significant canal function deficits as early as 1-month of age. Notably, these mice exhibited higher rVOR gains (4Hz) at 1 month compared to 6 and 12 months, indicating a progression of canal dysfunction. In contrast to rVOR, USH1C mice exhibited similar tVOR gains as WT mice at 1 and 6 months, although with larger phase lags. However, by 12 months-of-age, USH1C mice exhibited lower tVOR gains compared to age-matched WT mice. Single-unit recordings of vestibular afferents revealed that USH1C mice exhibited substantially reduced spontaneous firing rates, a decrease in the proportion of regular afferents, and reduced sensitivities to head rotation or translation at 6 and 12 months.
Conclusions: These preliminary data suggest the vestibular dysfunction in USH1C mice progresses over time and is end organ specific. Additionally, these data suggest that antisense therapy significantly improves long-term fine motor coordination and balance behavior in USH1C mice and support the efficacy of ASO treatment in restoring vestibular function in Usher syndrome.

SU215. Effects of Lifetime Noise Exposure on Irregular Afferent Function and the Near VOR Response

Natela Shanidze*[1], Anca Velisar[1]

[1]*The Smith-Kettlewell Eye Research Institute*

Category: Vestibular: Basic Research and Clinical

Background: Prolonged noise exposure has been implicated in noise-induced hearing loss and more recently in damage to the vestibular periphery, although studies to date, particularly in humans, have shown mixed results. A likely barrier to detection and diagnosis of noise-induced vestibular loss is the difference in susceptibility to damaging noise exposure of certain components of the vestibular system. Of the three classes of vestibular afferents, calyx-only afferents exhibit irregular firing rates and phasic responses related to head acceleration and as they are sound sensitive, they may be susceptible to noise overstimulation. Irregular afferents are also key for VOR gain adaptation to near viewing.

Methods: In this pilot study we examined changes in VOR gain between near and far viewing in participants with varying degrees of noise exposure. Participants (13, 7F, 50.15±21.3 years) were asked to fixate LED targets at far (2 m), intermediate (0.5 m) and near distances (point of fusion, 0.12-0.3m) while actively rotating their heads in yaw to a metronome set at 0.5 and 1 Hz. Eye movements were recorded using the EyeSeeCam Sci eye tracking goggles. VOR gain (as compared to the ideal gain at each viewing distance) and vergence were assessed for each participant at each viewing distance. Noise Exposure Structured Interview (NESI) was used to assess noise exposure history. Pure tone audiometry was performed and hearing thresholds in the 4KHz frequency band (worst ear thresholds, between-ear threshold difference) were used for analysis.

Results: While VOR gain was not different between participants at the two farther viewing distances (2m: 1.05±0.03, 0.5m: 1.17±0.04) there was a variation in the amount of VOR gain error (0.13±0.1) at the closest viewing distance (VORnear). We found a significant correlation between VORnear error and the between-ear threshold difference at 4KHz ($p = 0.012$, $\rho = 0.68$). To further probe the relationship between noise exposure and VOR gain error, we constructed a linear model that took into account the age, log-transformed NESI score, and 4KHz audiometric difference. We found that both log(NESI) and between ear threshold difference were significant predictors ($p = 0.008$ and $p = 0.031$ respectively; model $R2 = 0.69$).

Conclusions: Our results suggest that noise exposure likely affects irregular afferent function. While it does rely on participant recall, the NESI likely accounts for overall noise exposure history of the participants, while the audiometric notch reflects the factors that may contribute to the deleterious effects of noise, such as age and susceptibility. Irregular afferents play a specialized and critical role in maintaining head and body posture during abrupt perturbations, which makes it possible to maintain center of gravity and avoid falling. Accordingly, damage to this particular class of vestibular primary afferents may be particularly implicated in increased fall risk due to vestibular loss.

Young Investigator Symposium 4 - New Approaches and Technologies in Auditory Neuroscience

1:45 p.m. - 3:45 p.m.

Grand Ballroom Salon E

New Approaches and Technologies in Auditory Neuroscience

Chair: Bonnie K. Lau*, University of Washington*

Co-Chair: Giovanni Di Liberto*, Trinity College Dublin*

Understanding Speech Processing in Children and Adolescents Using Intracranial Recordings

Liberty Hamilton

The University of Texas at Austin

Individual Abstract: Direct brain recordings used in surgical epilepsy monitoring provide a window into understanding speech processing at high spatiotemporal resolution. Such recordings have allowed for a

detailed delineation of brain regions responsive to spectrotemporal modulations, onsets, phonetic, and higher order linguistic content in speech during perception and production. However, few groups have used intracranial recordings to understand the development of neurophysiological responses to speech in children, partly due to the unique challenges faced in the in-patient setting. Here, I will detail our efforts to understand the neural underpinnings of speech in children and adolescents using a combination of naturalistic and controlled tasks in the epilepsy monitoring unit. We recorded brain responses from implanted intracranial electrodes while participants listened to and watch movie trailers and compared them to brain responses to sentences from the TIMIT acoustic phonetic corpus. Using both noninvasive scalp EEG and invasive intracranial recordings, we show that acoustic and phonetic receptive fields derived from these very different stimulus sets are both similar and generalizable to out of set stimuli. Importantly, the use of more engaging audiovisual stimuli has led to our ability to record much larger datasets from children as young as four years old, which will allow us to investigate variation in response properties and acoustic to phonetic mappings across age.

Using EEG, Pupillometry and Information Modelling to Track Musical Expectations in Non-Human Primates

Roberta Bianco

Istituto Italiano di Tecnologia

Individual Abstract: Music has often been regarded as uniquely human. Yet, growing evidence of spontaneous musical abilities in animals and infants raises questions about the cognitive building blocks that supported the evolution of music. Studies in non-human primates reared without musical exposure can help to distinguish phylogenetically conserved musical abilities from those that are driven by enculturation. In this study, we tested whether rhesus monkeys generate musical expectations by examining monkeys' pupil dilation and neural (EEG) activity in response to music as a function of musical predictability. Statistical features relative to pitch and timing were extracted from the stimuli using a predictive model of musical structure based on Markov chains. We assessed the contribution of statistical versus acoustic musical features to the neural signal with temporal response functions. We report that, beyond plain acoustic processing, monkeys engage with musical melodies more than with shuffled (control) melodies. Further, by comparing human and monkey neural responses to the same melodies, we find that timing-based expectations are conserved across species but are based on reduced musical context in monkeys. Conversely, pitch-based expectations are found only in humans. These results highlight structural processing capacities in monkeys, and they distil homologous from human-specific underlying mechanisms. Moreover, using cutting-edge analytical approaches and computational modelling, this work demonstrates that musical abilities can be determined non-invasively in non-human passive listeners. Overall, combining neuroscience and comparative musicology holds great potential for tracing the evolutionary pathways undertaken by music in humans and other species.

From Whole-Brain Decoding to Single Neuron Spiking: Uncovering the Code of Speech Comprehension

Laura Gwilliams

Stanford University

Individual Abstract: Our science lives in an exciting technological age. Advances in machine learning have provided the field with powerful analysis methods, capable of capturing non-linear encoding schemes, and complex hierarchies of linguistic representations. At the same time, impressive hardware developments in neural recording techniques provide unprecedented ability to record hundreds of single neurons simultaneously across the cortical depth. In this talk, I will present two studies that showcase each of these advances for understanding speech processing in the human brain. First, I will present results from whole-brain magneto-encephalography recordings, using machine learning methods to reconstruct phonetic and lexical sequences from individuals listening to short stories. Our findings provide evidence that each element in the sequence is maintained in parallel with its neighbours, and embedded in a dynamic coding scheme whereby information locally reconfigures over space as a function of time. Second, I will present results from Neuropixels probes, where we recorded over 700 single neurons from superior temporal gyrus while patients listened to spoken sentences. The results reveal that a rich diversity of speech features are encoded in a single

cortical column, permitting local access to short timescale properties such as acoustic-phonetic features, and longer timescale properties such as prosody and pitch. Together, these two lines of work demonstrate the powerful synergy between technological progress -- in both software and hardware -- and scientific discovery, and the importance of integrating these advances for furthering our understanding of auditory neuroscience.

Hierarchical Encoding of Natural Sound Mixtures in Ferret and Human Auditory Cortex
Yves Boubenec

Ecole Normale Superieure

Individual Abstract: Using functional Ultrasound imaging, we measured hemodynamic activity in the auditory cortex of ferrets passively listening to continuous mixtures of background and foreground natural sounds and to their components in isolation. We examined responses in three different stages of auditory cortex, from primary to tertiary areas. We found foreground- and background-representing voxels throughout auditory cortex, but invariance to background sounds increased along the hierarchy, with non-primary areas hosting more invariant foreground representations. These results are consistent with a recent study in humans (Kell et al, 2019), suggesting cross-species generic mechanisms. We then predicted human and ferrets cortical responses using a model of frequency and spectrotemporal modulation tuning and show that predictions recapitulate these properties. Differences in tuning between areas combined with the acoustic differences between foregrounds and backgrounds could partly account for hierarchical effects. Thus, we show a large-scale hierarchical organization of invariance to background noise in auditory cortex, inherited from tuning to canonical acoustic features.

The Organization of Neural Integration Windows in the Human Auditory Cortex
Samuel Norman-Haignere

University of Rochester

Individual Abstract: Natural sounds such as speech and music are structured across many different temporal scales, and the structures that compose them (e.g., phonemes and words) vary widely in duration. The auditory cortex must therefore have mechanisms to flexibly integrate across diverse timescales to derive meaning from sound. However, identifying the integration timescales of auditory neural populations has been challenging, in part due to their complex, nonlinear tuning for natural sound structure. In this talk, I will describe a method we have developed for estimating neural integration windows from nonlinear systems using natural sounds (the temporal context invariance paradigm). Our method is conceptually simple and general, and thus applicable to virtually any brain region, sensory domain, or temporally precise recording modality. By applying this method to intracranial recordings from human neurosurgical patients, we have found that the human auditory cortex integrates hierarchically across diverse timescales spanning approximately 50 to 400 ms, with substantially longer integration windows in non-primary regions, bilaterally. Moreover, we have found that neural populations with short and long integration windows exhibit distinct functional properties: short-integration electrodes (less than ~200 ms) show prominent spectrotemporal modulation selectivity, while long-integration electrodes (greater than ~200 ms) show prominent category selectivity. Preliminary results suggest that these integration windows predominantly reflect physical time (e.g., 200 ms) rather than the duration of sound structures, such as phonemes or words, even in non-primary regions with long timescales.

Investigating Auditory Cognition With Natural Speech and Music
Giovanni Di Liberto

Trinity College Dublin

Individual Abstract: That cortical activity tracks the dynamics of auditory stimuli is reasonably well established. In speech and music perception, this phenomenon produces reliable coupling between various features of the sound input (e.g., envelope) and the corresponding cortical responses. In this talk, I will present

recent work attempting to measure that phenomenon with neurophysiology, attempting to isolate neural signatures at progressively more abstract properties of speech and music. I will also present recent developments availing of deep learning models to more accurately probe human communication. This brief presentation (~5 min) is planned at the end of this young investigator symposium and aims to prompt a discussion on the great potential and possible risks of using such methodologies for bridging interpretations and results across species.

Investigating Maturation of Auditory Cortical Processing in Infants Using Magnetoencephalography
Bonnie K. Lau

University of Washington

Individual Abstract: Human infants rely on listening in noisy, real-world environments to acquire language, appreciate music, and to learn. However, many aspects of the neural mechanisms that support infant sound processing are not well understood due to the limited neurophysiological methods appropriate for use with infants. In this talk, I will present results from a study that employed magnetoencephalography (MEG) with recent advancements in movement compensation to obtain functional measures of auditory processing in awake infants. We recorded MEG responses longitudinally at 3, 6, and 11 months of age to both speech and complex tones and the neural generators of the MEG signals were determined using an equivalent current dipole (ECD) model. High quality MEG data with good signal-to-noise ratios were recorded in infants at all three timepoints and ECD field maps showed focal sources in the brain. Our results show that MEG in combination with advanced movement compensation offers a temporally precise method of investigating cortical sound processing in awake infants during the first year of life. This brief presentation will prompt a discussion on the challenges in bridging the interpretation of results across methods and populations.

Podium Session 7 - Regeneration

1:45 p.m. - 3:45 p.m.

Platinum Salon 5

Endogenous Progenitor Cells in the Adult (Human and Mouse) Cochlea

Natalia Smith-Cortinez*[1], Joep Kuppers[1], Huib Versnel[1], Hans G.X.M. Thomeer[1], Robert J. Stokroos[1], Louise Straatman[1]

[1]*University Medical Center Utrecht*

Category: Regeneration

Background: Supporting cells (SCs) in the cochlea give rise to hair cells (HC) in embryonic development. It has been recently demonstrated that SCs in adult mice express progenitor cell markers like SOX2 and LGR5. Promoting hair cell (HC) regeneration in vivo using transgenic animal models has been the focus of extensive research. Neonatal SCs have increased progenitor potential compared to adult and only a few studies (including ours) have shown survival of SCs with progenitor cell markers after severe HC loss in adult mice. In mammals, there is no evidence for spontaneous HC regeneration in adulthood. However, three-dimensional cultures have allowed the expansion and experimentation of human (and mouse) inner ear organoids. Here, we evaluated HC differentiation from cochlear- and vestibular organ- organoids from adult patients undergoing surgery for skull-base tumors and from adult normal-hearing and deafened mice.
Methods: Adult patients undergoing surgery for skull base tumors were included. Sensory epithelium of the cochlea and vestibular organ was collected in medium and tissue was digested to single cell suspension. Adult (postnatal day 40-300) Lgr5-eGFP-IRES-creERT2 heterozygous mice were used. Mice were deafened with a single dose of 100 mg/kg i.v. furosemide in combination with kanamycin (males: 700 mg/kg and females: 900 mg/kg) injected subcutaneously. Before deafening and seven days after deafening, auditory brainstem responses (ABRs) were recorded to click stimuli. Cochleas were harvested and digested to single cell suspension. Cells (derived from inner ear from patients or mouse cochleas) were filtered, mixed with Matrigel and 3D drops were made. Cells were grown on expansion medium (EM, contains histone deacetylase inhibitor

and Wnt activator) for 10 days and differentiation medium (DM, contains Notch inhibitor) for 3-10 days. Organoids were fixed, permeabilized and processed for immunofluorescence and whole-mounted for imaging in a confocal microscope.
Results: Vestibular-organ-derived organoids were generated in EM from all five patients so far included. Cochlea-derived organoids were generated in three out of five patients. After exposure to DM, vestibular organ-derived and cochlea-derived organoids produced MYO7A+ HC-like cells. Cochlear organoids from normal-hearing mice expressed LGR5 and Ki67 in EM and after differentiation newly produced MYO7A+ HCs were visible. Significantly less cochlear organoids were produced from deafened mice; however these organoids reached similar size as NH-cochlear organoids, expressed LGR5 and Ki67 in EM and differentiated to HCs after differentiation.
Conclusions: Cochlear and vestibular tissue from adult patients (and adult normal-hearing and deafened mice) possess progenitor potential and the capacity to generate inner ear organoids in vitro. After differentiation, HCs were visible in tissue derived from human cochlea, human vestibular organ, and adult mouse cochlea. The adult inner ear has regenerative capacity and can produce new MYO7A+ HCs.

"Turning off" Hippo Signaling Induces Cochlear Hair Cell Regeneration Primarily via Direct Trans-Differentiation

Huiqian Yu*[1], Xiaoling Lu[1], Jiaoyao Ma[1], Kunkun Wang[1], Shan Sun[1], Huawei Li[1], Xiaoling Lu[1]

[1]*Eye and ENT Hospital, Fudan University*

Category: Regeneration
Background: The mammalian sensory hair cells have limited capacity to regenerate.
Methods: Here, we show that turning off the Hippo signaling pathway can regenerate HCs in mammalian cochlea. As the effector molecule of Hippo signaling, YAP staining showed a punctate distribution in virtually all of the HCs and supporting cells (SCs), and turning off the Hippo pathway led to the nuclear accumulation of YAP, especially in SCs, which initiated HCs regeneration in vivo. More importantly, our results demonstrated that YAP nuclear translocation could replenish the damaged HCs. Specifically, over 80% of the increased HCs were non-proliferative upon turning off of Hippo signaling. Mechanistically, these effects of Hippo signaling worked synergistically with the Notch/Jag1 pathway, with genes functioning downstream of the Hippo signaling pathway. Finally, our data indicated that the regenerated HCs possess similar stereocilia structures as the original HCs and are able to form neural connections to auditory regions in vivo, which strongly supports the hypothesis that the regeneration of HCs in situ attracts new neurites to make new connections between HCs and neurons.
Results: Turning off Hippo signaling, accompanied by YAP nuclear translocation, could induce HCs regeneration and replenish the damaged HCs.
Conclusions: Taken together, turning off Hippo signaling provides new strategies for promoting cochlear SC proliferation, HC regeneration, and reconnection with neurons in mammals.

The Phoenix Platform as Novel Tool to Unveil Regenerative Pathways in Presenescent Auditory Neuroprogenitors

Francis Rousset*[1], Stéphanie Sgroi[1], Lucie Oberhauser[1], Rebecca Sipione[1], Giulia Schilardi[2], Sonja Kleinlogel[2], Vincent Jaquet[1], Pascal Senn[1]

[1]*University of Geneva,* [2]*University of Bern*

Category: Regeneration
Background: Hearing loss affects over 466 million people worldwide and is a major socioeconomic burden. Both genetic and environmental factors (i.e. noise overexposure, ototoxic drug treatment or ageing), promote irreversible degeneration of cochlear hair cells and associated auditory neurons, leading to sensorineural hearing loss. In contrast to birds, fish or amphibians, the mammalian inner ear is virtually unable to regenerate due to the limited stemness of auditory progenitors and no causal treatment is able to prevent or reverse hearing loss.

Methods: We have previously identified and characterized the phoenix auditory neuroprogenitors (ANPGs) as highly proliferative progenitor cells isolated from the A/J mouse cochlea. In the present study, we aimed at identifying signaling pathways responsible for the intrinsic high stemness of phoenix ANPGs. A transcriptomic comparison of traditionally low stemness ANPGs, isolated from C57Bl/6 and high stemness phoenix ANPGs was performed. Based on the differentially expressed pathways, we sought to reprogram the stemness of presenescent ANPGs, with the ultimate goal of recapitulating the proliferative phenotype seen in phoenix ANPGs.
Results: A strategic pharmacological combination of a WNT agonist and TGFβ/Smad inhibitors resulted in a remarkable increase in the growth of presenescent neurospheres, effectively allowing the expansion of ANPGs on an extensive scale. The so-called stemness-induced ANPGs exhibited the unique property of being freezable and thawable, facilitating their distribution to other research facilities. Importantly, even after more than 20 generations, stemness-induced ANPGs retained their capacity to differentiate into electrophysiologically active type I-like auditory neurons.
Conclusions: Both the stemness-induced and phoenix ANPGs represent a significant breakthrough in addressing a major bottleneck in auditory research. They offer an efficient, high-throughput, cost-effective, and 3R compatible approach for in vitro screening of potential otoprotective and otoregenerative drug candidates. This study may also open new avenues in the field of inner ear regeneration.

Mechanistic Insights Into the Regenerative Ability of Greater Epithelial Ridge Cells.

Marie Kubota*[1], Paul K. Lee[1], Taha A. Jan[2], Stefan Heller[1]

[1]*Stanford University School of Medicine,* [2]*Vanderbilt University Medical Center*

Category: Regeneration
Background: Adult mammalian cochlear cells are quiescent and lack regenerative potential. The greater epithelial ridge (GER; Kolliker's organ) is a cell population that transiently exists in neonatal mammals. Recent studies have suggested that GER cells have the ability to give rise to hair cells and supporting cell subtypes in murine damage models in vivo. We have demonstrated that GER cells have robust proliferative capacity, and they are the principal otic organoid-forming progenitors that give rise to new hair cells and supporting cells in vitro. The aim of this study is to identify molecular mechanisms that contribute to the regenerative capacity of GER cells. We plan to exploit these findings to develop strategies for induction of regenerative proliferation in supporting cells of the adult mouse cochlea.
Methods: To characterize the proliferation of GER cells, we used an organoid assay. GER cells were collected at greater than 90% purity from cochlear duct cells of postnatal day 2 (P2) Fgfr3-tdTomato/Sox2-GFP transgenic mice using fluorescence-activated cell sorting (FACS), and were cultured in media conducive for the organoid growth. The organoids were harvested on culture days 1, 3, and 7, followed by single-cell RNA-seq sequencing (SmartSeq2 protocol).
We computationally identified cell groups at the onset of GER cell proliferation, and gene expression changes associated with growth-promoting signals were identified. To narrow down the prioritized signaling pathways, we performed a screen using inhibitors and activators with organoid growth as a readout. Identified pathways essential for organoid growth were further tested on cochlear duct cells isolated from P2 (GER exists) and P14 (GER does not exist) using adeno-associated virus (AAV)-based overexpression of specific genes.

Results: We identified putative cell growth-promoting effectors, such as integrins, endogenous opioids, cytoplasmic calcium-binding proteins, and galectins. Inhibitors of galectin-1 and galectin-3 were particularly effective in attenuating organoid formation. We also identified specific transcription factors upregulated at the onset of GER cell proliferation. AAVs encoding these transcription factors, in addition to galectin-1 and galectin-3 were individually created and P2 and P14 cochlear duct cells were transduced with these AAVs followed by assaying organoid formation and cell proliferation. We plan to present the results of our ongoing experiments including a comparative validation of our manipulations with respect to P2 and P14 cochlear duct cells.
Conclusions: We identified proliferating otic progenitors in single-cell RNA-seq of GER-derived organoids. Galectins are potential effectors that contribute to the GER cells' strong ability to proliferate. We are in the process of investigating the functional aspect of galectins. We are also validating whether specific

transcription factors contribute to the stemness of GER cells, and if these transcription factors are capable of inducing proliferation in P14 or older cochlear supporting cells.

Progenitor Cell Pools in the Chick Auditory Epithelium and Their Response to Hair Cell Loss

Marie Takeuchi*[1], Mami Matsunaga[2], Ryosuke Yamamoto[2], Tomoko Kita[2], Koichi Omori[2], Takayuki Nakagawa[2]

[1] *Kyoto University,* [2] *School of Medicine, Kyoto University*

Category: Regeneration

Background: In contrast to mammals, the avian auditory epithelium is capable of hair cell (HC) regeneration through direct conversion or mitotic division of supporting cells (SCs). Single-cell RNA sequencing has been applied to studies of HC regeneration in chick and zebrafish auditory epithelia, which contributed to the subtyping of SC populations. In zebrafish neuromasts, SC subtypes are well defined according to their roles in HC regeneration. In particular, the presence of a stem cell population was identified in zebrafish neuromasts, whereas its presence is unknown in the chick auditory epithelia. Previously, we performed single-cell RNA sequencing of an explant culture model for chick HC regeneration, which indicated the presence of a stem or progenitor pool in chick basilar papillae (BP) as a sub-population of SCs (Matsunaga et al., 2023). The expression patterns of CD44 and FGFR1, markers of this SC population, indicated their localization in the neural and abneural edge of BP. The aim of this study was to further characterize this SC subtype and investigate its response to HC loss.

Methods: We used an explant culture model of chick BP for HC regeneration (Matsunaga et al., 2020), in which a 48-h exposure to streptomycin induced total HC loss followed by HC regeneration via SC direct conversion. To illustrate activated signaling pathways in SC subtypes, we performed a signaling pathway analysis using the dataset from our previous study (Matsunaga et al., 2023). We verified the expression patterns of molecules related to activated signaling pathways in the SC subtype annotated as progenitor pools.

Results: Signaling pathway analysis demonstrated that EPHB and FGF signaling pathways were the most upregulated pathways in the SC subtype of progenitor pools. According to differentially expressed genes of the SC subtype of progenitor pools, we focused on the expression patterns of EPHA2 and EPHB2. EPHA2 expression exhibited a gradient decrease from the neural to the abneural edges in BP, suggesting its contribution to the formation of the niche for progenitor pools in the neural portion. EPHB expression was observed in the neural and abneural edges of BP, similar to FGFR1 and CD44. Total HC loss induced no alteration in the expression patterns of EPHA2 and EPHB2 in BP.

Conclusions: The present results indicate that EPHA and EPHB signaling may play a role in the maintenance of progenitor populations in chick BP.

Combinatorial Transcription Factor Gene Therapy Induces Hair Cell Regeneration on Both Sides of the Basilar Membrane

Yujie Liu[1], Lin Yang[1], Sunita Singh[2], Lisa Beyer[1], Diane Prieskorn[1], Donald Swiderski[1], Hanna Erhardt[1], Andrew Groves[2], Yehoash Raphael*[1]

[1]*University of Michigan,* [2]*Baylor College of Medicine*

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 ear, using two lesion models. One model was the flat epithelium (FE), a condition characterized by the loss of both HCs and supporting cells and the transformation of the organ of Corti into a simple flat or cuboidal epithelium, which was induced in guinea pigs. The other model was the DTR mouse, where an injection of diphtheria toxin (DT) leads to degeneration of all HCs leaving behind supporting cells that appear morphologically differentiated.

Methods: Guinea pigs were deafened by injecting neomycin (15%) which produced FE. Mature DTR mice were deafened by injecting DT (20 ng/g). In both species, an adenovirus vector containing gene inserts for Gfi1, Atoh1, Pou4f3, Six1 (GAPS) and a venus reporter, or the reporter alone, was injected via scala media. Guinea pigs were given the vector one week after deafening; mice were given the vector either at the same time as deafening or one month later. At 1 or 2 months after adenovirus injection, cochlear whole-mounts were labeled with antibodies against Myosin VIIa, as a hair cell marker, and Sox2. Presence of new hair cell like cells (HCLC) was assessed and quantified using epi-fluorescence or confocal microscopy.
Results: All groups treated with the GAPS vector exhibited large variability in the number of HCLC with some animals displaying no new cells. In guinea pigs, the FE was negative for Sox2, and HCLCs could be detected in the mesothelial layer of cells residing in the scala tympani side of the basilar membrane. In DT-deafened DTR mice, HCLCs appeared in the area of the organ of Corti, in the adjacent Hensen's cell and inner sulcus areas, and in the mesothelial layer. Mice given the vector at deafening had nearly 50% more HCLCs (70.0 vs 48.4) than mice given the vector a month after deafening, which was not statistically significant ($t=1.38$, $p=0.17$). However, giving the vector at deafening produced three times as many HCLCs in the organ of Corti (55.4 vs 16.3) as giving it on month later, which was significant ($t=4.08$, $p=0.0002$).
Conclusions: GAPS cocktail of transcription factor transgenes can convert mesothelial cells to HCLCs in guinea pigs with FE. In deaf DTR mice, HCLCs can be generated in the organ of Corti and flanking epithelial regions, as well as in mesothelial cells. In epithelial cells, presence of Sox2 may be needed for transdifferentiation into HCLCs.

Supported by the HRP and NIH-NIDCD grant R01-DC014832

Unexpected Dynamics of Vestibular Hair Cell Differentiation During Normal Development and Local Regeneration

Shahar Kasirer[1], Olga Loza[1], David Sprinzak*[1]

[1]*Tel Aviv University*

Category: Regeneration
Background: The vestibular sensory system contains five sensory domains with alternating patterns of sensory hair cells (HCs) and non-sensory supporting cells (SCs). These alternating patterns are considered to emerge through the process of Notch mediated lateral inhibition. Many non-mammalian species have the capability to regenerate lost HCs by the proliferation and differentiation of nearby SCs. In contrast, in mammals the capacity to regenerate HCs quickly diminishes with age (in mice it is significantly reduced after the first postnatal week). However, little is known about the factors controlling regenerative processes in the vestibule and how lateral inhibition is employed to induce trans-differentiation of from SCs to HCs following HC death. Moreover, it is unclear why the capacity to regenerate diminishes with developmental age.
Methods: We developed a live imaging assay for mouse utricle explants that can be used to track normal development and regeneration. To track cell morphology and differentiation, we use mice containing ZO1-EGFP (marking apical boundaries) and Atoh1-mCherry (marking differentiation of HCs). We track the dynamics of the developing utricle explants at different developmental stages and under different perturbations for 48-72 hours. We perform quantitative analysis of the morphological and regulatory events (delaminations, divisions, and differentiation) in the tissue. We also track the local regeneration dynamics (proliferation and differentiation) following laser ablation of single HCs at different developmental stages. Finally, we look at the effect Notch inhibition (using DAPT) and mechanical perturbation (using Rho kinase inhibitor) on vestibular differentiation and regeneration.
Results: Our live imaging results of E17.5 and P0 utricle explant, reveal that utricle development is a highly dynamic process, where SCs exhibit cell divisions, delaminations, and differentiation events. SCs to HCs differentiation events at E17.5 typically obey the rules of lateral inhibition, namely, that newly differentiating cells do not have any HC neighbors. In contrast, at P0 differentiating cells typically have one HC neighbor, a behavior that does not align with standard lateral inhibition models. Moreover, a similar behavior is observed during local regeneration experiments. At E17.5, following laser ablation of a single HC, we observe trans-differentiation of a SC, with no HC neighbors, into a HC (as expected from lateral inhibition). In contrast, at P0, trans-differentiating cells typically have one HC neighbor (does not match lateral inhibition). Finally, we

also show that treating the explants with Rho Kinase inhibitor dramatically affects the dynamics of HC differentiation and trans-differentiation.
Conclusions: Live imaging of vestibular explants reveals complex dynamics of HC differentiation during normal development and regeneration following single cell ablation. Our findings suggest that classic lateral inhibition model cannot account for the differentiation and trans-differentiation dynamics observed at more advanced developmental stages. Our results suggest that local factors, including cell mechanics, may play an important role during HC regeneration.

Lsd1 Knockout Promotes Atoh1-Mediated Conversion of Supporting Cells to Hair Cells in Mouse Cochleae

Jackson Diers*[1], Yan Zhang[1], Shu Tu[1], Zhenhang Xu[1], June Li[1], Huizhan Liu[1], Mi Zhuo[1], Guanfang Xie[1], Litao Tao[1], Jian Zuo[2]

[1]*Creighton University School of Medicine,* [2]*Creighton University School of Medicine and Ting Therapeutics LLC*

Category: Regeneration
Background: Atoh1 is the master regulator dictating hair cell fate in the cochlea during development, and Atoh1 overexpression induces supporting cell transdifferentiation in mouse cochleae at neonatal ages. However, Atoh1 fails to exert fate conversion in mature supporting cells in terms of conversion rate and transcriptome profile, suggesting potential epigenetic barriers blocking the transcription activation of Atoh1 target genes. Lysine specific demethylase 1 (Lsd1) is a histone demethylase that is known to play a key role in cell fate determination by silencing lineage-specific genes. Previously, we knocked out Lsd1 while simultaneously overexpressing Atoh1 in Lgr5+ supporting cells at P0/P1 and found that Lsd1 KO led to significantly more induced hair cell-like cells compared to Atoh1 overexpression alone. Here, we elucidate the phenotype of hair cell-like cells and the underlying molecular mechanism using scanning electron microscopy, immunohistochemistry, and Cut and Run sequencing.
Methods: Animals: Tamoxifen was injected at P0/P1 in four groups of compound transgenic mice: (1) Lgr5-CreER:Atoh1-HA: Lsd1flox/flox:TdTomato(LALT); (2) Lgr5CreER, Atoh1-HA, tdTomato (LAT); (3) Lgr5CreER, Lsd1flox/flox; tdTomato (LLT), and (4) Lgr5CreER; tdTomato (LT) mice.
Immunohistochemistry: At P7 the cochleae were harvested, fixed, and immunolabeled for hair cell specific markers.

Cut and Run: Tamoxifen was injected at P0/P1 in LT and LLT mice. At P7, the cochleae were dissected and Lgr5+ supporting cells purified using fluorescence activated cell sorting were used for Cut and Run.

Results: Lsd1 knockout with Atoh1 overexpression in Lgr5+ cochlear supporting cells at P0/P1 results in significantly more hair cell-like cells than with Atoh1 overexpression alone, primarily in the inner hair cell (IHC) region. Newly converted hair cell-like cells are derived from Lgr5+ supporting cells. Using Cut and Run, we profile genome-wide LSD1 binding sites and changes in element priming by H3K4me1 after knockout of Lsd1.
Conclusions: Lsd1 knockout at P0/P1 promotes Atoh1-mediated conversion of supporting cells to hair cells at P7 presumably by alleviating the epigenetic silencing of hair cell gene elements. Our data suggests that Lsd1 KO might help drive Atoh1-mediated conversion by preventing the removal of element priming histone modifications, subsequently allowing Atoh1 to access its target genes more easily. These findings help shed light on the role of epigenetics in the reprogramming of differentiated sensory cells.

Podium Session 8 - Binaural Hearing and Sound Localization

1:45 p.m. - 3:45 p.m.

Platinum Salon 6

Does Hearing Experience With Jittered Input Affect the Temporal Weighting of Binaural Stimuli in Cochlear Implanted Rats?

Nicole Rosskothen-Kuhl*[1], Tim Fleiner[1], Emily Becker[1], Alexa N Buck[2], Susan Arndt[1], Jan W Schnupp[3]

[1]*University of Freiburg, ,* [2]*City University of Hong Kong,*

Category: Binaural Hearing and Sound Localization

Background: Sound localization is one of the major challenges for bilateral cochlear implant (CI) users, especially if they suffer from early hearing loss. Their ability to use binaural cues, particularly interaural time differences (ITDs), falls below that of normal hearing peers. Our recent work on neonatally deafened (ND), CI implanted rats has demonstrated that very good ITD sensitivity with remarkably low thresholds (~50 µs) can be developed even in the absence of early sensory input if the CIs are synchronized. However, these hearing inexperienced rats do appear to have abnormal temporal weighting functions (TWFs), with a much less pronounced "onset dominance" (precedence effect) than is observed in normally hearing humans or rats. Here we investigate the effect of hearing experience with jittered binaural input on the temporal weighting of pulses in ITD perception of ND CI rats.

Methods: Neonatal rat pups were deafened using kanamycin and bilaterally implanted with CIs as young adults. Subsequently, rats learned to lateralize ITDs and interaural level differences (ILD) over the normal physiological range of ±120 µs and ±5 dB, respectively, using a two-alternative forced choice task, with each single pulse of a burst receiving a random jitter between ±60 µs. Biphasic electrical stimuli with a pulse rate of 250 pps were delivered via CIs. After 6 weeks of ITD/ILD lateralization training with varying jitter on each single pulse, the rats performed a temporal weighting test similar to that developed by Brown and Stecker (2010). During testing, each pulse in a stimulus burst had a different, randomly selected ITD between ±120 µs. TWFs were calculated using multiple regression to determine the perceptual weight of each pulse.

Results: In contrast to the onset weighting of ITDs observed in normal hearing rats and ND rats with synchronized CIs, our CI rats with jittered input during training showed no perceptual up-weighting of only the first pulse, in other words, they showed no measurable precedence effect. Instead, TWF analysis revealed that all pulses in the train contributed significantly and with similar weight to the animals' lateralization judgments.

Conclusions: In summary, the behavior results demonstrate that jittered input during sound lateralization training affects the temporally weighting of ITD cues in a way that is different from the temporal weighting previously described for normal hearing listeners and rats with binaurally synchronized CIs. All CI rats showed significant weighting of all pulses, which may indicate that the auditory system averages ITDs across all pulses to make a lateralization decision.

How Sensitive is the Deaf, Cochlear Implanted Auditory System for Interaural Time and Interaural Level Differences and How Do They Interact in Spatial Hearing?

Sarah Buchholz*[1], Heika Hildebrandt-Schoenfeld[1], Theresa A Preyer[1], Henrike Budig[1], Susan Arndt[1], Jan W Schnupp[2], Nicole Rosskothen-Kuhl[1]

[1]*University of Freiburg,* [2]*City University of Hong Kong*

Category: Binaural Hearing and Sound Localization

Background: Sound localization in the horizontal plane is based on two binaural cues: interaural level differences (ILDs) and interaural time differences (ITDs). For bilateral cochlear implant (biCI) users, spatial hearing is one of the major challenges, and ITD sensitivity is particularly poor, with most early deaf biCI patients relying almost exclusively on ILDs. However, our recent work demonstrates that neonatally deafened rats with exclusively precise temporal information in the timing of the electrical stimulation pulses (synchronized) biCIs can develop excellent ITD sensitivity. Here, we investigate whether ND rats provided with synchronized biCI input from the outset can also develop good ILD sensitivity and how sensitivities to ILDs and ITDs compare and interact.

Methods: Nine neonatally deafened biCI rats were trained under synchronous stimulation conditions to lateralize pulse trains at a pulse rate of 900 pps containing ITDs in a range of ±120 µs and ILDs in a range of ±5 dB. After the training period, rats were tested on their ITD and ILD sensitivity, respectively. Subsequently, stimuli containing either congruent or incongruent ITDs {±100, 80, 60, 0} µs and ILDs {±6, 4, 1, 0.5, 0} dB

were presented. Thereby, trials in which ITDs and ILDs varied independently from each other were used to determine the relative strength and interaction of these two spatial cues.
Results: Our neonatally deafened biCI rats developed both excellent ITD and ILD sensitivities. When presented congruently the two types of cues interacted additively. Importantly, very small pulse timing ITDs of ~80 µs could influence an animal's lateralization judgment as powerfully as relatively very large electrical pulse amplitude ILDs of ~4 dB, resulting in a mean time-intensity-trading-ratio of 18 µs/dB over all animals.
Conclusions: Our results show that under synchronous CI stimulation, the hearing inexperienced mammalian auditory system, at least in rats, can develop very good ITD and ILD sensitivities. Under this condition, ITDs can be so strong compared to ILDs that they would interfere even with the perception of large ILDs. Since biCI patients do not receive informative pulse timing ITDs, it could be that they need to become ITD-insensitive to perceive ILDs. This could explain why poor ITD sensitivity is common in human biCI patients with current CI processors. These findings add to the growing evidence that the inability of CI processing strategies in current clinical use to encode auditory information with precise pulse timing is a very significant technical limitation.

Role of Inhibition in Shaping Interaural Time Difference Tuning in the Medial Superior Olive

Ben-Zheng Li*[1], Matthew Ridenour[1], Tim Lei[2], Achim Klug[1]

[1]*University of Colorado Anschutz Medical Campus,* [2]*University of Colorado Denver*

Category: Binaural Hearing and Sound Localization
Background: During the sound localization process, the auditory brainstem analyzes the interaural time difference (ITD) in the medial superior olive (MSO) by integrating precisely-timed excitatory and inhibitory inputs from both ipsilateral and contralateral sides. The peak location of the ITD tuning curve can be effectively shifted by adjusting the relative timing and strength of excitatory inputs to MSO neurons. The role of inhibitory inputs in ITD tuning, however, remains incompletely understood and requires further investigation.
Methods: In this study, in-silico simulations were first conducted to estimate the effects of contralateral inhibition on ITD coding, using a computational model of the auditory brainstem. Subsequent in-vivo optogenetic experiments were performed to measure the ITD tuning characteristics of MSO neurons receiving inhibitory input from medial nuclei of the trapezoid body (MNTB), which were modulated through optical stimulation.
Results: The simulation results indicated that both the action potential timing and the synaptic strength of contralateral inputs from the MNTB played key roles in shaping the width of the ITD tuning curves and in refining the precision of ITD detection at the microsecond level. Moreover, our preliminary experimental findings aligned with the model prediction, showing that increased contralateral inhibition led to a reduction in the width of the ITD tuning curve. This narrowing increased the contrast for differentiating small symmetric ITDs, thereby enhancing detection precision.
Conclusions: The study reveals how contralateral inhibition shapes ITD tuning and improves detection precision during the sound localization process. It further suggests that alterations or demyelination in the MNTB could potentially lead to deficits in spatial hearing ability.

Binaural-Bimodal (Electric/Acoustic) Stimulation Degrades Neural Coding of Interaural Time Differences

Maike Vollmer*[1], Merle Berents[1], Andreas Schulz[2], Frank W. Ohl[2]

[1]*University Hospital Magdeburg; Systems Physiology of Learning, Leibniz Institute for Neurobiology, Magdeburg,* [2]*Systems Physiology of Learning, Leibniz Institute for Neurobiology*

Category: Binaural Hearing and Sound Localization
Background: Unilateral cochlear implants (CIs) allow the functional restoration of binaural hearing in subjects with single-sided deafness (SSD). However, speech perception in noise and directional hearing in

SSD-CI users is typically poorer than in normal hearing listeners, indicating suboptimal binaural integration of unilateral CI stimulation and contralateral acoustic hearing.
Methods: In order to characterize the limitations in the neural representation of binaural cues in bimodal (combined electric/acoustic) stimulation, we quantitatively compared phenomenological aspects (tuning curves) and functional efficacy (Fisher information) of interaural time difference (ITD) coding of single neurons in the inferior colliculus in response to unimodal (bilateral acoustic) and bimodal stimulation. Mongolian gerbils were implanted unilaterally or bilaterally with round window electrodes. This approach allowed electric stimulation of auditory-nerve fibers while maintaining acoustic sensitivity of the implanted ears.
Results: The incidence of ITD-sensitive neurons was similar for unimodal acoustic and bimodal stimulation. However, despite within-ear balancing of response strengths to acoustic and electric stimulation, responses to bimodal stimulation demonstrated a lower degree of ITD sensitivity (signal-to-total variance ratio), broader ITD tuning, and lower Fisher information when compared to unimodal stimulation. Correcting for temporal offsets in bimodal stimulation sharpened averaged bimodal ITD tuning functions and shifted them towards the physiological range of ITDs. Although maximum Fisher information in bimodal stimulation remained lower than in unimodal stimulation, the described shifts led to a significant increase of Fisher information in comparison to unshifted data.
Conclusions: Our results demonstrate that balancing binaural level cues and correcting for temporal offsets between unimodal acoustic and bimodal ITD tuning can improve binaural benefits in bimodal stimulation and, thus, suggest strategies for the future development of improved cochlear implants supporting binaural processing in bimodal stimulation.

Supported by DFG VO 640/2-2.

Speech-In-Noise and Sound Localization in Single Sided Deafness and Cochlear Implantation: Differences in Complex Head Movements, Localization, and Speech Performance in Noise

Nadine Ibrahim*[1], Gerilyn Jones[2], Obada Abdulrazzak[2], Madison Epperson[1], Chioma Anidi[2], Carolyn Kroger[2], Jackson Graves[2], Renee M. Banakis Hartl[1]

[1]*University of Michigan Hospitals,* [2]*University of Michigan*

Category: Binaural Hearing and Sound Localization
Background: Patients with single-sided deafness (SSD) have difficulty with sound localization and understanding speech-in-noise (SIN), resulting in increased listening effort. Though many studies have outlined the potential benefits of cochlear implant (CI) use in this population, only a few studies have examined changes in compensatory head movements for patients with SSD who use CI. When binaural hearing is restored with a CI, patients may continue to rely on monaural cues, and as such, individuals with SSD and a CI may not be deriving optimal binaural benefit. Improved understanding of the impact of SSD on listening behavior may yield improvements in personalized device performance, functions of binaural hearing, and ultimately quality of life. The objective of this study is to analyze the impact of SSD on listening behavior by quantifying and comparing the compensatory head movements and ear-specific sound information in normal hearing individuals to those with SSD, both with and without a CI.
Methods: This is a non-randomized, prospective study in which subjects with normal hearing or SSD and a CI undergo a novel combination speech-in-noise (SIN) and localization task in a dark, semi-anechoic chamber with a 24-speaker array equally spaced in a 360-degree configuration. An orienting stimulus followed by Harvard IEEE sentences is presented from one of 12 speakers in diffuse background pink noise with varying SNR (-10 to +10 dB). Subjects are asked to move naturally while wearing a head tracker and to indicate target speech source. In-ear microphones measure ear-specific acoustic input during the task. This novel paradigm is designed to closely mimic real-world human behavior in daily complex listening tasks.
Results: Individuals with SSD will demonstrate decreased localization accuracy (root-mean square error and linear best-fit characteristics across targets), increased response time (ms), increased absolute total head displacement (degrees), and increased movement delay (ms) compared with controls, with CI use resulting in a relative limited improvement across domains. SIN performance will be characterized by percent correct.

Acoustic cues measured at each ear reflect signal-to-noise ratio (SNR) optimization in the better-hearing ear in SSD in both aided and unaided conditions compared with a more equal SNR between ears for those with normal hearing.
Conclusions: Individuals with SSD both with and without CI conditions demonstrate decreased localization accuracy and distinct patterns of compensatory head movements and acoustic cue generation when compared to controls. This study supports the relationship between SSD and specific listening behaviors; further study will utilize this data to assess the impact of compensatory head movements on device programming, efficacy, and use. Our findings, using a paradigm that models real-word complex listening experiences, may lay the foundation for improved device programming and device benefit, an important contribution to the growing practice of cochlear implantation for SSD.

Effects of Preceding Exposure on Distance Perception in Varying and Fixed Virtual Environments

Norbert Kopco*[1], Myroslav Fedorenko[1]

[1]*P.J. Safarik University, Kosice*

Category: Binaural Hearing and Sound Localization
Background: Previous research showed that listeners calibrate to the acoustic environment when judging distance in real reverberant rooms, resulting in gradually improving performance even without explicit training. In contrast, similar enhancements are not observed in anechoic environments, suggesting that the improvements are due to tuning to specific reverberation-related cues in a given room. The mechanisms underlying this "room learning" are not well understood. A previous study [Schoolmaster, Kopčo, and Shinn-Cunningham, J Acoust Soc Am 113, 2285, 2003], examined 1) how consistency in simulated room presentation affects performance, and 2) whether previous exposure to consistent vs. inconsistent simulation influences the ability to interpret the distance cues in different rooms. It showed that distance perception is more accurate when the simulated environment is consistent than randomly chosen from trial to trial. Here, we further analyze the data to examine how they depend on type of simulated room (anechoic vs. reverberant) and the direction of stimulus presentation (frontal vs. lateral) and, we develop a model that proposes how subjects combine available distance cues in different contexts.
Methods: Three environments were simulated in the experiment, anechoic, center of a classroom and corner of a classroom. Participants were divided into two groups. The first group started with 6 sessions in a fixed environment (each session maintaining a consistent room condition in a random order) and then proceeded to 6 sessions in a mixed environment (room conditions randomly selected from trial to trial). The second group proceeded in the reverse order. Each session comprised eight runs, each consisting of 45 trials. Nine distances ranging from 15 to 170 cm were presented randomly within each run. Each run kept the direction of simulation (frontal vs. lateral) fixed.
Results: Overall performance, evaluated using the correlation coefficient, showed a complex pattern of results. The group that started with inconsistent room generally performed poorly, and even when later exposed to consistent simulation only improved performance slightly. On the other hand, the group starting with consistent simulation performed very accurately in each room and, when switched to inconsistent simulation, its performance deteriorated differentially depending on simulated room and direction. Analysis of response biases showed that these results are consistent with a model that assumes that 1) the listeners use room-specific weighting parameter values to combine the distance cues in consistent simulation, but that 2) they use one non-specific weighting parameter set when simulation is inconsistent.
Conclusions: These results show that listeners use past experience when calibrating to specific environments and that they might not be able to use an optimal room-specific tuning even for dramatically different environments (like anechoic vs. reverberant) when the environments switch rapidly.

[Work supported by Horizon Europe HORIZON-MSCA-2022-SE-01 grant N° 101129903 and VEGA 1/0350/22]

Providing a Binaural Advantage by Improving the Signal-To-Noise Ratio in the Acoustically Better Ear

Enrique Lopez-Poveda*[1], Almudena Eustaquio-Martín[1], Milagros J Fumero[1], Fernando M. San-Victoriano[1]

[1]*University of Salamanca*

Category: Binaural Hearing and Sound Localization

Background: The recognition of speech in noise is better when listening with two ears rather than one. This binaural advantage (BA) is reduced for users of bilateral hearing devices (hearing aids or cochlear implants) probably because the two devices provide interaurally uncorrelated signals. To provide a BA, some efforts are directed to designing bilateral stimulation systems with greater interaural correlation. Here, we investigate whether a BA can be provided more simply by improving the signal-to-noise ratio (SNR) in the acoustically better ear.

Methods: 16 people with normal hearing were asked to recognize the key words of sentences presented in competition with five masking sounds. Stimuli were delivered via loudspeakers mounted on a ring in a room with low reverberation (t60=20 ms). The target sound source (female speaker, 65 dB SPL) was located in front of the listener (0-deg. azimuth). Four of the maskers (international female fluctuating maskers, 60 dB SPL) were located at +/-45-deg. and +/-135-deg. azimuth. The fifth masker (steady noise, 75 dB SPL) was randomly located in the arc between +/-120-deg. for each sentence presentation. This made it uncertain what the better ear would be. Participants performed the task while listening with two ears, or with the left or the right ear alone. To make this possible with life sounds, stimuli were picked up via an acoustic manikin placed in the center of ring and sent to the listener, who was sitting in a separate room. Sounds were either unprocessed or processed through a binaural algorithm that can improve the SNR in the acoustically better ear by attenuating low-frequency contralateral sounds (Lopez-Poveda et al., 2022, Hear Res 409:108469).

Results: For unprocessed sounds, word recognition was better when listening with two ears than with either ear alone. This confirms that there was a BA even when recognition with the acoustically worse ear was zero. For processed sounds, recognition was equal when listening with one or two ears, i.e., a BA did not occur. This is probably because the algorithm distorted the inter-aural correlation. However, the recognition of processed sounds with one ear was as good as that of unprocessed sounds with two ears.

Conclusions: Listeners use the information in the acoustically better ear even when they are uncertain as to which ear that will be. A BA can be provided by improving the signal-to-noise ratio in the acoustically better ear, as if the tested algorithm mimics the binaural system. Future work will investigate the benefit of the algorithm for users of hearing devices. Work supported by MED-EL GmbH, the Spanish Ministry of Science and Innovation (PID2019-108985GB-I00), Junta de Castilla y León, and the European Regional Development Fund.

Cortical Patches and Map of Auditory Space

Chenggang Chen*[1], Xindong Song[1], Yueqi Guo[1], Xiaoqin Wang[1]

[1]*Johns Hopkins University*

Category: Binaural Hearing and Sound Localization

Background: Cerebral cortices have topographical maps of somatosensory space (somatotopy), visual space (retinotopy), and auditory frequency (tonotopy) but not auditory space. Previous studies have failed to identify any maps or patches of spatial representation in the mammalian auditory cortex. A prevailing hypothesis of cortical spatial processing is the distributed population coding, supported by the evidence that neurons respond broadly to sound locations on the contralateral hemifield. However, electrophysiology and fMRI methods have limited spatial resolution to evaluate the cortical representation of sound locations.

Methods: To search for an auditory spatial map, we took advantage of the flat brain of the marmoset and developed a wide-field calcium imaging method. Specifically, we 1) implanted an artificial dura window (9.5mm X 6.4mm) that covered the entire auditory cortex and neighboring multisensory region (medial superior temporal, MST), 2) injected AAV-DJ CaMKII-GCaMP6s virus into cortical layer 5 to uniformly label neurons and confirmed labeling efficiency and location through histology, 3) performed chronic (over three years for one animal and one year for two animals) imaging in the awake and sleep condition with

animals' arousal state (temperature, respiration, heart rate, and pupil diameter) and spontaneous movements (eye, face, trunk, and tail) monitored simultaneously.
Results: We found that most cortical areas preferred contralateral sound locations, but regions tuned to the front and ipsilateral locations formed five to ten patches. Next, we investigated whether spatial tuning of patches depends on interaural time and level differences (ITD and ILD) cues. We found patches that preferred low frequency were ITD cue dependent. In contrast, patches that preferred high frequency were cue-independent. Furthermore, a neighboring multisensory MST had weak sound-driven responses and was not topographically organized by sound frequency. Surprisingly, MST was organized topographically by sound locations that range from far-contralateral to front. We also identified a retinotopic map in the MST that largely overlapped with the auditory spatial map. Finally, auditory spatial patches and map were stable across years and sound levels, under different arousal states, and during sleep.
Conclusions: In summary, we found that auditory space, like somatosensory space and visual space, is represented in the cortex through patches and map.

Workshop: DMA Supportive Allyship and STEM

4:00 p.m. - 6:00 p.m.

Platinum Salon 5

Supportive Allyship in STEM

Chair: Jeffrey Cheng*, Mass. Eye and Ear/Harvard Medical School*
Co-Chair: Samantha O'Connell, *Keck School of Medicine of USC*

Presenters:

Samantha O'Connell, *Keck School of Medicine of USC*

Meg Warren, *Western Washington University*

Samantha O'Connell, *Keck School of Medicine of USC*

Matheus Macedo-Lima, *University of Maryland*

Jeffrey Cheng, *Mass. Eye and Ear/Harvard Medical School*

Overall Workshop Description: The Association for Research in Otolaryngology (ARO) is committed to fostering scientists and technical experts that welcome diverse people, approaches, and ideas. While ARO is becoming more diverse, it reflects a profile largely of Caucasian/white and male-identifying members that is characteristic of STEM fields. Thus, the ARO Diversity and Minority Affairs Committee (DMAC) is dedicated to increasing equity and inclusion within the ARO community with year-round educational programming for its members. Last year, the ARO DMAC hosted a well-attended MidWinter Meeting workshop that discussed microaggressions, allyship, and equitable mentoring focused on racial, ethnic and sexual identity minority groups. The culture of sexism, however, is still present in the STEM community. Workplace sexism is a source of stress for non-male-identifying individuals that leads to work dissatisfaction, burnout, and job departure. Allyship by male-identifying coworkers can be a powerful tool for mitigating the impact of sexism. Therefore, for the 2024 MidWinter Meeting, we propose to dive deeper into gender allyship. The main objectives of this workshop are to provide participants with a descriptive overview of allyship, help them identify situations when allyship is needed, and educate them on how to build allyship safely and supportively. This two-hour workshop will begin with a ~15-minute platform presentation by an ARO DMAC member on membership demographics followed by a ~30-minute presentation by Dr. Meg Warren from Western Washington University to introduce the topic of allyship. In the next ~45 minutes, Dr. Warren will provide evidence from her own research pertaining to allyship between male- and female-identifying members in STEM fields and will facilitate group discussions to share real-world situations of allyship in participants'

lives. Anticipated outcomes of this workshop include raising awareness about allyship within the ARO community and providing members with tools to promote effective allyship within their own academic and work organizations.

In Memoriam Symposium - Mechanics and Physiology of the Inner Ear Vestibular Organs: A Tribute to J. Wally Grant

4:00 p.m. - 6:00 p.m.

Platinum Salon 6

Mechanics and Physiology of the Inner Ear Vestibular Organs: A Tribute to J. Wally Grant

Chair: Jong-Hoon Nam*, University of Rochester*

Ultrafast Non-Quantal Vestibular Synaptic Transmission Probably Differentiates Vestibular From Cochlear Responses to Sound and Vibration: A Tribute to Wally Grant

Christopher Pastras

Macquarie University

Individual Abstract: Wally Grant was an outstanding engineer who made significant contributions towards understanding how sound or vibration can activate otolith irregular afferents from the striola of the utricular macula. His insight - that the otoliths can function both as an accelerometer and a seismometer - is a major step in the story of the otoliths. How far he has taken us - when he started studying otolith mechanics it was believed by engineers that the natural frequency of the otoliths was about 2Hz. Now it is widely accepted, thanks to Wally, that the natural frequency is around 400Hz and new evidence shows that sound and vibration at frequencies of greater than 2000Hz can activate what was regarded as sluggish sensory system.: indeed some otolithic afferents even outperform auditory afferents in temporal processing, almost certainly due to ultrafast synaptic transmission at the calyx shown by Contini et al (2020). Sound or vibration are now being widely used to investigate vestibular primary neurons - both by recordings of action potentials in individual otolithic neurons and by recordings of gross nerve responses to clicks (the vestibular or cochlear compound action potentials (vCAP or cCAP)). We have shown in guinea pigs it is possible to differentiate the responses of auditory vs vestibular systems to clicks because 1) the vCAP can be recorded after complete surgical or chemical cochlear ablation and 2) for transient stimuli, both forward masking or broadband noise cause a reduced cCAP but have negligible effect on the vCAP. Why the difference between auditory and vestibular responses? Each cochlear afferent neuron forms a bouton ending on the inner hair cell receptor whereas each irregular vestibular afferent forms a unique calyx ending on an otolithic type I receptor. For both cochlear and vestibular synapses the transmitter is glutamate, but after glutamate blockade by CNQX, cCAPs are lost but vCAPs remain - a result that suggests that it is ultrafast non-quantal transmission at the calyx synapse (effectively electrical resistive coupling) which is the main generator of the vestibular compound action potential.

Vestibular Biomechanics in Health and Disease

Richard Rabbitt

University of Utah

Individual Abstract: In a paper entitled "The fluid mechanics of the semicircular canals" published with W.C. Van Buskirk and Y.K. Liu in the Journal of Fluid Mechanics (1976, 78(1): 87-98), J. "Wally" Grant applied first principles of fluid mechanics to predict responses of the semicircular canals to angular motion.

Results demonstrate that fluid displacement relative to the temporal bone in response to sinusoidal angular acceleration of the head is proportional to angular velocity of the head over a broad bandwidth. In subsequent work, he applied first principles of mechanics to predict responses of the utricle to linear gravito-inertial acceleration, and responses of vestibular hair bundles to applied forces. Here, I review how first principles of mechanics can be applied to understand, diagnose, and treat common disorders of the semicircular canals including benign paroxysmal positional vertigo (BPPV), Tullio phenomena, and third window syndrome. Mechanical and afferent neural responses to the Head Impulse Test (HIT) and audio-frequency sound and vibration (e.g. VEMP and VsEP stimuli) are also described.

Supported by: R01-DC006685 (Rabbitt) and R01-DC01919 (Zhu and Zhou)

Does the Distribution of Hair Cell Morphologic Polarization Vectors Provide Evidence of Spatial Tuning in the Mammalian Utricle?

Larry Hoffman

Geffen School of Medicine at UCLA

Individual Abstract: The mechanical characteristics of the utricular otolithic membrane fascinated Wally Grant and were a focus of his intellectual curiosity. Using finite element analyses based on otolithic membrane material properties derived from serial histologic sections, Julian Davis and Wally proposed that the otolithic membrane of the turtle utricle exhibited mechanical characteristics that resulted in asymmetric static mechanical gain characteristics. This suggested that the axis of maximal mechanical gains was aligned in the posterior-lateral direction, reflecting a form of spatial tuning that favored linear acceleration stimuli applied along that axis. The objective of the present study was to test whether other features of utricular direction coding also exhibited characteristics of spatial tuning in the wild-type mouse utricle. This was achieved by applying an immunohistochemistry protocol designed to label alpha-spectrin for illuminating the apical hair cell at the level of the cuticular plate, acetylated tubulin to illuminate kinocilia, calretinin to positively identify the utricular striola, and beta3-tubulin to label the calyces of all type I hair cells. The apical-most alpha-spectrin immunolabeling of all striolar hair cells and the location of their respective kinocilia were manually segmented. The centroids of each segmented region were determined and provided the basis to compute the morphologic polarization vector (MPV) of each hair cell. A simulation of stimulus-evoked depolarization of each hair cell was then resolved by computing the dot product of simulated unit stimulus vectors with each hair cell MPV and adding resultant simulated "response magnitudes" for striolar hair cells at each stimulus vector. Stimulus vectors were applied in one-degree increments from 0° (directly caudal) to 180° (directly rostral). Data collected thus far indicates that the distribution of striolar hair cell MPVs supports the existence of spatial tuning that resulted in enhanced aggregate responses in the posterior-lateral direction. An analysis of the murine otolithic membrane mechanics comparable to that reported for the turtle by Grant and colleagues has not been conducted, and therefore it is not known how the present results would directly integrate with the mechanics of the murine otolithic membrane. However, these results serve to underscore Wally's seminal contributions to understanding how the natural properties of mechanophysiological systems contribute to sensory coding by peripheral vestibular epithelia, for which we are all grateful.

Transduction in Mammalian Otolith Organs: In Memory of Wally Grant

Ruth Anne Eatock

University of Chicago

Individual Abstract: J. Wallace (Wally) Grant studied how otoliths in the vestibular inner ear translate linear headmotions into hair bundle deflections. He was interested in the exuberant differentiation of hair bundles with cell type and epithelial zone, and the implications for shaping the receptor potential. Wally noted how different bundles should extract different features of head motion, in part through their own structure but also by differential coupling to the overlying otolith. When you expose an otolith organ under the dissecting microscope, the striola appears as a stripe in the otoconial mass, where the crystals are smaller and more transparent. In the surrounding extrastriola, hair bundles have long kinocilia and tall stereocilia that extend

into the gel layer supporting the crystals. A step of linear acceleration displaces the otolith, delivering a step of displacement to the embedded extrastriolar hair bundles, which experimentalists can mimic with a step displacement of a rigid probe positioned against the bundle. In the striola, in contrast, hair bundles delicately attach just at their tips to the otolithic gel layer and otherwise are more freestanding in liquid endolymph. Striolar bundles therefore receive a complex stimulus, with both displacement and velocity components imparted by the gel layer and the endolymph motion, respectively. Wally thought that step deflection of a flexible glass probe might come closest to approximating the striolar stimulus during a step of linear acceleration. Thus, by the design of their accessory structures, otolith organs reveal the division of labor across the sensory epithelium: as we know from research by Jay Goldberg and colleagues, striolar afferents report a more differentiated (adapting) version of the linear acceleration step than do extrastriolar afferents, as expected based on the differential coupling of the accessory structure to hair bundles. But even when we remove the otolith and directly displace individual hair bundles with a simple rigid probe, we see zonal differences in the evoked transduction currents, as if the transduction machinery, like the macromechanical response, is more dynamic in the striola. In the mouse utricle, striolar type I hair cells have a more prominent very fast adaptation (time constant less than 1 ms) and less slow adaptation (time constant greater than 10 ms) than do extrastriolar type I hair cells or type II hair cells from either zone.

In remembrance of Wally and his insights into the miniature mechanics of the inner ear, which he shared with generosity and humor.

Supported by DC018304

Activity of the Efferent Vestibular Nucleus Neurons in Mice During Self-Generated, High Acceleration Head Movements

Alan Brichta

The University of Newcastle

Individual Abstract: Curthoys, Grant, et al., (2019) reviewed the mechanical and synaptic processes underlying otolith transduction of sound and vibration for clinical VEMP testing. In the paper they highlighted the importance of high precision phase-locking by mammalian irregular afferents synapsing on striolar type I hair cells by calyx terminals. The presence of a group of vestibular afferents sensitive to high frequency head movements would imply that an efferent feedback mechanism aimed at modulating their output needs to be similarly attuned to high frequencies. Our recent studies of the mammalian Efferent Vestibular Nucleus (EVN) in awake behaving mice suggests the approximately fifty neurons that constitute the EVN are preferentially active during self-generated, high acceleration, head movements. We therefore conjecture one function of the EVN, which has been the subject of much speculation over the years, is to modulate specialised high precision, high frequency sensitive irregular vestibular afferents. In our study we used in vivo calcium imaging of EVN neurons in mice during natural active head movements as well as during moderate passive motion (provocative motion). This was done using miniaturized brain-implanted miniscope system to record real-time calcium activity in a transgenic Chat-gCaMP6f mouse strain. This strain expressed the fast variant of the genetically encoded GCaMP6 calcium sensor protein in cholinergic neurons, including the EVN. In addition, we collected simultaneous 3D accelerometer and gyroscopic data and video capture of mouse behavior. These data allowed us to correlate EVN neuronal activity to behavior and specific head movements. Our results showed EVN increased activity during self-generated, high acceleration, head movements, but not during moderate, passive, head motion. Increased neuronal activity, as indicated by calcium peaks, in some cases anticipated large head movements by as much as 50 – 100 ms. Taken together, our results suggest EVN neurons are ideally placed to modulate activity of high acceleration-sensitive vestibular afferents during large volitional head movements and can do so on a short time scale.

Understanding the Effects of Vestibular Efferent Stimulation on Afferent Responses to Vestibular Stimuli: A Nod to Wally Grant

Joseph Holt

University of Rochester

Individual Abstract: In what may be a surprise to many, Dr. Wally Grant and I had a lot in common including receiving our PhDs from Tulane University, an intense fascination with the peripheral vestibular system, and a deep appreciation for the utility of turtle inner ear preparations for understanding vestibular mechanics and physiology. Given these connections, our paths crossed often, but our deepest conversations centered on data suggesting that activation of vestibular efferents might affect hair bundle mechanics in type II vestibular hair cells. Wally's thoughts on the underlying processes were quite insightful and he imagined early on how one might provide direct evidence for this phenomenon. My presentation will review these data and serve as a nod to Wally's collegiality, intellectual contributions, and infectious scientific enthusiasm. Electrical stimulation of cholinergic vestibular efferents gives rise to multiple effects on the background discharge of vestibular afferents. These effects include inhibitory and/or excitatory responses that operate along varying time scales and are attributed to different synaptic mechanisms on both hair cells and afferents. Pharmacological evidence in multiple vertebrate species reveals that: (1) Efferent-mediated afferent inhibition requires activation of 9 10 nicotinic acetylcholine receptors (nAChRs) and SK2 potassium channels in type II hair cells, (2) Efferent-mediated fast excitation involves engaging 4 6 2*nAChRs on vestibular afferents, and (3) Efferent-mediated slow excitation relies on the activation of muscarinic AChRs and closure of KCNQ potassium channels in calyx-bearing afferents. To understand how these distinct efferent mechanisms modulate afferent responses to vestibular stimuli, we employed afferent recordings from the turtle posterior crista, a reliable lab workhorse where both efferent and vestibular stimulation can be easily combined. We measured the gain and phase of each afferent's response to sinusoidal canal indentation before, during, and after the delivery of efferent stimuli. Predictably, efferent-mediated inhibition and fast excitation were both associated with reductions in afferent sensitivity to vestibular stimuli while efferent-mediated slow excitation was associated with an enhancement, consistent with parallel changes in input impedances of hair cells and/or afferents. Surprisingly, while afferent response phases to vestibular stimuli did not change with either efferent-mediated fast or slow excitation, significant phase lags of nearly ~15˚ were observed during efferent-mediated inhibition, implying α9α10nAChRs/SK activation is "sensed" by the transduction apparatus in type II hair cells. Similar efferent-mediated phase lags are seen in other species and, in alignment with Wally's earlier ruminations, recent work in the frog saccule clearly demonstrates this efferent mechanism alters hair bundle mechanics.

Engineering in a Dish: A Hokie Journey From Mechanics to Molecules

R. Keith Duncan

University of Michigan

Individual Abstract: The legacy of Dr. J. Wallace "Wally" Grant extends into many academic domains, from pioneering work in otolith mechanics to the care and integrity brought to teaching and mentoring. Through personal stories and scientific narratives, this presentation will focus on Dr. Grant's legacy embodied in the life of an undergraduate engineer from Virginia Tech inspired to consider the wonders of the inner ear. Undergraduate research can be a gateway toward a long-term research career, but the path is often closed to those with limited experience and resources. In a seminar on balance and the vestibular system, Dr. Grant spun a scientific story that illustrated the beauty of the inner ear and the interesting mechanics questions yet to explore, capturing my imagination and inspiring an engineer with no life science training to shift their perspective. He was undaunted when approached with the novel idea to use finite-elements to model hair bundle micromechanics. His mentoring approach fostered creativity and independence with guardrails of kindness, trust, and humor. In this, he opened a gateway that would launch a research career that continues to find touchstones in the vestibular system and a love for mentoring that has led to a focus on diversity, equity, and inclusion. This presentation will incorporate some of the mentoring lessons learned from Dr. Grant and link them to the lasting legacy of research now focused more on molecules than mechanics and on the creation of several mentoring programs at the University of Michigan.

Monday, February 5, 2024

Symposium 5 - Advanced Imaging Methods and Applications for Inner Ear Studies

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

Platinum Salon 5

Advanced Imaging Methods and Applications for Inner Ear Studies

Chair: Katie Kindt*, NIH/NIDCD*
Co-Chair: Uri Manor*, University of California, San Diego*

Session Description: The goal of this symposium is to bring greater awareness to inner ear researchers on the latest advances in imaging techniques, along with the discoveries brought about by these techniques, as these techniques be useful for their own research labs or as collaborations. The inner ear is an extraordinary organ housed within the intricate bony labyrinth of the temporal bone. The cochlea has long been a favorite structure for highlighting the power and beauty of biological imaging techniques, due to its exquisite spatial organization and morphology. An important step towards comprehending inner ear health, function, development, and pathology lies in better understanding the morphology and spatial distribution of key molecules at the tissue, cellular, and subcellular level. Thus, to gain a comprehensive understanding of both normal and impaired inner ear function, it is imperative to develop and apply imaging technologies that are able to visualize these features across scales. To study the inner ear researchers are applying cutting-edge imaging techniques to visualize and understand this complex organ. This symposium highlights a subset of ongoing imaging research. This includes clinical studies using advanced MRI and micro-OCT to visualize the inner ear in the context of hearing disorders along with other pathological conditions. In parallel, scientists are employing high-resolution imaging methods to examine cellular and subcellular structures within sensory hair cells, such as electron microscopy (FIB-SEM, tomography) along with deep-learning approaches to reconstruct these structures. Complementing these efforts, studies exploring the movement and assembly of molecules in living tissues are providing a more comprehensive understanding of the inner ear. Together the knowledge gained from these imaging studies will serve to better understand the inner ear and provide foundational knowledge to develop better therapies in the future to treat hearing or balance disorders.

How Does Maturation and Aging Change the Morphological Vesicle Pools of Auditory Synapses?

Carolin Wichmann

Molecular Architecture of Synapses Group, Institute for Auditory Neuroscience, InnerEarLab and Center for Biostructural Imaging of Neurodegeneration, University Medical Center Göttingen

Individual Abstract: In mammals, the cochlear inner hair cell (IHC) ribbon synapses are the key structures for sound encoding. Indefatigable release is enabled by their specific structure, the synaptic ribbon that tethers dozens of synaptic vesicles. Previously, morphological synaptic vesicle pools were described to consist of different sub-pools, determined by variations in the tethering and location of the synaptic vesicles. It has been shown that the number of ribbon synapses declines upon aging but a comprehensive overview of fine structural changes on the level of vesicle pools is still lacking to date. We raised the hypothesis that vesicle pools or vesicle tethering might be affected prior to synapse and fiber degeneration. We used a combination of high pressure freezing and freeze substitution (HPF/FS) with electron tomography to assess the ultrastructure of murine IHC ribbon synapses with highest resolution in a near-to-native state. Furthermore, we employed focused ion beam-scanning electron microscopy (FIB-SEM) to determine changes upon aging on the IHC level. In this study, we investigated three different genotypes throughout a mouse lifespan: (i) CBA/J wild-type mice with a normal onset of hearing-loss with aging, (ii) C57Bl6 mice that show an early onset of progressive hearing loss and (iii) congenitally deaf animals, lacking the protein otoferlin, which is essential for IHC ribbon synapse exocytosis. Additionally, we analyzed morphological changes of endbulb of Held

active zones upon maturation towards adulthood. Overall, using advanced electron microscopic techniques, our work aims to provide fundamental insights into structural changes upon maturation and aging of two auditory synapses.

Dynamic Structural Plasticity Determines Developmental Maturation of the Cochlear Inner Hair Cell Ribbon Synapse

Roos Voorn

Medical University Innsbruck, Auditory Neuroscience Group

Individual Abstract: In mammals, sound detection occurs in the cochlea, where sensory inner hair cells (IHC) accurately convert auditory stimuli into neural code. At their presynaptic active zones (AZ), IHCs harbor electron-dense specializations – so-called 'ribbons' – that facilitate ultrafast, temporally-precise and indefatigable exocytosis of ribbon-tethered synaptic vesicles (SV) onto postsynaptic spiral ganglion neurons. During synapse assembly and subsequent maturation, IHC ribbons increase in volume and SV tethering capacity. This volume accumulation of the developing ribbon is thought to result from the aggregation and fusion of multiple smaller ribbon precursors at the presynaptic AZ in a maturation process that likely also involves plastic structural remodeling in the run-up to hearing onset. However, to date, the molecular drivers of precursor transport towards the AZ as well as the structural dynamics of the ribbon scaffold have remained largely elusive. In the present study, we now established a novel method for long-term triple-color live-cell imaging of the organ of Corti in vitro and examined the plastic assembly processes and mode of transport of ribbon precursors to the AZ of developing IHCs. We found that during early postnatal development, ribbon precursors are highly dynamic and subject to a period of bidirectional plasticity at the synapse. Hereby, ribbon material was not only synaptically recruited, but also disassembled and redistributed to neighboring synaptic contacts within the same IHC. This dramatic remodeling at and between distinct presynaptic AZs was found to depend on targeted transport by the interwoven networks of the actin and microtubule cytoskeleton. Moreover, ribbon displacement appeared to be coordinated by spontaneous bouts of presynaptic SV release and pharmacological inhibition of CaV1.3-dependent Ca2+ influx negatively impacted on ribbon precursor mobility at the AZ. In summary, our data provide novel insights into AZ remodeling during auditory ribbon synapse maturation and suggest the ribbon to be a strikingly dynamic molecular scaffold.

Longitudinal Quantification of Inner Ear Fluid Spaces and Endolymphatic Hydrops on Contrast-Enhanced Delayed Flair MRI: Reliability and Correlation With Changes in Hearing

Julia Telischi

University of Miami Miller School of Medicine

Individual Abstract: Background: Hearing instability (HI) disorders, including Meniere's disease, autoimmune inner ear disease, and sudden sensorineural hearing loss, can be characterized histopathologically by endolymphatic hydrops (EH), an expansion of the endolymphatic space. Fluid sensitive magnetic resonance imaging (MRI) techniques combined with gadolinium-based contrast agents (GBCAs) have been used to differentiate different fluid spaces in the labyrinth as such agents preferentially accumulate in the perilymph but not in the endolymph. While several studies have quantified these fluid spaces and EH in the inner ear based on such MRI techniques, few studies to date have attempted to quantify them in a longitudinal fashion. Furthermore, the utility and efficiency of MRI processing pipelines may be optimized and further automated. Here we characterize longitudinal variations in EH on contrast-enhanced delayed fluid attenuated inversion recovery (CED-FLAIR) MRI and its correlation to changes in hearing in patients with HI with the help of a semi-automated pipeline.
Methods: CED-FLAIR MRI was performed 4-8 hours following intravenous gadoteridol (0.2 mmol/kg) in a cohort of HI patients and healthy volunteers at 3-month intervals, under a deep phenotyping protocol. MRI were performed at 3.0 T with an 8-channel head coil using 3D FLAIR and short tau inversion recovery (STIR) sequences with 0.8 mm isotropic resolution requiring ~ 12 and 4 minutes, respectively. Based on STIR (representing combined perilymph and endolymph fluids) and delayed FLAIR (representing perilymph fluid)

MRI sequences, a custom-developed MRI processing and analysis pipeline was utilized to quantify the volume of the perilymph and endolymph in the inner ear.

Results: Changes in EH volume were quantified in a longitudinal fashion and correlated with clinical measures of hearing. Repeated STIR MRI's establish normal expected variability of using MRI in imaging inner ear fluid spaces.

Conclusions: Longitudinal assessment of patients with HI utilizing contrast-enhanced delayed FLAIR MRI allows for detection of quantifiable changes in EH that correlate with changes in hearing. If combined with deep learning, this methodology has the potential to monitor HI more efficiently over time and help better evaluate potential treatments for HI in which EH is present.

High-Resolution Imaging of Human Inner Ear

Konstantina Stankovic

Stanford School of Medicine

Individual Abstract: Sensorineural hearing loss (SNHL) is a major public health issue, disabling 466 million people today, and projected to affect 900 million by the year 2050. Advances in cellular-level diagnostics and therapeutics for SNHL have been impeded by the current inaccessibility of human cochlea for in vivo imaging due to its small size, complex three-dimensional anatomy, and encasement in dense bone. To address this unmet medical need, we have developed a sub-millimeter-diameter, flexible endoscopic probe interfaced with a micro-optical coherence tomography imaging system. This probe is capable of imaging the cochlea's sensory epithelium at micron scale in cadaveric human temporal bones. To expedite validation of micro-optical coherence tomography images and further our understanding of the cellular underpinnings of otologic disorders, we have established a pipeline for collection and processing of human temporal bones. We will discuss key steps in this process, including the design of a temporal bone plug sawblade that can be attached to an autopsy saw to collect specimens from autopsy donors, and expedited decalcification by drilling down a temporal bone to the level of the otic capsule or by combining ethylenediaminetetraacetic acid with microwave tissue processing and periodic bone trimming.

A Population of Short Actin Filaments Contributes to Stereocilia Widening

Benjamin Perrin

Indiana University, Purdue University

Individual Abstract: Actin regulation plays a crucial role in stereocilia development and maintenance. The primary structure of stereocilia consists of a large bundle of long, parallel actin filaments, which establish the length and width of stereocilia. These core actin filaments are oriented with their barbed ends at the stereocilia tips and pointed ends at the base. We stained developing stereocilia using probes specifically designed to label the pointed ends of actin filaments, which resulted in robust staining of the stereocilia tips. This observation suggests a distinct population of short actin filaments localized at the tips, separate from the barbed-end-presenting F-actin core. We refer to these as 'tip filaments'. Overexpression of either EGFP-MYO3A or EGFP-MYO15, proteins implicated in stereocilia widening and elongation respectively, led to an increased level of these tip filaments. This result is consistent with earlier findings suggesting that MYO15 can nucleate actin filaments, thereby implying that both myosins could facilitate the production of tip filaments essential for stereocilia growth. Notably, overexpression of EGFP-MYO3A led to an increased width of stereocilia, suggesting a role for these short tip-localized actin filaments in the widening process. Correspondingly, live imaging showed EGFP-actin initially incorporating at the stereocilia tips before migrating down towards the base. Subsequent expansion microscopy and super-resolution imaging revealed that this newly incorporated EGFP-actin formed a peripheral layer around the pre-existing, stable core of the stereocilia. This suggests a mechanism where these new actin filaments, originating from the tip, contribute to increasing the stereocilia diameter by augmenting the existing core structure.

A Deep-Learning Approach at Mitochondria Morphological Analysis in the Outer Hair Cell

Christopher Buswinka
Harvard University

Individual Abstract: Sensory hair cells contain hundreds of mitochondria, the health and dynamics of which are critical for cell function. Mitochondria can be visualized at high resolution by 3D EM techniques, such as focused ion beam scanning electron microscopy (FIB-SEM), and datasets can contain multiple cells and thousands of mitochondria. However, morphological analysis on this scale represents a significant challenge. There is therefore great interest in facilitating this analysis by automated algorithms using deep neural networks. Unfortunately, mitochondria in whole cell 3D EM datasets are not well suited for existing automated analysis tools. We have developed a novel deep-learning analysis approach, which quickly and accurately extracts morphological information from mitochondria, imaged by volumetric electron microscopy, through instance segmentation. "Skeleton oriented object segmentation" (SKOOTS); efficiently handles large, densely packed mitochondria in 3D, a previously difficult segmentation task. We trained this approach on a dataset of over 700 manually annotated, 3D outer hair cell mitochondria, and demonstrate superior accuracy compared to other instance segmentation algorithms. Finally, we have expanded the functionality of our approach to the segmentation, and morphological analysis, of hair cells imaged by confocal microscopy. Overall, this work enables the execution of previously prohibitive experimental design, removing the significant barrier of manual analysis. Through this tool, we might better understand the underlying mitochondrial dynamics in normal and pathological conditions.

New Uses for Local Shape Descriptors in Image Analysis

Uri Manor
University of California

Individual Abstract: Segmentation of microscopy images is often plagued by difficulties determining borders between objects, whether they be plasma membrane borders in electron microscopy images of brain tissues, fluorescence-labeled organelles moving past one another in living cells, or synaptic punctae in immunofluorescence-stained 3D confocal images of whole mount organ of Corti tissues. Deep learning-based approaches have shown great promise in these kinds of tasks. One of the biggest bottlenecks in deep learning-based segmentation approaches is the requirement for large amounts of human-annotated/proofread training data, which is extremely time-intensive and therefore expensive to generate. Here we show that utilizing local shape descriptors ("LSDs", Sheridan et al., 2023) as an auxiliary learning task we can significantly reduce the required amount of annotations in order to generate useful 3D segmentation results. We also show that transformer-based architectures can be useful for improved tracking of organelles in live cell videomicroscopy data. We speculate that a combination of LSD-based segmentation and transformer-based tracking approaches will serve as a useful tool for a broad range of biological imaging applications, including inner ear studies.

Podium Session 9 - Auditory Research Insights: Ear Morphology, Hearing Tests, and Tinnitus Treatments in Animal Models

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

Platinum Salon 6

Efficacy of AC102 in a Noise-Induced Tinnitus Model in Mongolian Gerbils

Konstantin Tziridis[1], Monika Kwiatkowska[2], Holger Schulze[1], Reimar Schlingensiepen*[2]
[1]*University Hospital Erlangen, ENT Hospital, Experimental Otolaryngology,* [2]*AudioCure Pharma GmbH*

Category: Tinnitus

Background: Tinnitus is a common symptom of many diseases including hearing loss. Loss of inner hair cell synapses and cochlear nerve degeneration are major contributors to the development of tinnitus. The small molecule AC102 has been shown to exert both preventive and regenerative properties on auditory neurons and synaptic connections in a guinea pig noise-induced hearing loss model. Therefore, we investigated AC102's capability in the treatment of acute tinnitus in a well-established noise-induced tinnitus model in gerbils and compared the efficacy of single and multiple applications of AC102.
Methods: To induce tinnitus, 38 Mongolian gerbils where exposed to noise (2kHz, 115dB SPL, 75min) before being assigned to treatment groups: (a) multiple dosing (three local drug applications one week apart) and (b) single dosing. AC102 was formulated in a thermosensitive gel and injected onto the round window membrane. Treatment with either AC102 or vehicle started 1h after noise exposure. Hearing thresholds were assessed by ABR measurements and tinnitus development via behavioural Gap-Prepulse Inhibition of the Acoustic Startle Reflex (GPIAS) test over five weeks, followed by immunohistochemical quantification of ribbon synapses.
Results: Mild transient threshold shifts completely recovered to baseline levels within one week in all groups. Treatment with AC102 significantly inhibited the development and reversed existing tinnitus. Only 2 out of 11 animals had behavioral signs of tinnitus one week after noise trauma. In comparison, 8 out of 12 animals developed tinnitus in the vehicle-treated group. The repressing effect on tinnitus development was maintained by AC102 up to 5 weeks. Ribbon synapse counts further revealed a significant reduction of synaptopathy in the AC102 group compared to vehicle. Repeated and single dosing with AC102 had similar effects on the inhibition of tinnitus as well as on synaptopathy.
Conclusions: This study yielded proof-of-principle that a single, local application of AC102 into the middle ear is effective in treating an acute tinnitus percept in vivo. These findings also confirm AC102's action on synaptopathy and functional hearing improvement seen in other species and indications, suggesting the potential of AC102 for the treatment of hearing disorders. Currently, AC102 is being evaluated in a Phase II clinical study for the treatment of idiopathic sudden sensorineural hearing loss (ISSNHL). The obtained data suggest that AC102 might be an effective treatment in acute tinnitus both as a single symptom and as a co-symptom as seen frequently in ISSNHL patients.

Differences in External Morphology and Implications for Auditory Function in Two Closely Related Mouse Species (Peromyscus Leucopus and Peromyscus Maniculatus)

Casey Sergott*[1], Katelynn Rodman[1], Luberson Joseph[1], Emily Margaret New[1], Genesis Alarcon[1], Elizabeth McCullagh[1]

[1]*Oklahoma State University*

Category: Middle and External Ear
Background: External morphological structures such as the head and pinna are the starting point of auditory communication by receiving sounds from the environment and play an important role in how an animal perceives sounds. Particularly, the presence of the pinna contributes to front/back and vertical discrimination of acoustic signals. The ability to accurately discriminate where sounds are coming from is directly dependent on the monaural and binaural hearing ability of an individual. While monaural hearing refers to the use of one ear to detect sounds, binaural hearing refers to the use of both ears to localize sounds. In order for the brain to assess the approximate location of a sound, it relies primarily on two cues: interaural time differences (ITDs), which are the differences in the arrival time of a sound between two pinnae, and interaural level differences (ILDs), or the difference in sound level between the two pinnae. Therefore, it has been postulated that variability in the size and shape of the pinnae will cause variability in the detection of acoustic signals.
Methods: Here, we focus specifically on the sound reception process in two closely related species (Peromyscus leucopus and Peromyscus maniculatus) in relation to external morphological structures such as the head and pinna. We measured the dimensions of the head and pinna of over 1,000 preserved specimens of P. leucopus and P. maniculatus and performed head-related transfer functions (HRTFs) on several individuals. Specimens were collected from all over the U.S. and were provided to us by the Collection of Vertebrates at Oklahoma State University. Our measurements included pinna size (length and width), distance between pinnae (inter pinna length), and distance from nose to pinna.
Results: Our preliminary data show differences in the dimensions of the pinnae across the two species, which could be related to differences in binaural hearing as well.

Conclusions: Since HRTFs are unique to every animal, they will give us insight into correlations between pinna size and functional differences in sound localization ability. Not only will this study bridge a knowledge gap between external morphology and auditory function, but it will also bring insight into the use of museum specimens for auditory research as a whole.

Audiometric and Vestibular Testing of a Murine Model of Down Syndrome

Cong Tian[1], Gabrielle Merchant[2], Sarath Vijayakumar[1], Gini George[1], Katherine Waugh[3], Kelly Sullivan[3], Joaquin Espinosa[3], Peter Steyger*[1]

[1]*Translational Hearing Center, School of Medicine, Creighton University,* [2]*Center for Hearing Research, Boys Town National Research Hospital,* [3]*Linda Crnic Institute for Down Syndrome, University of Colorado Anschutz Medical Campus*

Category: Middle and External Ear

Background: The Dp16 mouse model of Down Syndrome has a large segmental duplication of mouse chromosome 16 leading to triplication of ~120 protein-coding genes orthologous to those on human chromosome 21, all on a B6 background. Elevated ABR thresholds were observed in 6-8-old-month-old Dp16 mice backcrossed to a CBA/J background to avoid early onset of age-related hearing loss due to the ahl gene in B6 mice (Chen et al., 2022). Our goal was to comprehensively characterize peripheral auditory function in Dp16 mice on a B6 background.

Methods: Comprehensive characterization of peripheral auditory function was achieved through both middle and inner ear assessments of auditory and vestibular function, plus histology. Middle ear function was characterized using noninvasive wideband acoustic immittance (WAI) measurements previously unreported in mice. WAI data of Dp16 and littermate control mice were obtained using an Interacoustics Titan device, with 3-5mm probe tips, across the human frequency range (0.25-10 kHz). ABR and DPOAE thresholds were collected with a Tucker-Davis Technologies RZ6 system following the method described in Liu et.al., 2021. Utricle and saccule functions were assessed by vestibular sensory evoked potentials (VsEPs) following methods described in Vijayakumar et al., 2015. Intact temporal bullae were excised after transcardial perfusion fixation, embedded in paraffin wax and sectioned before staining and microscopy.

Results: We replicated CBA/J auditory dysfunction in 2-month-old Dp16 mice compared to age-matched controls. Dp16 mice exhibited (i) 45-25 dB higher ABR thresholds (4–40kHz) compared to controls, with threshold shifts decreasing at increasing stimulus frequencies, and (ii) reduced DPOAE responses compared to controls, typical of conductive hearing loss (N=4). WAI from Dp16 mice show significantly reduced absorbance in the 4-10 kHz frequency range compared to controls, consistent to humans with otitis media and indicative of at least a conductive component to the hearing loss. This was supported by histopathological changes including thicker epithelia with inflammatory infiltration, thickened tympanic membranes, and variable effusion in the middle ear cavity (N=4). Vestibular function in Dp16 mice appeared impaired yet was variable. VsEP threshold was profoundly elevated in one mouse (-1.5 dB re: 1g/ms). Mean VsEP threshold was 4 dB higher in the Dp16 mice compared to age-matched WT controls. The mean VsEP P1-N1 amplitude was also lower in Dp16 mice (N=3).

Conclusions: The Dp16 mouse model appears to replicate human auditory and vestibular phenotypes of Down Syndrome, with mixed conductive and sensorineural hearing losses, as well as otitis media and vestibular deficits commonly found in clinical assessments. Further work to optimize our novel non-invasive middle ear assessment to the frequency range of the Dp16 mouse using a custom software and hardware setup are ongoing.

Up-Regulation of Gaba Type a Receptor α1 in the Dorsal Cochlear Nucleus of Rats With Noise-Induced Tinnitus and Evidence of Therapeutic Mitigation With NHPN-1010

Xiaoping Du*[1], Weihua Cheng[1], Qunfeng Cai[1], Jianzhong Lu[1], Zachary Yokell[1], Don Nakmali[1], Richard D. Kopke[1], Matthew West[1]

[1]*Hough Ear Institute*

Category: Tinnitus

Background: Traditionally, it has been postulated that the etiology and the maintenance of tinnitus is the result of reduced inhibitory neurotransmission in the central auditory system. However, in one of our previous studies, we found that up-regulation of both glutamate receptor 2 (GluR2) and GABA type A receptor α1 (GABAA-Rα1) in the DCN of rats exposed to blast correlated with an 85% incidence rate of behavioral evidence of tinnitus.
Methods: To further study the mechanisms of GABAARα1 expression in the DCN in a more targeted fashion, we developed a temporary threshold shift model of noise-induced tinnitus in rats (8-16 kHz OBN, 2h, 108 dB SPL for 2h). Tinnitus assessment by acoustic startle reflexes and GABAA-Rα1 immunostaining in the DCN were conducted at 4 or 12 weeks after noise exposure. In this context, We also studied the treatment effects of NHPN-1010, a Phase II-ready combination drug composed of HPN-07 and NAC, on the noise-induced up-regulation of GABAA-Rα1 in the DCN and the tinnitus incidence rate. NHPN-1010 has previously been shown to be effective for mitigating tinnitus. Rats with established chronic tinnitus identified at four weeks post-noise exposure were treated with either NHPN-1010 (300 mg/kg twice a day) or saline for 14 days.
Results: Using this model, behavioral evidence of chronic tinnitus assessed by acoustic startle reflexes was detected in 61% of rats at four weeks after noise exposure. Significantly more GABAA-Rα1-positive cells were measured in the DCN of noise-exposed rats (with or without tinnitus) compared to naïve control rats (all p less than 0.001). However, significantly fewer GABAA-Rα1-positive cells were measured in the DCN of rats with tinnitus compared to rats without tinnitus (p less than 0.001). NHPN-1010 treatment significantly reduced the tinnitus incidence rate (77.8%) compared to rats treated with saline (94.4%). Significantly more GABAA-Rα1-positive cells were found in the DCN of rats treated with NHPN-1010 compared to rats treated with saline or naïve control rats.
Conclusions: These results appear to indicate that up-regulation of GABAA-Rα1 is a generalizable response of the central auditory system to excessive noise exposure, but the extent to which inhibitory GABAA-Rα1-containing receptors predominate in the DCN may be linked with an adaptive mechanism that suppresses the development of a chronic tinnitus percept. These results also suggest that increases in GABAA-Rα1-containing inhibitory ion channels in the DCN may represent a modulatory attribute of NHPN-1010's efficacy in treating tinnitus.

Ebselen Attenuates Noise-Induced Tinnitus in Older Mice With Age-Related Hearing Loss

Annie Jia[1], Ryan Longenecker[1], Rende Gu[1], Celia Escabi[2], Jonathan Kil*[1]

[1]*Sound Pharmaceuticals, Inc.,* [2]*UT Dallas, Sound Pharma*

Category: Tinnitus
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 studies have shown that ebselen treatment can prevent acute NIHL in young adults with normal hearing (4 days) and improve hearing and tinnitus deficits in aged adults with Meniere's disease (21 and 28 days). We previously shown in a 3- to 6-month-old mouse model of NIT, that behavioral evidence of tinnitus was temporarily reversed following 4 days of ebselen treatment. The goal of this study was to determine if 14 days of ebselen treatment can permanently reverse NIT in aged mice (12 to 18 months) where hearing loss and tinnitus are more prevalent.
Methods: Two cohorts of CBA/CaJ mice were utilized: 14–18-month-old cohort (N=20) and 12-month-old cohort (N=20). Each cohort was divided into two groups: Group 1 (N=4) served as an unexposed control with no occlusion; Group 2 (N=16) received a temporary unilateral occlusion of an external ear canal before a brief narrowband noise exposure. Baseline ABRs and Gap-Induced-Prepulse-Inhibition of the Acoustic Startle Reflex (GPIAS) were assessed prior to and three months after noise exposure to assess gap detection deficits (behavioral marker for NIT). Animals that developed NIT were treated with a 14-day course of ebselen (10/mg/kg/d/po) and reassessed at 1, 10, and 30 days post-treatment. Cochlear histopathology was performed using immunofluorescence labeling to identify inner and outer hair cell numbers.
Results: Three months after noise-exposure, permanent threshold shifts were observed in the open ear 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=32). After 14-days of ebselen treatment, gap

detection deficits were significantly reduced to pre-exposure levels in 6 of the 7 NIT mice at 1 day. This significant reduction in NIT was observed in 3 of 6 mice at 10 days. Gap detection deficits remained below pre-treatment levels at 30 days in these 3 mice, although this difference was not statistically significant. Cochlear histology for IHCs and OHCs is ongoing.
Conclusions: A 14-day course of ebselen treatment can durably reverse gap detection deficits to baseline or pre-noise levels in 50% of aged mice that developed NIT. This work is the first demonstration of an anti-inflammatory reversing NIT after the development of age-related hearing loss that is further exacerbated by acute NIHL. Longer ebselen treatment durations are being tested to determine if this reversal can be improved and results in the repair of injured cochlear hair cells. This data provide promising implications for ebselen as a treatment for noise-induced tinnitus in older adults.

Bone Conducted Ossicular Sound Transmission in Elephant and Human Middle Ears

Caitlin O'Connell-Rodwell*[1], Jodie Berezin[1], Xiying Guan[2], Anbuselvan Dharmarajan[1], Alessio Pignatelli[4], Sunil Puria[5]

[1]*Harvard Medical School, Eaton Peabody Lab, Massachusetts Eye and Ear Infirmary,* [2]*Wayne State University,* [4]*Johns Hopkins University,* [5]*Harvard Medical School, Mass. Eye and Ear Infirmary*

Category: Middle and External Ear
Background: Elephants, the largest terrestrial mammal, communicate over long distances using vocalizations with a fundamental frequency in the 10-20 Hz range. The middle ear influences the ability to hear these vocalizations through both air conduction (AC) and bone conduction (BC) pathways. Elephant hearing is more sensitive than human hearing below 100 Hz and less sensitive above 1 kHz (Heffner and Heffner, 1982), having ossicles (the largest among terrestrial mammals) that are approximately 10 times more massive (Hemila et al, 1995). This provides a natural experiment to determine the extent to which ossicular mass impacts ossicular sound transmission due to BC stimulation.
Methods: We measured 3D ossicular motions and the bony promontory in response to vibration stimulation termed 'BC'. Measurements were at auditory frequencies (7-13,000 Hz) in cadaveric middle ears of both African and Asian elephants (n=4) and compared them to humans (n=3). We determined umbo and stapes velocities in their functionally relevant piston directions to compute BC input at the umbo, and the stapes which was a proxy for cochlear stimulation.
Results: Upon BC stimulation, elephant umbo velocity was about an order of magnitude greater than human below about 50 Hz, with elephant stapes velocity being approximately an order of magnitude greater than human up to about 1 kHz. There is very little difference between the different responses above about 1 kHz. Elephant stapes velocity phase was like that in human.
Conclusions: In our previous findings, we showed that elephants have an order of magnitude better AC sensitivity below resonance of about 300 Hz (O'Connell-Rodwell et al., 2023 bioRxiv). In this study, we showed that elephants relative to humans have almost similarly better BC sensitivity up to 50 Hz at the umbo and up to 1 kHz at the stapes. These findings have implications for understanding how elephants communicate over long distances through ground vibrations and a path to understanding ways for enhancing BC sensitivity in humans. [Supported by K01 DC017812 from the NIDCD of NIH and the Amelia Peabody Charitable Fund.]

Therapeutic Effects of Near-Infrared Light (NIR) on Tinnitus in Rats

Jinsheng Zhang*[1], Po Hung Chiang[1], Paul Morse[1], Tasnim Arroum[1], Maik Huttemann[1]

[1]*Wayne State University*

Category: Tinnitus
Background: Tinnitus results from noise trauma that triggers oxidative stress and inflammation in the cochlea. The peripheral de-afferentation results in maladaptive neuroplasticity in the forms of hyperactivity, hypersynchrony, lost balance of inhibitory and excitatory processes, leading to tinnitus percept. Thus, reducing oxidative stress and inflammation to mitigate acoustic trauma-induced peripheral deafferentation should reduce the maladaptive neuroplasticity and relieve tinnitus. Our team has demonstrated that near-infrared light (NIR) laser serves as a cytochrome c oxidase inhibitor to "inhibit" mitochondrial respiration to down-regulate

the production of damaging reactive oxygen species, thus reducing oxidative stress and inflammation. The aim of this study was to demonstrate that NIR laser administration has therapeutic effects on noise-induced hearing loss and tinnitus.
Methods: Thirty-two SD rats were used, among which, 14 were used to study the effect on hearing loss. Four rats were used for a MitoSOX superoxide test, and 6 for a COX activity test. Eight rats were used to study the effects on tinnitus using our optimized conditioned licking suppression behavioral paradigm (CLS, Pace and Zhang, 2016). To induce hearing loss and tinnitus, rats were exposed with a loud noise (8–16 kHz, 105-116 dB SPL, 1.5-2 hours). Under anesthesia, rats received 4 hours NIR laser treatment on day 0 after noise exposure and 1 hour for additional 6 consecutive days.
Results: ABR thresholds were significantly lower in the ear that received laser treatment compared to the ear without treatment (4 kHz, $p = 0.02$; 12 kHz, $p = 0.004$; 16 kHz, $p = 0.003$; 20 kHz, $p = 0.02$; 28 kHz, $p = 0.04$; click, $p = 0.002$). DPOAE results showed that the ear with laser treatment exhibited significant recovery at the 20 kHz region compared to the ear without treatment ($p = 0.006$, $n=6$). The ear with laser treatment had a significant lower hair cell loss rate than the ear without treatment (apical, $p = 0.02$; middle, $p = 0.01$; basal, $p = 0.04$, $n = 4$). Laser treatment also significantly reduced Mitosox (+) hair cells compared to without treatment (apical, $p = 0.0013$; middle, $p = 0.0004$; basal, $p = 0.019$). The noise trauma induced 52% increase in cytochrome c oxidase (COX), which was reduced to 22% following laser treatment. Results of CLS behavioral assays showed that NIR laser treatment significantly reduced the number of licks during silence trials in noise-exposed rats, compared to rats without laser treatment, indicating suppression of behavioral evidence of tinnitus.
Conclusions: The study demonstrated that NIR laser administration has significant therapeutic effects on noise-induced hearing loss and tinnitus by inhibiting cytochrome c oxidase to reduce oxidative stress and inflammation.

In-Vivo Middle Ear Imaging via Transbullar Approach for Topical Delivery of Rhodamine Liposomes

Ki Wan Park*[1], Stella Yang[1], Roya Sadabad[1], Tulio Valdez[1]

[1]*Stanford University School of Medicine*

Category: Middle and External Ear
Background: Liposomes have previously been demonstrated as a method for topical drug delivery to the middle and inner ear. These quantification methods, however, rely on histopathology and there is a lack of in-vivo of imaging tools to demonstrate the ability of liposomes to cross the tympanic membrane. We aimed to establish a novel, in-vivo method to image the middle ear through a cervical transbullar surgical approach.
Methods: Murine models and imaging were established with 5 Balb/c mice. After mice were anesthetized, a large cervical incision was made and the bulla identified. A very wide bullectomy was performed to accommodate a 3 mm endoscope for transbullar imaging. The tympanic membrane and middle ear bones were visualized without issues. Following imaging, 50 ul of 20 mg/ml rhodamine liposomes was topically applied to external ear canal and imaged serially at 15-minute intervals up to 1 hour. We then applied vibrations into the external auditory canal to see if there were any differences in rhodamine liposome distribution.
Results: Intact tympanic membrane and middle ear ossicles were successfully imaged via the transbullar approach with a rigid endoscope. We successfully demonstrate that rhodamine liposomes cross the tympanic membrane into the middle ear in-vivo within 15 minutes. Vibrations expectedly affected the rate of distribution of rhodamine liposomes through the middle ear.
Conclusions: We demonstrate the feasibility of in-vivo imaging of the middle ear through the cervical transbullar method. We also demonstrate the ability of rhodamine-liposomes to cross the tympanic membrane within 15 minutes.

Podium Session 10 - Brainstem: Structure and Function

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

Grand Ballroom Salon E

Spatial Transcriptomic Analysis of Mouse Cochlear Nucleus

huihui liu*[1], Weijun Zhou[1], Xingle Zhao[1], Cai Ruijie[1], Hongchao Liu[1], Hao Wu[1]

[1]*Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine*

Category: Brainstem: Structure and Function

Background: The cochlear nucleus (CN) serves as the first relay station in the central auditory nervous system, responsible for receiving cochlear input. It also plays a critical role in auditory processing and the integration of multiple sensory modalities through the coordinated action of different neural circuits and subsequent transmission to the brain. Much of our current knowledge about its architecture is mainly based on morphologic and physiologic studies. However, the molecular basis underlying its cells' heterogeneity and auditory stimuli -related plasticity remains largely unknown.

Methods: In this study, we combined snRNA-seq, spatially enhanced resolution omics sequencing (Stereo-seq), congenital hearing loss animal models to generate a comprehensive cellular and molecular atlas of the CN and uncover the cellular and molecular changes associated with auditory sensory input.

Results: 1) We have identified molecular subregions of the cochlear nucleus and revealed the spatial heterogeneity of cell types within it, highlighting its functional specialization. 2) We have generated a transcriptome atlas of different cell types, annotated CN-specific neuron types, and identified marker genes for these neurons. 3) We found that the bushy cell, especially the bushy cell, is the major cell type that exhibits transcriptomic expression patterns upon auditory stimuli.

Conclusions: Overall, these findings emphasize the importance of determining the molecular and spatial signatures of the CN and studying their dynamics.

Heksor: A Novel Framework for Conceptualizing Olivo-Cochlear Efferent System Functions

Anthony Cacace*[1]

[1]*Wayne State University*

Category: Brainstem: Structure and Function

Background: Many hypotheses have been put forth concerning the functional properties of the descending olivo-cochlear (OC) efferent system. Prominent theoretical considerations include improving acoustic signal detection, localization proficiency, and enhanced speech perception in the presence of background noise. Facilitated through antimasking effects and mechanisms associated with selective attention, these particular operations are considered "adaptive behaviors" that allow individuals to function effectively in challenging environments. Overall, these functions fit well with the novel concept of "Heksor;" a distributed network of neurons and synapses that involve processes necessary to maintain key features of adaptive behaviors (Wolpaw and Kamesar, J. Physiol, 2022).

Methods: Using the Heksor framework, the functional effects attributed to OC efferent function consider whether single or multiple neural assemblies are involved in maintaining the stability and function of these various behavioral effects. In this regard, it has been proposed that a "negotiated equilibrium" is required to enable each Heksor to maintain pertinent features of its own adaptive function to avoid disarray or chaos within the system,

Results: Heksor represents a distributed network of neurons and synapses that changes as needed to maintain key adaptive behaviors, including those elements that make the behavior satisfactory for adapting to specific events, circumstances, or environments. With respect to the functional properties of OC efferent function, several important questions emerge that need to be addressed: specifically, 1) how were these behaviors acquired, and 2) how are they maintained throughout the lifespan? To put this area in perspective and to address these various issues, a general framework is considered which incorporates plastic changes to the neural substrate that mediate and maintain these effects.

Conclusions: The current challenge is to explore how the Heksor framework operates within the medial and lateral OC systems. Indeed, it has been argued that Heksor negotiations are among those functions that enable the distributed, potentially overlapping, and continually changing neural networks to respond to different

conditions, circumstances, and environments. Currently, how these intervening factors subserve this complex neural network remains unknown. These areas will be examined and explicated in this review.

A Striking Source of Giant Synapses in the Auditory Thalamus: The Olivo-Thalamic Projections.

Héctor Rincón*[1], Mario Gómez-Martínez[1], Marcelo Gómez-Álvarez[1], Enrique Saldana[1]

[1]*University of Salamanca*

Category: Brainstem: Structure and Function

Background: Giant synaptic terminals are characteristic of the lower auditory pathways, exemplified by the endbulb of Held in the ventral cochlear nucleus, the calyx of Held in the medial nucleus of the trapezoid body or the calyx-like input from octopus cells to the ventral nucleus of the lateral lemniscus. These large synaptic contacts seem to transmit temporal information about the sounds with exquisite precision and speed. Another sensory-related region, the thalamus, also exhibits this type of nerve terminal in the afferents that the visual thalamus receives from the retina and the somatosensory thalamus receives from the brainstem and the barrel cortex. We have observed a population of giant synaptic terminals in the auditory thalamus with two surprising sources: the medial superior olive (MSO) and the lateral superior olive (LSO) of the superior olivary complex.

Methods: To study the projections of the MSO and LSO to the medial geniculate body of the thalamus (MGB) of the rat we injected unilaterally the bidirectional tracer biotinylated dextran amine (BDA) into these nuclei. We analyzed statistically the morphology of the giant terminals using image analysis software.

Results: We observed that the MSO and LSO sent direct projections to the MGB, bypassing the inferior colliculus (IC). The MSO innervated the ventral division of the ipsilateral MGB (MGBv) and the LSO innervated the contralateral MGBv. The morphology of the projections of both nuclei were equivalent: olivo-thalamic fibers entered medially into the MGBv and terminated in compact nests formed by striking terminals that resembled giant boutons. Around 70% of the olivo-thalamic terminals are larger than 3 microns in diameter, many of them reaching 10 microns. In fact, their mean area in coronal slices is more than twice the mean area of the IC synaptic terminals innervating the MGBv. Some of these giant boutons form appositions with the somas of MGBv neurons, indicating that they made somatic synapses.

Conclusions: Given that information coded in MSO and LSO is less processed than that coded in the IC, the MGB could receive rapid signals from the olives that are then refined by subsequent signaling from IC. This less processed information is sent via giant boutons, known for their extreme precision transmitting information. Furthermore, considering their size they could contain a large number of synaptic release sites and this makes them more likely to generate action potentials in the MGBv neurons. This observation may change the way we understand the ascending auditory pathway and how auditory information about sound localization is integrated in the MGB.

Discovering Neuron Types and Constructing Synaptic Maps Utilizing High Throughput Segmentation of Volume Electron Microscopy Images

Timothy Fawcett*[1], Emily Amick*[1], Audrey Gabbard[1], Matthew Madany[2], Christine Kim[2], Mark Ellisman[2], Paul Manis[3], George Spirou*[1]

[1]*University of South Florida,* [2]*University of California, San Diego,* [3]*University of North Carolina, Chapel Hill*

Category: Brainstem: Structure and Function

Background: A major goal of neuroscience is to specify neuron types and construct synaptic maps of their connectivity that preserves the location of contacts on the cell surface and which incorporates functional information about each contact. We describe here improvements to our workflow to image, segment, reconstruct and study neurons of the cochlear nucleus. We have implemented modifications to serial blockface scanning electron microscopy (SBEM) that permit high resolution imaging with improved signal to noise, and tiling of fields of view to construct individual sections.

Methods: Tissue sections (200 μm thickness) were processed using our established rOTO technique (Holcomb et al. 2013), and examined using X-ray tomography. From these images an ROI containing ventral cochlear nucleus (VCN) and the and granule cell layer that caps the VCN region underlying the dorsal cochlear

nucleus (DCN) was selected for SBEM. An imaging run of 60 days yielded a 3.5TB image volume of dimension of 228 x 336 x 116 µm. The tiles for each section were stitched together and the sections were aligned. Multilabel segmentation utilized a 3D U-net architecture to detect membranes (which includes membrane prediction to fix gaps in staining), myelin, and intracellular organelles (nuclei, mitochondria, synapses). These predictions were overlaid onto the original images, and painting tools in the software VAST were used for rapid object segmentation.
Results: We selected for segmentation a large neuron in the middle of the image volume, which had an irregularly shaped soma and was part of a group of neurons with similar ultrastructural characteristics. Sections with well-predicted membranes led to a 10X speed increase for segmentation over manual tracing; regions with membrane breaks or light staining maintained a 3X speed increase. The 3D reconstruction was imported into Blender for smoothing, and exported for visualization using virtual reality software (syGlass, Inc.). Three primary dendrites of varying thickness emerged from the cell body. The thickest dendrite branched little until, at its distal end, it branched multiple times into a large tuft of processes. A portion of this tuft extended into the dorsal cap area of the VCN. The second-thickest dendrite was a scaled down version of the first dendrite, and the thinnest dendrite did not branch. The neuron did not match published cells from the VCN of mice nor other species. We will report on our pipeline for tissue processing, imaging, auto-segmentation, constructing the synaptic map, cell classification and, following procedures from our previous work, converting the neuron into a 3D compartmental model for biophysical modeling of intrinsic excitability and responses to synaptic activation.
Conclusions: This approach creates cellular representations that can be used to explore the establishment of parallel processing networks in the cochlear nucleus, which is the initial processing stage of auditory information in the CNS.

A Critical Period for Synaptic Refinement in Octopus Cells of the Mammalian Cochlear Nucleus

Lauren J. Kreeger*[1], Sydney Maeker[1], Lisa V. Goodrich[1]

[1]*Harvard Medical School*

Category: Brainstem: Structure and Function
Background: During development, refinement of synaptic inputs and maturation of intrinsic electrophysiological properties occur simultaneously to finely tune neural circuits. Before sound onset, tonotopically-patterned spontaneous activity in the cochlea produces synaptic activity centrally that results in precise tonotopic organization from initial stages of wiring. The onset of sound-driven activity has been shown to further refine tonotopic maps through the elimination of synapses and pruning of auditory nerve collaterals. For octopus cells (OCs) of the mammalian cochlear nucleus, precise tonotopic maps are critical for their submillisecond coincidence detection computations. Adult OCs have extraordinarily fast membrane properties and lack the mechanisms required for NMDA-mediated functional plasticity. In addition, excitatory synapses found on the smooth dendrites of adult OCs are not known to undergo structural plasticity. Given the lack plasticity in adult OCs, correct experience-dependent refinement during development would be critical for accurate spectrotemporal coincidence detection computations once the circuit is firmly wired.
Methods: We targeted OCs for in vitro whole-cell current clamp recordings while electrically stimulating synaptic inputs (P8-35 mice, 35°C). We quantified membrane properties, spike properties, and responses to spontaneous activity before stimulating auditory nerve inputs with 100Hz trains that evoked spikes in OCs. We then passively monitored these properties for 30+ minutes. We also made 3D reconstructions of sparsely labeled OCs (P6-28 mice) and measured dendritic arborization patterns.
Results: We show that before hearing onset, OC intrinsic properties are relatively mature such that EPSPs and spikes do not reach voltages sufficient to fully remove magnesium block of NMDA receptors and spontaneous activity from the periphery is unlikely to reach necessary rate of depolarization to produce spikes. We elicited action potentials in prehearing OCs to determine if activity-dependent plasticity was possible despite these challenges. We found a 10-fold change in input resistance approximately 20 minutes after spikes were elicited at 100Hz. This change in excitability suggests that after sound elicits the first action potentials in OCs, intrinsic properties could now mediate functional synaptic plasticity. We also observe filopodia on

OC somas and dendrites around the time of hearing onset (P8-P14), suggesting a spine-like substrate for structural plasticity.
Conclusions: We have identified structural and functional mechanisms for transient synaptic plasticity in OCs and suggest that the onset of auditory input is a critical period for OC circuit development. Future studies will address the molecular and functional consequences of developmental disfunction on this specialized cell population which requires anatomical and synaptic stability in adults.

Perinatal Nicotine Exposure Disrupts nAChR Functional Expression and Impairs Glutamatergic Synaptic Transmission in the Mouse Auditory Brainstem

Mackenna Wollet*[1], Abram Hernandez, Kaila Nip[1], Jason Pugh[1], Jun Hee Kim[3]

[1]*UT Health Science Center at San Antonio,* [3]*University of Michigan*

Category: Brainstem: Structure and Function
Background: Maternal smoking leads to chronic nicotine exposure in utero, increasing the risk of persistent auditory processing deficits in the developing brain. However, the impact of chronic nicotine exposure during the critical period on glutamatergic synaptic transmission and auditory processing in the brainstem remains poorly understood.
Methods: We thoroughly investigated the functional expression of nicotinic acetylcholine receptors (nAChRs) at both post- and presynaptic terminals in the medial nucleus of the trapezoid body (MNTB) and assessed the impact of perinatal nicotine exposure (PNE) on nAChR mediated currents, glutamate release, and auditory brainstem responses in juvenile mice.
Results: Our findings show a switch in functional nAChR expression from primarily postsynaptic at MNTB neurons to primarily presynaptic expression at Calyx of Held terminals around hearing onset. Chronic nicotine exposure prior to hearing onset resulted in abnormally increased nAChR-mediated postsynaptic currents in MNTB neurons and compromised glutamatergic neurotransmission at the calyx-MNTB synapse. Notably, PNE significantly reduced readily releasable pool size and release probability, indicating that chronic activation of nAChRs during early postnatal development critically impacts presynaptic neurotransmitter release in the auditory brainstem. Furthermore, PNE mice exhibited increased auditory brainstem response (ABR) thresholds and reduced ABR peak amplitudes, suggesting impaired auditory processing without alterations in cochlear function.
Conclusions: In summary, our findings reveal that PNE disrupts glutamatergic synaptic transmission at the calyx of Held-MNTB synapse and impairs auditory processing, providing novel insights into the underlying mechanisms of auditory deficits following chronic developmental nicotine exposure.

Ipsilateral Noise Effect on Human Electrocochleography Latencies

Lavinia Slabu*[1], Grégory Gerenton[1], Céline Quinsac[1], Paul Avan[1]

[1]*Institut Pasteur, Université Paris Cité, Inserm, Institut de l'Audition*

Category: Brainstem: Structure and Function
Background: Electrocochleography (ECochG) is an evoked potential that measures the electrical activity of the cochlea and auditory nerve from subpopulations of neurons with different spontaneous activities, activation thresholds and saturation levels. While the highly synchronous low-threshold neurons when damaged have a noticeable impact on the ECochG, the less synchronized, yet more vulnerable high-threshold cochlear neurons can be selectively impaired without observable changes in ECochG thresholds, and likely, not even in suprathreshold ECochGs which are dominated by low-threshold neuron contributions. The term 'hidden' auditory neuropathy has been used to describe conditions, hypothetical in humans, in which ECochGs seem normal although high-threshold neurons may no longer contribute.
Methods: We tried to isolate the contributions of high-threshold fibers in ECochGs by eliminating the influence of low-threshold fibers. For this purpose, we used white noise at a level sufficient to swamp the activity of low-threshold fibers all along the cochlea. The use of the ipsilateral white noise at a level of 85 dBSPL facilitated the suppression of the ECochG responses to 80 dBpeakSPL click stimuli. By definition, all masked fibers, saturated by the white noise, could no longer contribute to the ECochGs when the click stimulus

level was set higher, 90, 100, 115 dBpeakSPL, even in the presence of the unchanged level of the white noise. The re-emerging ECochGs (re-ECochGs) must therefore have come from higher-threshold fibers, thus serving as biomarkers of these fibers.
We used an Elios EcochG system (Echodia, Electronique du Mazet, France) with inserted Etymotic gold tip trode active electrodes to record (Re-)ECochG from the two ears of 11 normally hearing human volunteers (type-A tympanogram, otoacoustic emissions present, tone audiometry threshold ≤25 dBnHL at all frequencies between 250 Hz and 12.5 kHz) to monaural stimulation. The opposite ear was masked with white noise (-50 dB relative to the dBpeakSPL click).

Results: By using a paired t-test (RStudio 2022.07.2), it was consistently observed that the interpeak intervals, which followed a normal distribution (Shapiro-Wilk test), were longer in Re-ECochGs than ECochGs: for the I-III interval (t = 11.021, df = 21, p = 3.442e-10), III-V (t = -2.5881, df = 21, p = 0.01716) and I-V (t = -5.8602, df = 21, p = 8.121e-06). The latency intervals I-V were systematically longer in Re-ECochGs than ECochGs, by 0.3 ms (range [3.94, 4.75] vs. [3.56, 4.47]) at 115 dBpeakSPL clicks.
Conclusions: The results suggest that Re-EcochGs may probe different auditory brainstem circuits than ECochGs, thus orienting the diagnosis toward specific functions of high-threshold neurons. One should expect Re-ABRs in some humans at risk of cochlear synaptopathy to be affected. Who these subjects may be and how to diagnose them using standard audiology will be the next crucial step for validating the Re-ABR paradigm.

Serotonin Excites Medial Olivocochlear Efferent Neurons

Kirupa Suthakar*[1], Catherine JC Weisz[1]

[1]*National Institute on Deafness and Other Communication Disorders (NIH/NIDCD)*

Category: Brainstem: Structure and Function
Background: Descending feedback circuitry to the cochlea via auditory efferent neurons provides the brain with a means to adjust auditory nerve sensitivity to sound. Medial olivocochlear (MOC) efferent neurons are located in the brainstem and project to outer hair cell (OHCs) where they inhibit electromotility, ultimately decreasing cochlear amplification via a unique inhibitory acetylcholine synapse. Proposed functions of MOC circuitry include protection from acoustic trauma, signal extraction in noisy environments, and selective attention. In addition to primary afferent input from the cochlear nucleus, MOC neurons also receive descending auditory input and putative input from non-auditory brain regions. We are interested in investigating the non-auditory neuromodulation of MOC activity by serotonin (5-hydroxytryptamine, or 5-HT). Serotonin has been implicated in protection from noise overexposure, but currently, no physiological data addressing the effect of 5-HT on intrinsic response properties of MOC efferent neurons exist.
Methods: ChAT-IRES-Cre;tdTomato mice were used to identify cholinergic MOC neurons in the auditory brainstem for both anatomical and electrophysiological experiments. We first confirmed the presence of serotonergic terminals in close apposition to MOC efferent neurons by combining retrograde tracer injections into the cochlea with immunohistochemistry for 5-HT or TPH2 (tryptophan hydroxylase 2, the rate limiting step in serotonin synthesis) in fixed brain sections. In-vitro patch clamping from brain slices was combined with exogenous application of 100µM 5-HT to demonstrate serotonergic responses in MOC neurons. Pharmacological experiments were also performed using ZD7288 to block hyperpolarization activated cyclic nucleotide gated (HCN) channels, which have been mechanistically implicated in 5-HT signaling in other auditory and vestibular neurons.
Results: Immunohistochemical data have validated the existence of serotonergic terminals in close apposition to both retrogradely-labeled and genetically-identified MOC neurons in mouse. Serotonin increased MOC excitability as evidenced by increased action potential (AP) firing rate, decreased rheobase and decreased AP threshold. Additionally, less stimulation was required to evoke a given action potential rate in MOC neurons. The magnitude of the serotonin effect was heterogeneous between MOC efferent neurons. Preliminary pharmacological experiments suggest a mixed mechanism of action, as 5-HT mediated increases in MOC excitation were retained when HCN channels were blocked by ZD7288. Experiments investigating activation of MOC neurons by endogenously released serotonin using optogenetic stimulation of serotonergic terminals are ongoing.

Conclusions: We have shown that serotonin plays a role in modulating MOC neuron excitability in-vitro. Current experiments are focused on optogenetically driving endogenous release of 5-HT and exploring the mechanism of action underlying the increase in MOC neuron excitability. These data will aid in our understanding of central auditory processing and eventually improve our understanding of how factors such as mood and attention are involved in modulating MOC responses in complex listening situations such as in the presence of background noise.

Symposium 6 - The Leaky Pipeline: How Can Mentors Better Support, Advocate, and Encourage Women and Underrepresented Minorities to Remain in the Sciences?

10:15 a.m. - 12:15 p.m.

Platinum Salon 6

The Leaky Pipeline: How Can Mentors Better Support, Advocate, and Encourage Women and Underrepresented Minorities to Remain in the Sciences?

Chair: Melanie Barzik, *National Institutes of Health*
Co-Chair: Jeffrey Cheng*, Mass. Eye and Ear/Harvard Medical School*

Session Description: The "leaky pipeline" metaphor describes the phenomenon that leads to a progressive loss of women and underrepresented minorities in science, technology, engineering, and math (STEM) disciplines at each stage of the educational system. Women in academia face many barriers to workplace equality, and although they represent more than half of all doctoral recipients in biology-related fields, they only make up 27% of the STEM workforce. Correspondingly, people of color, with disabilities, LGBTQIA+, and from low socioeconomic backgrounds often encounter harassment, prejudice, stereotype, and bias in form of microaggressions, stereotype threat, and imposter syndrome, which might contribute to reduced career achievements. The COVID-19 pandemic has further intensified the leaky pipeline and the underrepresentation of women and minorities in STEM. Numerous studies have shown that this continuing disproportionately low representation of women and minorities impedes innovation and discovery by systematically excluding individuals with the ability to make significant contributions to the scientific enterprise. The goals of this symposium are to provide tools to promote diversity in STEM and create opportunities for women and underrepresented minorities. For this, we have invited three distinguished speakers to discuss the societal factors that contribute to the leaky pipeline phenomenon, explore strategies for fostering inclusivity and equitable opportunities within institutions, share insights on empowering marginalized communities through mentorship and support networks, and present recommendations for creating inclusive workplaces that retain diverse talent.

Following a 10-minute topic introduction there will be a 30-minute keynote presentation by Dr. Cendrine Robinson, followed by presentations from Drs. Barbara Shinn-Cunningham, and Lisa Cunningham (15-min presentations each). After their presentations, all invited speakers will join for an open Q&A session with the audience. The symposium will inspire ARO members to act towards a more inclusive future for underrepresented individuals and help improve the communication of ARO programs supporting diversity in membership.

Podium Session 11 - Inner Ear: Ototoxicity and Otoprotection

10:15 a.m. - 12:15 p.m.

Platinum Salon 5

Live Imaging of Intracellular Calcium Waves in Mature Hearing Cochlea After Noise Exposure

Yesai Park[1], Noura Ismail[1], Ian Matthews[1], Peu Santra[1], Dylan Chan*[1]

[1]*University of California, San Francisco*

Category: Inner Ear: Damage and Protection

Background: Intracellular Ca2+ signaling (ICS) waves, in which endoplasmic reticulum (ER)-cytosolic Ca2+ spikes propagate across supporting-cell networks in response to neuronal injury, activate cell regulatory pathways that can result in neuronal apoptosis, migration, and regeneration. In the cochlea, ICS waves occur in neonatal cochlea, where they impact cochlear development. Mechanical trauma has also been shown to induce ICS activity in neonatal cochleae, suggesting that ICS waves may be involved in the cochlea's response to acoustic overstimulation and could affect hair-cell fate. ICS activity is thought to become quiescent shortly after the onset of hearing, but methodological limitations have precluded investigation of ICS activity in the mature, hearing cochlea.

Methods: Sox2Cre or Myo15Cre mice were bred with Ai95D mice for expression of GcAMP in supporting or hair cells, respectively. P4 neonatal cochlear explant cultures, as well as a novel juvenile mature-hearing cochlear explant preparation, were used for live imaging, performed on an upright Nikon A1R confocal microscope with a 60x water-immersion objective and stagetop incubator. Fluorescence timecourses were measured from 6.5-µm square regions of interest (ImageJ), and Ca2+ spike activity was captured using a custom in-house script (Matlab). Mice were exposed to 98 or 106 dB SPL 8-16 kHz octave-band noise prior to imaging, which respectively cause cochlear synaptopathy without hearing threshold shift or hair cell loss, and permanent threshold shift and hair-cell death.

Results: In neonatal cochleae, spontaneous ICS waves occurred frequently in supporting cells, but not hair cells. ATP induced non-propagating Ca2+ spikes in hair cells. Thapsigargin, which inhibits ER Ca2+ uptake, and vanadate, which inhibits cytosolic Ca2+ clearance, both caused large, significant increases in the decay time of Ca2+ spikes to baseline as well as increases in steady-state cytosolic Ca2+ levels. In mature-hearing cochleae, spontaneous ICS waves were not seen in either supporting cells or hair cells. However, after noise exposure, abundant ICS activity was observed in supporting cells that was affected by the noise level and time interval after noise exposure. 106-dB SPL noise, which causes permanent threshold shifts and hair-cell death, elicited persistent ICS activity 24h after noise exposure, whereas 98-dB SPL noise did not.

Conclusions: Sox2Cre- and Myo15Cre-GcAMP mice are effective tools to perform live imaging of intra- and intercellular Ca2+ activity in cochlear supporting and hair cells, respectively. In neonatal cochleae, live imaging confirms prior findings on the underlying mechanisms of ATP-induced and spontaneous ER-to-cytosolic Ca2+ release, reuptake, and extrusion. In a novel mature hearing explant model, ICS wave activity was quiescent, but reactivated after noise exposure is a dose- and time-dependent manner. This suggests that ICS wave activity is triggered by acoustic trauma and could be an important, and modifiable, control mechanism governing the cochlea's response to noise.

Age-Related Hair Cell Loss in Zebrafish: Decoupling of Homeostasis Vs. Regeneration

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Category: Inner Ear: Damage and Protection

Background: Age-related hearing loss (ARHL) is a debilitating disorder for millions of adults worldwide. While there are multiple underlying causes of ARHL, one common factor is loss of sensory hair cells. In mammals, new hair cells are not produced postnatally and do not regenerate after damage, leading to permanent hearing impairment. By contrast, fish produce hair cells throughout life and robustly regenerate these cells after toxic insult. Despite these regenerative abilities, zebrafish still experience features of ARHL. Here, we examined hair cell loss and homeostatic cell addition in the inner ear of zebrafish from different age classes. We also asked if regenerative abilities differed with age.

Methods: We used both male and female zebrafish aged 3.5-7 months (classified as young) or over 24 months of age (classified as old). Using cellular assays we quantified inner ear and lateral line hair cell number and

inner ear cell proliferation and cell death. We also used a transgenic fish line to quantify inner ear macrophages as an assay for inflammatory state during the aging process. Finally, we used bulk RNA-Seq to determine relative changes in gene expression between ears from young vs. old fish.
Results: We found that zebrafish aged two years or older exhibited significant hair cell loss in all three inner ear epithelia (saccule, utricle, lagena), coincident with decreased cell proliferation. Ears from aged fish showed increased expression of pro-inflammatory genes in ears and had significantly more macrophages than ears from young adult animals. Aged zebrafish also had fewer lateral line hair cells then young animals, although these hair cells still robustly regenerated following acute toxin exposure.
Conclusions: Our work demonstrates that zebrafish exhibit key features of auditory aging and suggest a decoupling of homeostatic hair cell addition from regeneration following acute trauma. Future work is needed to determine the signaling mechanisms responsible for the age-related loss of hair cell addition in this system.

Macrophage Ablation Protects Against Cisplatin-induced Hearing Loss Without Affecting the Tumor Efficacy of Cisplatin in Mice

Cathy Yea Won Sung*[1], Bryce Hambach[2], Haley Ogbemudia[3], Katharine Fernandez[1], Mark Warchol[4], Lisa Cunningham[1]

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Category: Inner Ear: Damage and Protection
Background: Cisplatin is a widely used anti-cancer drug that leads to the death of mechanosensory hair cells in the cochlea, resulting in permanent hearing loss. Macrophages, the major resident immune cells in the cochlea, play an important role in driving both inflammatory and tissue repair responses. We have used a clinically relevant mouse model of cisplatin-induced ototoxicity coupled with selective macrophage ablation using PLX3397, an inhibitor of colony-stimulating factor 1 receptor. Remarkably, we found that macrophage ablation provided near-complete protection against cisplatin-induced hearing loss. As PLX3397 was recently approved by the FDA to treat tenosynovial giant cell tumor, this raises the possibility of repurposing PLX3397 to prevent cisplatin-induced ototoxicity in cancer patients receiving cisplatin. To examine the feasibility of using PLX3397 to reduce cisplatin-induced ototoxicity in the clinic, we further determined whether PLX3397 altered the chemotherapeutic efficacy of cisplatin in tumor-bearing mice.
Methods: To determine the effect of PLX3397 in cisplatin-induced ototoxicity, mice underwent three cycles of cisplatin treatment, each cycle consisting of a once-daily cisplatin injection for 4 days followed by a 10-day recovery period. Selective macrophage ablation was achieved by treating subsets of mice with PLX3397 seven days prior to and throughout the cisplatin treatment. Hearing sensitivity was assessed using auditory brainstem responses (ABR).
To establish the tumor-bearing mouse model, TC-1 tumor cells were subcutaneously injected into the hind flank of adult mice. Simultaneously, mice received control or PLX3397 chow to ablate macrophages. Seven days later, when palpable tumors were detected, mice were administered either saline or cisplatin while continuing to receive PLX3397. Tumor volume was measured daily.

The four experimental groups employed were Saline/Vehicle, Saline/PLX3397, Cisplatin/Vehicle, and Cisplatin/PLX3397 in wild-type mice or mice implanted with TC-1 tumor cells.

Results: ABR threshold shifts were significantly reduced in mice treated with Cisplatin/PLX3397 compared to mice treated with Cisplatin/Vehicle, indicating that macrophage ablation protected against cisplatin-induced hearing loss. Quantification of hair cells suggests that this protective effect of macrophage ablation by PLX3397 treatment was due to the protection of outer hair cells.
Cisplatin/Vehicle treatment significantly reduced tumor volume by approximately 63% (25 days after tumor implantation) compared with the Saline/Vehicle-treated group. Importantly, no significant difference in tumor volume was observed between mice receiving Cisplatin/PLX3397 versus those treated with Cisplatin/Vehicle, indicating that PLX3397 did not compromise the anti-tumor efficacy of cisplatin.

Conclusions: Our data indicate that macrophage ablation using PLX3397 provides robust protection against cisplatin-induced hearing loss. Importantly, in a tumor-bearing mouse model, we observed no reduction in the

anti-tumor efficacy of cisplatin in mice that were also treated with PLX3397. As PLX3397 was approved by the FDA to treat tenosynovial giant cell tumor, our present study provides evidence that PLX3397 can likely be repurposed to prevent cisplatin-induced hearing loss in cancer patients receiving cisplatin.

Pharmacological Downregulation of TRPV1 Channels Protects From Aminoglycoside-Induced Hearing Loss

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Category: Inner Ear: Damage and Protection

Background: Hearing loss is a major health concern in our society, affecting over 400 million people worldwide. Aminoglycoside therapy causes permanent hearing loss in 40-60% of treated patients. To date, no drugs have been approved by the Food and Drug Administration for protection from aminoglycoside-related hearing loss (AGIHL). Most candidate compounds currently in pre-clinical and clinical trials are related to antioxidants, vitamins, and glutathione metabolism. We have conducted a high-throughput screening of bioactive natural compounds employing zebrafish as our platform for aminoglycoside ototoxicity and identified piperlongumine, an alkaloid extracted from the long pepper Piper Longum L., as an important therapeutic molecule for aminoglycoside-induced hair cell death.

Methods: Piperlongumine's pharmacochemical properties were assessed in vitro and in vivo in zebrafish larvae. Inner ear protection was determined in a mouse model for AGIHL. Hearing function (auditory brainstem responses [ABRs] and distortion product otoacoustic emissions [DPOAEs] was measured before and 4 weeks post-treatment. The number of outer hair cells (OHCs), pre-synaptic ribbons, and neuronal fibers was quantified at different frequencies by confocal microscopy. To characterize piperlongumine's mechanism of action, the activity of various kinases was assessed using a kinome platform and confirmed by immunoblot and immunohistochemistry analysis. Molecular dynamics (MD) between piperlongumine and putative targets is currently being performed.

Results: . Dose-response curves in a zebrafish model for kanamycin ototoxicity showed piperlongumine can protect with an EC50 of ~125 pM. Similarly, experiments using the mouse inner ear cell line, HEI, showed a LD50 for piperlongumine of ~25 μM. Additionally, we found that piperlongumine can protect kidney function from aminoglycoside nephrotoxicity and does not show any drug-drug interactions. Studies in a mouse model for AGIHL demonstrated that piperlongumine can protect hearing function when given as a single dose for 17 consecutive days. While kanamycin alone resulted in elevated ABR and DPOAE thresholds, the co-administration of piperlongumine reduced them to almost normal levels. OHCs and pre-synaptic ribbons were protected by piperlongumine treatment. Kinome analysis of organ of Corti extracts suggested that piperlongumine mechanism of action is, at least in part, mediated by the regulation of retinoblastoma proteins. Finally, because there is evidence that piperlongumine can regulate the transient receptor potential vanilloid (TRPV) channels and given that TRPV1 is upregulated in the inner ear during inflammation and that the TRPV1 knockout mouse is resistant to aminoglycoside ototoxicity, we decided to look at TRPV1 expression in kanamycin- and kanamycin+piperlongumine-treated animals. While TRPV1 was upregulated by aminoglycoside, the co-administration of piperlongumine resulted in a down-regulation of the channel in the stria vascularis and hair cells. Moreover, computational assessments are currently being performed to address piperlongumine TRPV1 dynamic interaction.

Conclusions: Piperlongumine can protect from AGIHL by regulating the activity and/or expression of the retinoblastoma proteins and TRPV1 channel.

Higher Platinum Signal is Detected in the Inner Hair Cells Than in the Outer Hair Cells After Systemic Cisplatin Administration

Xiaodong Tan*[1], Prerita Pandya[1], Devin Thomas[1], Robert Fuentes[1], Chail Koo[1], Olga Antipova[2], Luxi Li[2], Yang Yang[3], Claus-Peter Richter[1]

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Category: Inner Ear: Damage and Protection

Background: Cisplatin is delivered intravenously (i.v.) in chemotherapy but intraperitoneal (i.p.) in most animal studies. This raises the question of whether ototoxic effects and target structures are the same for the two different delivery methods. We reported an ongoing project in the last ARO that platinum signal in the cochlea is different between i.v. and i.p. administration. Using a state-of-the-art technique, X-ray fluorescence microscopy (XFM), we found that a higher platinum signal was observed in the cochlea after cisplatin i.v. injection. A high platinum deposit was observed in the stria vascularis and nerve tissue, although an obvious platinum signal was also shown in the organ of Corti. We report here the follow-up studies using higher resolution XFM (300 nm) to show platinum signal detected in cochlear structures on the cellular level.

Methods: Male and female mice between 2-3 months (weight 20-30 grams) are randomly assigned to Cis_IP and Cis_IV groups receiving cisplatin (10 mg/kg) through i.p. or i.v. injection, respective. Thresholds of auditory brainstem responses (ABRs) served as the outcome measure for hearing and were measured before (baseline) and after (1, 7, and 14 days) the treatment. Animals are euthanized and cardiac perfused at different time points after the treatment (1 hour, 1 day, and 14 days). cochleae were harvested for histology and XFM imaging and analysis. XFM was performed on cross-sections of cochlea, at Beamlines 2-ID-E and 2-ID-D at the Argonne National Laboratories and at Beamlines 3-ID and 5-ID at the National Synchrotron Light Source II. The number of mice in the study was justified by statistical analysis with G*Power using an alpha of 0.05 and a power of 0.08.

Results: The animal survival rate in the Cis_IP group (90%) is greater than that in the Cis_IV cisplatin group (60%). However, the ABR threshold shift is insignificant and does not show any difference between the two groups. XFM scans show that a high platinum signal is detected in the stria vascularis (SV) and spiral ganglia after day 7, and platinum concentration is higher in the Cis_IV group than in the Cis_IP group. The platinum signal is also shown in the organ of Corti. Surprisingly, a higher resolution XFM scan (300 nm) shows that the platinum signal in the inner hair cells (IHCs) is about 5 times higher than that of the outer hair cells (OHCs).

Conclusions: Cisplatin i.v. injection shows greater toxic effects than i.p. injection while its ototoxicity does not show any difference up to 14 days. The high platinum deposition in IHCs instead of in OHCs raises the question of what is the dominant mechanism underlying cisplatin ototoxicity. Further studies are ongoing to determine the mechanism of cisplatin ototoxicity.

Exploring Protective Strategies Against Aminoglycoside-Induced Ototoxicity Using Molecular Dynamics Simulations and Transcriptomic Analyses

Cheng Yu Hsieh*[1], Cheng-Yu Tsai[2], Chen-Chi Wu[3]

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Category: Inner Ear: Damage and Protection

Background: Aminoglycoside-induced ototoxicity is a pressing concern due to the widespread clinical use of these drugs. This condition is characterized by aminoglycoside entry into the endolymph and hair cells through specific channels, resulting in the generation of reactive oxygen species (ROS) and subsequent hair cell apoptosis. Current strategies primarily target the downstream effects of ROS. However, recent evidence suggests that inhibiting drug uptake by hair cells is an alternative and promising approach to address the upstream drug uptake pathway. Advances in structural biology have revealed the conformation of channels such as the mechanoelectrical transduction (MET) channel and transporters such as organic cation transporter 2 (OCT2), making them potential targets for innovative strategies. While some MET channel blockers have shown efficacy in zebrafish and mouse cochlear cultures, their safety for human use remains a concern.

Therefore, this study uses a combination of molecular dynamics (MD) simulations and transcriptomic analyses to elucidate mechanisms and develop safer otoprotective strategies against drug-induced ototoxicity.
Methods: This study has three integral components. First, we used MD simulations to refine the 3D structures of TMC1 (transmembrane channel-like protein 1) and explore potential binding sites for blockers. These simulations included screening drugs curated in the DRDOCK platform to identify potential otoprotective candidates. Second, transcriptomic analyses were performed using UB/OC-2 cells (in vitro) and cochlear explants (ex vivo) models. These models were subjected to gentamicin treatment followed by RNA extraction for comprehensive transcriptomic analysis to identify predominant pathways involved in ototoxicity. Finally, functional validations were performed and target drugs were selected based on the intersection of MD simulations and transcriptomic analyses. The protective effects of these drugs against gentamicin-induced ototoxicity were validated in both ex vivo and in vivo experimental models.
Results: We screened a pool of 5,856 curated drugs from the DRDOCK platform and identified 37 highly promising candidates based on preliminary data. Transcriptomic analyses revealed significant enrichment of upregulated genes associated with the PI3K-Akt and the MAPK pathways. A total of 680 Gene Ontology (GO) terms were analyzed, demonstrating increased expression of calcium channel-related genes and ion channel activity from a molecular function perspective. Among the 37 drug candidates identified by the DRDOCK platform, five drugs, specifically calcium channel blockers and selective estrogen receptor modulators, exhibited remarkable otoprotective capabilities against gentamicin-induced ototoxicity in in vitro assays. These results underscore the promising potential of our innovative strategy for identifying otoprotective drugs.
Conclusions: This study supports the inhibition of aminoglycoside uptake by hair cells as a promising strategy for targeting the drug uptake pathway. The integration of MD simulations and transcriptomic analyses holds great promise for identifying potent agents against aminoglycoside-induced ototoxicity, offering novel strategies and validating novel targets.

Vesicular Compartmentalization of Aminoglycosides in Zebrafish Lateral Line Hair Cells

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Category: Inner Ear: Damage and Protection
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 a considered standard treatment for patients. Our group has extensively explored the multiple adverse effects of AGs on zebrafish lateral line hair cells. Especially, the distinct hair cell death patterns that different AG generates. During uptake, neomycin accumulates mostly in the cytoplasm of hair cells generating an acute death of the hair cell. On the other side, AGs like, gentamicin or G418, accumulate mostly in vesicles within hair cells, and they generate a delayed hair cell death. These observations led us to hypothesize that vesicular compartmentalization and endosomal maturation could be influencing a delayed hair cell death. Our ultimate goal is to understand the mechanisms that participate in AG uptake and vesicular compartmentalization, and the role that the vesicular network exerts over AG-induced hair cell toxicity.
Methods: In order to follow AG compartmentalization and vesicle maturation over time we generated zebrafish transgenic lines expressing Rab5 and Rab7 fluorescent probes in hair cells to label early and late endosomes, respectively. Furthermore, we also expressed mutated forms of Rab5 and Rab7 to disrupt the normal maturation process of vesicles inside hair cells. Using super-resolution imaging and dose-response curves we analyzed the accumulation of AGs in the cell, as well as their influence over hair cell death.
Results: Our results support the previous observation that most G418 accumulation happens in late endosomes/lysosomes, labeled by Rab7. Interference of the Rab7 vesicular maturation doesn't appear to affect accumulation of the AG, as well as hair cell toxicity. Interestingly, a small fraction of G418 does show a transient passage through Rab5-positive vesicles. Forcing accumulation of G418 into Rab5-positive vesicles

makes hair cells more susceptible to AGs at lower concentrations of the drug. This condition does not affect neomycin ototoxicity, which accumulates mostly in the cytoplasm and triggers an acute cell death.
Conclusions: Our approach allows us to conclude that compartmentalization of AGs into vesicles is participating in delayed cell death. Moreover, accumulating AGs in early endosomes is detrimental for hair cell survival. Overall, our results suggest that compartmentalization and location of AGs within the vesicular network plays an important role during delayed hair cell death.

Designing a Tumor-Bearing Mouse Model of Cisplatin-Induced Hearing Loss to Test Potential Therapeutics

Bryce Hambach*[1], Cathy Yea Won Sung[2], Haley Ogbemudia[3], Katharine Fernandez[4], Lisa Cunningham[4]

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Category: Inner Ear: Damage and Protection
Background: Cisplatin is an anti-cancer drug that is used to treat millions of cancer patients each year. Significant, permanent hearing loss has been reported in 50-60% of patients receiving cisplatin. Previously, our lab has developed a multi-dose, multi-cycle mouse model of cisplatin administration to induce a robust, reliable, hearing loss similar to the type and severity observed clinically. Various tumor mouse models exist for the purposes of studying the efficacy of cancer therapies, but there is not yet a model that incorporates hearing loss in a tumor-bearing mouse. Here, our goal is to incorporate our existing cisplatin ototoxicity protocol into a tumor-bearing mouse model to establish a platform for evaluating potential therapeutics that may reduce cisplatin-induced hearing loss without interfering with cisplatin's efficacy.
Methods: 40 (20F; 20M) adult C57BL/6 WT mice received lung epithelial tumor cells (TC-1 cells) subcutaneously in the right hind flank. Mice were assigned to one of four treatment groups receiving either cisplatin or saline and co-treatment with either PLX3397 drug or vehicle. Anesthetized mice underwent auditory brainstem response (ABR) and distortion product otoacoustic emissions (DPOAE) testing pre- and post-cisplatin treatment. Tumor growth, weight, and health conditioning score were assessed daily. Mice were removed from the experiment when they reached pre-specified endpoint health condition criteria.
Results: Tumors in cisplatin-treated mice grew significantly slower compared to tumors in saline-treated mice (2-way ANOVA, p less than 0.05). Co-treatment with PLX3397 did not significantly change the tumor growth trajectory compared to those treated with vehicle for both cisplatin (2-way ANOVA, p greater than 0.05) and saline-treated groups (2-way ANOVA, p greater than 0.05). Weight loss observed in all groups was minimal, with a reduction of less that 20%. Kaplan-Meier survival analysis shows reduced survival rate in saline-treated mice compared to cisplatin-treated mice. ABR and DPOAE thresholds were similarly elevated across all treatment groups. Significant hearing loss was found in a subset of mice during baseline testing, consistent with characteristics of C57BL/6 WT mice that can develop early-onset age-related hearing loss.
Conclusions: Cisplatin-treated mice had significantly reduced tumor growth compared to saline-treated mice and co-treatment of PLX3397 did not alter cisplatin's anti-tumor efficacy. In developing this model, we found that mice reached endpoint criteria prior to the timeline of the original ototoxicity protocol. Additionally, the strain of mice used was prone to early onset hearing loss. This complicated the interpretation of auditory testing in mice. Moving forward, our goal will be to move the protocol into a strain of mice not susceptible to premature hearing loss and to optimize the cisplatin dose as well as administration schedule to induce a hearing loss in a time frame adequate to assess tumor growth.

Podium Session 12 - Hearing Loss: Implication for Cochlear Implantation and Cross-Modal Plasticity

10:15 a.m. - 12:15 p.m.

Grand Ballroom Salon E

Impact of Inner Ear Malformation and Cochlear Nerve Deficiency on the Development of Auditory-Language Network in Children With Profound Sensorineural Hearing Loss

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[1]*Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine*

Category: Hearing Loss: Consequences and Adaptation

Background: Profound congenital sensorineural hearing loss (SNHL) prevents children from developing spoken language. Cochlear implantation (CI) and auditory brainstem implantation (ABI) can provide partial hearing sensation, but language development outcomes can vary, particularly for patients with inner ear malformations and/or cochlear nerve deficiency (IEM and CND). Currently, the peripheral auditory structure is evaluated through visual inspection of clinical imaging, but this method is insufficient for surgical planning and prognosis. The central auditory pathway is also challenging to examine in vivo due to its delicate subcortical structures. Previous attempts to locate subcortical auditory nuclei using fMRI responses to sounds are not applicable to patients with profound hearing loss as no auditory brainstem responses can be detected in these individuals, making it impossible to capture corresponding blood oxygen signals in fMRI.

Methods: In this study, we introduced a novel pipeline that employs both structural and diffusion MRI to map the auditory pathway. We used a fixel-based approach to examine the development of the auditory-language network in children under six with profound SNHL, as well as in normal hearing controls. Our investigation encompassed: 1) changes in fiber metrics within the auditory-language network; 2) the influence of IEM and CND on the network's pre-implant structural development; and 3) the correlation between pre-implant structural characteristics of this network and post-implant auditory-language outcomes in CI or ABI recipients.

Results: We successfully segmented subcortical auditory regions and reconstructed the auditory and language pathways in vivo. Our findings showed decreased fibre density and fibre cross-section mainly in the inferior part of the central auditory pathway and the left language pathway in patients with profound SNHL. Additionally, we discovered that the correlation between language pathway fibre metrics and peripheral vestibulocochlear nerve tissue density is stronger and more significant than that in the central auditory pathway, and that the peripheral nerve tissue density negatively moderated the developmental trajectory of the central auditory and language pathways. Preoperatively evaluating the structure of the auditory-language network helps predict postoperative audiometric and qualitative language outcomes.

Conclusions: This study provides structural evidence that highlights the importance of early intervention for profound SNHL children to provide timely speech inputs, particularly stressing the need for special attention for patients with IEM and CND. It also introduces a promising comprehensive pre-surgical evaluation framework for the auditory-language network in children with severe-to-profound SNHL to assist with surgical planning and prognosis.

Hearing Loss in Adult Rats Leads to Less Ultrasound Vocalization During Social Interaction, Cognitive Disturbances in Visuospatial Attention and Learning Behavior

Mariele Stenzel[1], Mesbah Alam[1], Jonas Jelinek[1], Joachim K. Krauss[1], Kerstin Schwabe[1], Marie Johne*[1]

[1]*Hannover Medical School*

Category: Hearing Loss: Consequences and Adaptation

Background: Hearing loss in the elderly has been associated with difficulties in speech comprehension, cognitive decline and as a possible risk factor for dementia. We already showed in adult rats that hearing loss leads to reduced neuronal activity in the medial prefrontal cortex (mPFC). To investigate the impact of hearing loss on cognitive function and communication, we tested adult rats in behavioral paradigms for motor activity, attention, and impulse control, as well as social interaction, including ultrasonic vocalization (USV).

Methods: In a cohort of adult male Sprague Dawley rats, hearing loss was induced under general anaesthesia with intracochlear injection of neomycin (n=11) and was verified after surgery with auditory brainstem response (ABR) measurement. Naive (n=10) and sham-operated rats (n=7) served as control. The rats were tested for motor activity (Open Field), motor coordination (Rotarod), and social interaction before surgery and at week 1, 2, 4, 8, 16, and 24 after surgery. From week 8 onwards, the rats were tested in the Five Choices Serial Reaction Time Task (5CSRTT) for visuospatial attention, impulse control, learning, and memory. In

this paradigm, rats have to react to a light stimulus in one of five holes of the aperture, which is shortened from session to session in the training phase.
Results: In the Open Field, deaf rats moved significantly faster and a longer distance in total than the naive and sham-operated controls (both p less than 0.05). Although social interaction was not altered, the frequency of USV was significantly lower in deaf rats compared to the control group (p less than 0.05). Learning the 5CSRTT paradigm was significantly impaired in the deaf group during the first training session (p less than 0.05). Looking at the omission rate, the deafened rats appeared to be less likely to miss a trial on a significant level (p less than 0.05), but also showed at least a tendency for more incorrect hits (p=0.076). Retesting in weeks 20 and 24 did not indicate a long-term memory deficit in the deaf group. For the training phase and retesting it is worth mentioning that the deafened rats had a significantly shorter latency between correct response and getting the reward (p less than 0.05).
Conclusions: Hearing loss in adult rats leads to hyperlocomotion and less USV during social interaction. Furthermore, these rats exhibit deficits in initial visuospatial attention as evidenced by a lower accuracy rate and delayed comprehension of the new task. These cognitive impairments may be associated to compromised neuronal activity in the mPFC, as shown in a prior publication. Therefore, this model could serve as a valuable tool for investigating the effect of neuromodulatory stimulation on cognitive decline attributed to hearing impairment.

Visual and Auditory Implicit Learning in Adult Cochlear Implant Users: A Cross-Modal Contribution to Speech Recognition?

Ranin Khayr[1], Riyad Khnifes[1], Talma Shpak[2], Karen Banai*[3]

[1]*University of Haifa and Bnai-Zion Medical Center,* [2]*Bnai-Zion Medical Center,* [3]*University of Haifa*

Category: Hearing Loss: Consequences and Adaptation
Background: Speech recognition in challenging conditions varies widely across listeners. Studies suggest that implicit learning is one variable that may contribute to this variability. The current study focuses on visual and auditory implicit learning and explores their unique contributions to individual differences in the recognition of challenging speech in cochlear implant users (CI) in comparison to normal-hearing (NH) listeners.
Methods: 36 adult CI users (ages 35-77, M=55) and a control group of 36 NH individuals (ages 36-77, M=52) completed a comprehensive battery of challenging speech recognition tests (words and sentences in noise and natural-fast speech), cognitive measures (vocabulary, working memory, attention, and verbal processing speed), two visual learning tasks (statistical and incidental) and a rapid auditory perceptual learning task (time-compressed speech). Accuracy in the most challenging speech tasks for each group was modelled with a series of generalized mixed linear models as a function of cognitive factors and learning to isolate the unique contribution of each index of learning to speech recognition.
Results: Both groups exhibited comparable performance in the two visual learning tasks: statistical and incidental. In the rapid auditory perceptual learning task, while both groups exhibited significant learning, learning was significantly poorer in CI than in the NH group (NH: M(slope) = 0.06, CI: M(slope) = 0.01; t (70) = -5.86, p less than 0.001). The contribution of visual and auditory learning to speech recognition was evidenced only in the CI group; both visual statistical and rapid auditory perceptual learning contributed to the recognition of natural fast speech (OR = 44% and 40% respectively), but only visual statistical learning contributed to the recognition of sentences in babble noise (OR = 64%). Working memory and attention also emerged as contributing factors to the recognition of challenging speech in the CI group. In the NH group, working memory was the only significant predictor but note that this group had a near ceiling performance in the speech tasks which were designed to be possible for CI users.
Conclusions: First, while visual learning in CI users is similar to that of their NH peers, their auditory learning still lags behind. Second, based on the findings in CI users, the contribution of implicit learning to speech recognition seems to increase and involve both modalities as listening becomes more challenging. Third, as learning in both modalities contributed to speech recognition in CI users, integrating a visual learning index into the pre-implantation evaluation should be explored as a way to establish post-implantation rehabilitation milestones and goals for CI candidates who are expected to benefit from post-implantation support.

Differences in Neural Correlates of Auditory Working Memory Between Cochlear Implant Users and Typical Hearing Controls

Priyanka Prince*[1], Joseph Chen[2], Trung Le[2], Vincent Lin[2], Andrew Dimitrijevic[3]

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Category: Hearing Loss: Consequences and Adaptation

Background: A common concern for individuals with severe-to-profound hearing loss fitted with cochlear implants (CIs) is difficulty following conversations in noisy environments. Previous literature has alluded to a possible factor which is the differences in cognitive resource recruitment, such as working memory and attention. However, the neural basis for this relationship is not fully understood.

Methods: In this study, we investigated behavioural and neural correlates of auditory working memory in 14 CI users and typical hearing (TH) controls using high-density electroencephalogram (EEG) while participants completed an N-back task consisting of two conditions, 0-back and 2-back. While 0-back measured speech perception ability, the 2-back measured cognitive ability through working memory and attention.

Results: Behavioural results, during the 2-back condition, suggests a delay in identifying the targets in older CI users; a result not found in TH controls. Through EEG, we find differences between groups in sensory and neural oscillatory activity. CI users, overall, showed a lack of adaption to increasing task demands compared to TH controls and 2-back differences in theta and alpha/beta activations relate to age only in CI users.

Conclusions: These oscillatory differences suggest that compensatory mechanisms younger CI users use may lead to detrimental effects over time. Additionally, these results show neural differences in both bottom-up (encoding) and top-down (attention and working memory) processes in CI users, specifically older adults, which may contribute to issues with working memory and therefore, in social situations.

Neural Entrainment of a Naturalistic Conversation in Varying Working Memory Loads

Priyanka Prince*[1], Joseph Chen[2], Trung Le[2], Vincent Lin[2], Andrew Dimitrijevic[3]

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Category: Hearing Loss: Consequences and Adaptation

Background: In a noisy environment with auditory and visual distractions, selective attention to target stimuli can be cognitively demanding especially in individuals with a hearing impairment or using a hearing protheses such as a cochlear implant (CI). CI users have been shown to rely more on visual input for the understanding of speech stimuli; this can result in an increased listening effort and therefore, more resources utilized from a limited cognitive load. The neural basis of this relationship between cognition and speech perception and understanding is not fully understood.

Methods: In this study, using a high-density electroencephalogram (EEG) in CI users and normal hearing (NH) adults, we investigated the neural correlates of speech entrainment to two people having a conversation with background multitalker noise whilst visual numbers appeared on the screen around them in three memory loads: no numbers, three numbers and seven numbers. The task was to answer questions about the conversation and recall the numbers that were presented.

Results: Behavioural results showed that as visual load increases, performance on recall for the conversation decreases for CI users and in terms of the digits, performance remains stable however, for NH controls, the results are reversed. EEG results show that the degree of neural entrainment to the conversation was larger in CI users suggesting that CI users prioritize the allocation of their attentional resources towards the conversation. Also, theta and alpha/beta activation differences suggest cognitive impairment in older cochlear implant users and increased use of attentional resources in those who presented issues with speech perception.

Conclusions: Therefore, these results would suggest that the CI users that allocate more attentional resources perhaps allocate too much and therefore, results in detriments in speech perception in the real world. The allocation of more attentional resources might be a compensatory mechanism used for speech perception however, it may affect their cognitive integrity while aging resulting in cognitive decline. This data provides

evidence that natural conversations can be used as a stimulus when probing cognitive functions related to speech in noise listening and working memory.

Characterization and Mitigation of Hearing Damage Induced by Repeated Mild Blast TBI in Chinchillas

Shangyuan Jiang*[1], Rong Gan[1]

[1]*University of Oklahoma*

Category: Hearing Loss: Consequences and Adaptation

Background: Blast-induced mild traumatic injury (mTBI) is a critical health concern that has been frequently observed among Service members during training and combat. Sensorineural hearing loss shows a strong correlation with mTBI even though hearing protection devices are widely used. We have investigated the mTBI-induced hearing damage and the therapeutic function of liraglutide, an FDA approved drug for type 2 diabetes, on mitigation of hearing damage in animal model of chinchilla. However, the previous study was performed based on a single incident on one day blast exposure and the hearing damage in drug treated animal with the ears protected was mostly recovered on Day 4 after 3-blast exposure on Day 1. Thus, the long-term effect of the liraglutide treatment on mitigation of repeated blast-induced hearing loss in protected chinchillas is not clear. This study aims to characterize hearing function changes after repeated, multiple-day blast incidents, and evaluate the effect of liraglutide treatment under this condition.

Methods: Chinchillas were divided into two treatment groups: blast control (N=8) and post-blast liraglutide treatment (N=9). All animals were exposed to 3 blasts at a level of 15-25 psi (103-138 kPa) on Day 1 and Day 4. Earplugs (EPs) were tightly inserted into chinchilla ear canals during the blast exposures to ensure the tympanic membrane was intact after blasts. Animals were observed for 28 days and the auditory brainstem response (ABR), distortion product otoacoustic emission (DPOAE), and middle latency responses (MLRs) were measured pre- and post-blast on Day 1, post-blast on Day 4, and on Days 7, 14, and 28. The 7-day-long subcutaneous liraglutide injection (0.2467/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. Upon the completion of the experiments, cochlea and brains were collected for immunofluorescence studies to detect the pathological changes in the peripheral and central auditory system.

Results: Repeated blast mTBI induced significant hearing damage on Days 1 and 4, but the damage gradually recovered overtime and no significant ABR threshold shift was observed by Day 28. Liraglutide treatment reduced the severity of acute damage induced by mTBI as reflected by the ABR threshold shifts. The mTBI-induced changes and the therapeutic function of liraglutide were observed in the central auditory system as reflected by the MLR and histologic results.

Conclusions: Repeated mTBI induced more severe hearing damage than single blast incident especially in the central auditory system. The damage mitigation effect of liraglutide was most significant at the acute stage. Long-term functional and histological changes induced by mTBI and liraglutide in the central auditory system require further investigations.

Localizing Cross-Modal Plasticity: Sensory Border Dynamics and Implications for Cochlear Implantation in Adult-Onset Deafness

Mariele Stenzel[1], Alam Mesbah[1], Marie Johne[1], Peter Baumhoff[2], Wiebke Konerding[2], Kerstin Schwabe[1], Andrej Kral[2], Rüdiger Land*[2]

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Category: Hearing Loss: Consequences and Adaptation

Background: The ability of the adult brain to adapt to late-onset deafness has implications for the restoration of hearing with cochlear implants. Adaptive brain changes due to visual compensation, such as cross-modal plasticity, may interfere with restored auditory input with cochlear implants. The exact extent and location of these changes on the neuronal level remain unclear, as they are difficult to detect using non-invasive methods such as EEG.

Methods: We investigated deafness-induced changes across the auditory-visual cortical border of adult-deafened Sprague-Dawley rats. Adult rats were deafened by intracochlear injection of neomycin and received visual training on a visual choice task for 6 months. After the visual training, the rats were implanted with cochlear implants. We then used multisite silicon probes to record intracortical neural responses to combined cochlea-implant and visual stimulation across the auditory-visual cortex border in both deafened rats and hearing controls (acutely deafened and also implanted with cochlear implants).
Results: Deafness caused a shift in sensory borders, with visual activity expanding towards the auditory cortex in adult-deafened rats. However, this shift was limited to the sensory border zone, and no visual responses were observed toward the core auditory cortex. Cochlear implant-evoked responses within the auditory cortex were weaker, and especially the induced power of responses was affected by deafness, however, still comparable to the hearing controls. Bimodal combined cochlear implant and visual stimulation did not largely interfere with auditory activation.
Conclusions: Our findings show that 'cross-modal plasticity' in adult-onset deafness is primarily a local effect at sensory borders, with no spilling into core auditory regions in adult deafened rats. Additionally, intramodal changes occur within the auditory cortex after adult-onset deafness, but overall neural activity to cochlear implant stimulation remains comparable between the adult deafened and the hearing auditory cortex. These plastic changes after adult-onset deafness may be reversible by adequate cochlear implant rehabilitation.

Effects of Cognitive Load and Spatial Hearing on Postural Sway and Associations With Fall Risk in Older Adults With and Without Hearing Aids

Alexander Francis*[1], Shirley Rietdyk[1], Jeffrey Haddad[1], Sarah Burgin[2], Sharon Christ[1], Melissa Newell[1], Mia Schmetter[1], Abigail Foley[1]

[1]*Purdue University,* [2]*Indianapolis University School of Medicine*

Category: Hearing Loss: Consequences and Adaptation
Background: Hearing loss is associated with a significantly higher incidence of falling in older adults. Three potentially interacting mechanisms have been proposed to explain this relationship (Agmon et al., 2017; Carpenter and Campos, 2020; Heitz et al., 2019; Jiam et al., 2016; Lin and Ferrucci, 2012; Mick et al., 2018): (1) Comorbidity with vestibular dysfunction (Heitz et al., 2019); (2) Changes in spatial awareness affecting balance and gait (Vitkovic et al., 2016; Weaver et al., 2017); and (3) Increased demand on cognitive resources reducing capacity to maintain balance (Carr et al., 2020; Yardley et al., 2001). Here we present a study comparing fall incidence in daily life with postural sway measured under various listening conditions in older adults with and without hearing aids. Our goal is to determine how cognitive demand and spatial awareness contribute to the observed relationship between hearing loss and fall risk.
Methods: Forty-six adults aged 62-85 years participated in this study (33 women, 13 men; 19 hearing aid wearers, 27 not). Participants first completed assessment of cognition, dizziness, and hearing. During testing, participants stood for one minute in each of eight conditions on a hard surface in a sound attenuated booth with feet together, shoes off, and eyes closed and masked. Eight conditions imposed varying degrees of cognitive or spatial auditory demand: Noise vocoded short stories with four, eight, and sixteen channels (high, moderate, and low listening effort); environmental sounds presented from one vs. three spatially distinct locations; counting forward by ones and backward by sevens; and standing in silence. All stimuli were presented in free field. Hearing aid users wore their hearing aids in all conditions. Postural sway was recorded using inertial measurement units (IMUs) mounted on the forehead and back and quantified as root mean squared (RMS) acceleration in three dimensions. Subsequently, for four months participants reported daily on whether they had experienced a slip, trip, or fall using either an online survey or paper diary.
Results: Results for postural sway suggest that hearing aid users stand more rigidly (exhibit lower sway values) than non-users and show greater decrease in sway as a function of cognitive demand and spatial sound. Similarly, older age and poorer hearing, but not cognitive or dizziness test scores, are associated with greater rigidity irrespective of hearing aid use. The relationship between laboratory measures, hearing aid use, and fall risk will be assessed once all participants complete the four-month fall survey.
Conclusions: Preliminary results are consistent with hypotheses suggesting that hearing loss affects balance under listening conditions requiring increased cognitive effort or spatial awareness. When completed, this

study will be among the first to link laboratory measures of postural sway to detailed observational measures of fall risk in daily life.

Poster Session 3

1:15 p.m. - 3:15 p.m.

Marquis Ballroom

M1. Auditory Attention Decoding Using Epidural Electrodes

Desmond Mehta*[1], Vishal Choudhari[2], Nima Mesgarani[2]

[1]*Cold Spring Harbor High School,* [2]*Columbia University*

Category: Auditory Cortex and Thalamus: Human Studies

Background: Hearing-impaired individuals express difficulty in attending to a specific talker in the presence of noise, especially in a multi-talker environment. Cognitively-controlled hearing aids could address this problem by selectively amplifying an attended speech stream by directly measuring neural signals that reflect a listener's target of attention using auditory attention decoding (AAD) algorithms. However, currently proposed methods rely upon direct invasive recordings from the auditory cortex. Our study explores the possibility of AAD in simulated multi-talker settings using less invasive epidural and subcutaneous electrodes.

Methods: Subjects were patients undergoing epilepsy seizure monitoring with invasive electrodes. Electrodes were implanted into the intracranial and epidural spaces and neural activity was recorded as subjects listened to simulated two talker environments. Subjects were asked to attend to one talker for each 40 second trial while ignoring the other. Electrodes were localized using MRI and CT scans. Intraparenchymal, epidural and subcutaneous electrode recordings were examined for the ability to perform AAD.

Results: Extending upon our prior work showing the ability of intraparenchymal electrodes to perform AAD, we found that epidural electrodes could perform AAD with significant accuracy even in brief time windows (as short as 100 ms). For longer durations, the AAD algorithm achieved 70% accuracy within a 2-second window and 80% within an 8-second window. Although subcutaneous recordings were less consistent, they demonstrated promise for improved performance through optimization.

Conclusions: Our findings demonstrate the feasibility of a novel approach to AAD using less invasive techniques. This would meaningfully advance progress towards technology to assist those who are hearing-impaired by enabling them to control a speech stream-segregated "smart hearing aid" using less invasive surgical methods.

M2. Exploring a Possible EEG Marker of Anticipatory Processing in a Natural Speech-Based Selective Attention Task

Thomas Stoll*[1], Ross Maddox[1]

[1]*University of Rochester*

Category: Auditory Cortex and Thalamus: Human Studies

Background: The classic cocktail party experiment studies auditory attention by presenting two speech recordings to subjects while asking them to attend only one talker. This experimental paradigm has commonly been combined with EEG to study how cortical responses are affected by selective attention, but recent advancements in stimulus and analysis techniques allow for the same paradigm to be extended to investigate subcortical responses. While conducting such an experiment and looking at early time lags associated with subcortical responses, we observed an attentional effect at negative lags (i.e., prior to time zero). Previous studies have reported effects of attention at negative lags in the alpha band. Here, we examine a much slower pre-zero component which is affected by attention.

Methods: We examine multiple datasets, one collected within our lab, during which subjects engage in a cocktail party experiment. Two talkers (one male, one female) are presented to the subjects, and they are instructed to attend only one talker. We use a model of the auditory nerve to generate a regressor which allows us to examine both subcortical and cortical responses by using deconvolution. Causal filters are used at all

stages to prevent spurious response spread to earlier latencies. Time-based statistical analyses are not suitable to analyze these responses since the slow response components overlap with the fast components, and the attentional difference in the slow component is large enough that significant differences would be observed across a large time window. We employ a wavelet analysis technique to deconstruct the stimulus into different time-frequency components and perform permutation tests to examine differences in response amplitude and latency for each component.
Results: Our analysis revealed a slow negative-lag component affected by attention. This effect was strong in our own dataset and present in data from other labs as well. This component appears as a slow buildup which begins at around −200 ms lag and is maintained after zero, overlapping temporally with the auditory brainstem response and middle latency response, until ~100 ms, where the well-known cortical effects begin to appear. It appears to be a distinct component from the auditory brainstem response or cortical middle latency response, despite their temporal overlap.
Conclusions: We observed attentional effects in a component which begins before zero across multiple datasets. Since speech stimuli are information dense, this component may represent a predictive or anticipatory process. However, it is difficult to definitively rule out artifacts which may have been introduced through regression.

M3. Neural Correlates of Auditory Figure-Ground Segregation in Cochlear Implant and Hearing Aid Users

Nour Alsabbagh*[1], Phillip Gander[2], Bob McMurray[1], Timothy Griffiths[3], Inyong Choi[1]

[1] *The University of Iowa,* [2]*University of Iowa Hospitals and Clinics,* [3]*Biosciences Institute, Newcastle University*

Category: Auditory Cortex and Thalamus: Human Studies
Background: Effective formation of auditory objects facilitates speech-in-noise (SiN) perception. Binding acoustic components across time and frequency is essential for detecting auditory objects. This process may rely on bottom-up cochlear encoding of sensory information and top-down cognitive mechanisms that utilize a priori expectation about the object. In the presence of sensorineural hearing loss (SNHL), however, an important question arises concerning how SNHL affects the cochlear processing of acoustic spectrotemporal features, and whether those with SNHL exhibit different top-down processing. Additionally, as preservation of low-frequency acoustic hearing in cochlear implants (CI) is associated with better SiN outcomes, whether such subjects can detect auditory objects better than CI users who receive electric-only (E-only) stimulation remains unknown. Therefore, we investigated the neural signatures of auditory grouping in subjects with different auditory configurations in this study.
Methods: A total of 101 subjects were included in this study, which were divided into four groups: 20 normal hearing (NH) listeners, 44 electroacoustic (EAS) CI users, 23 bilateral CI users with E-only stimulation, and 14 bilateral hearing aid users with acoustic-only stimulation (A-only). Subjects performed the stochastic figure-ground (SFG) paradigm, a task that was developed to evaluate auditory grouping abilities that depend on temporal coherence. In this paradigm, subjects had to determine whether they heard figures, characterized by auditory pips presented coherently across time and frequency, popping out from a background of random noise. Electroencephalography (EEG) was recorded as the subjects performed the task. d' was calculated to determine the accuracy of figure detection. Analyses of EEG data included measuring event-related potentials (ERP), event-related spectral perturbations (ERSP), and intra-trial phase coherence (ITPC). Beamforming using the dynamic imaging of coherent source (DICS) method was implemented on induced power responses to look for brain sources of auditory figures.
Results: One-way ANOVA on d' showed significant differences between subjects, with the NH group demonstrating superior performance. ERP responses to auditory figures were absent in the SNHL groups that were otherwise present in the NH subjects. This finding was further supported by the ITPC results that did not exhibit responses to figures in the SNHL groups; however, ITPC responses in the theta frequency range were present in the NH group. Strong alpha-beta desynchronization and delta synchronization were observed in the NH and A-only groups, and to a lesser extent in the E-only and EAS groups. DICS on these induced responses in the NH group revealed brain sources in the frontocentral and primary motor cortices, which were absent in the SNHL groups.

Conclusions: The study findings reveal distinct neural signatures of SFG that depend on the auditory configuration of the subjects, supporting the idea that electric and acoustic hearing contribute differently to auditory object formation.

M4. Analyzing Sub-Groups of People Living With HIV in Tanzania Who Perform Below Average in Central Auditory Tests

Titilayo Mabogunje*[1], Christopher Niemczak[2], Samantha Leigh[2], Linda Zhang[2], Albert Magohe[3], Enica Massawe[3], Jay Buckey[2]

[1]*Geisel School of Medicine at Dartmouth,* [2]*Dartmouth-Hitchcock, Geisel School of Medicine,* [3]*Muhimbili University of Health and Allied Sciences*

Category: Auditory Cortex and Thalamus: Human Studies

Background: Although the life expectancy of people living with HIV (PLWH) has notably improved over the past three decades, disparities in quality of life persist when compared to people living without HIV. This study explores variations observed in central auditory tests, aiming to pinpoint sub-groups of PLWH who exhibit below average performance. Ultimately, the overarching goal is to elucidate the implications of any discrepancies and gain insights into the factors that may predispose certain PLWH to underperform in these auditory assessments.

Methods: Subjects were recruited from longitudinal pediatric and adult cohort studies in infectious disease centers in Dar es Salaam, Tanzania. Data from June 2016 to November 2022 were analyzed. Participants included 710 HIV-positive individuals and 460 HIV-negative individuals. All HIV-positive individuals were receiving antiretroviral treatment. Test scores from participants under the age of 10 years were excluded based on wide variability of scores that skewed the results of the larger data set. Test scores from subjects who were determined to have hearing loss (i.e. could not hear frequencies less than 20 dB) were also excluded as their scores were expected to trend differently compared to subjects without hearing loss. Auditory tests included signal-to-noise ratio (SNR) of the Triple Digit Test (TDT) and speech reception threshold (SRT) tests. Test results of people living without HIV were plotted against age of subject. A line of best fit and 95% confidence interval were calculated and plotted. A plot of the test results of PLWH against age of subject was superimposed onto the graph. All participants who scored above the upper 95% confidence interval were identified. A chi-squared test was used to compare the proportion of PLWH who scored above the upper 95% confidence interval to the corresponding proportion of people living without HIV. A p-value was calculated to understand whether the difference in proportions between the HIV groups was due to chance.

Results: Independent chi-squared tests comparing the proportion of HIV-positive and -negative individuals who performed below average in the auditory tests yielded significant results for each test studied (for SNR, $p = 0.0003$; for SRT, $p = 0.0006$). Thus, there was a statistically significant difference between the proportion of PLWH who scored above the upper 95% confidence interval (i.e. performed worse) as compared to the proportion of people living without HIV who scored above the upper 95% confidence interval for the same central auditory tests.

Conclusions: A significantly higher proportion of PLWH exhibit below average central auditory test performance compared to people living without HIV. These findings support the idea that disparities in auditory function persist between groups of individuals living with and without HIV. Further investigation is warranted to gain a deeper understanding of observed distinctions in auditory processing across the lifespan of PLWH.

M5. Decoding Maintenance and Replay Activity in the Human Auditory Cortical Mnemonic System

Ryan Calmus*[1], Zsuzsanna Kocsis[2], Yukiko Kikuchi[3], Hiroto Kawasaki[1], Timothy D. Griffiths[3], Matthew A. Howard III[1], Christopher I. Petkov[4]

[1]*University of Iowa,* [2]*Carnegie Mellon University,* [3]*Newcastle University,* [4]*University of Iowa and Newcastle University*

Category: Other, Auditory Cognition

Background: To understand how the auditory-cognitive system establishes internal models of the world, there is substantial interest in identifying neural signals that carry traces of the auditory sensory past or an expectancy about the future. To date, memory traces of sensory sequences containing regularities have been decoded in auditory cortex and the hippocampus in animal models, or in humans using non-invasive neuroimaging. However, outside of animal models we lack insights into how site-specific neurophysiological signals from the auditory cortical mnemonic system may carry information reflecting maintenance activity to sounds over delays, and on the signals that may characterize retrospective replay of regularities in a sensory sequence.
Methods: We conducted an auditory statistical learning task containing non-adjacent dependencies with 12 neurosurgery patients during presurgical intracranial monitoring of refractory epilepsy. Patients listened to sequences of 3 nonsense words drawn from sets (X, A and B), with regularities between relevant pairs of sounds (A-B) often separated in time by uninformative (X) words. We first analyzed site-specific local field potentials from auditory cortex, hippocampus and frontal cortex using traditional methods. We then computed time-resolved multivariate decoding waveforms at a high temporal resolution to reveal the latencies and timescales of sequence item representations in regions across this network, as well as undertaking decoding on the timescale of individual words.
Results: Site-specific local field potentials from auditory cortex, hippocampus and frontal cortex demonstrated engagement of the fronto-temporal network in the processing of the sequence regularities. Multivariate high-resolution decoding of neural signals in the hippocampus revealed evidence of auditory hippocampal replay, while time-resolved decoding of information in auditory cortex revealed that maintenance of sound representations within non-primary auditory cortex (superior temporal gyrus) appears prolonged relative to primary auditory cortex (Heschl's gyrus). Finally, decoding on the timescale of individual words revealed that prefrontal areas including precentral gyrus appear to maintain an ordered buffer of auditory item representations.
Conclusions: This represents to our knowledge the first human intracranial electrophysiological evidence of purely auditory-driven hippocampal replay, showing that time-compressed replay occurs before and after key sounds in the sequence. The results also provide richly detailed insights into the characteristics of auditory maintenance activity within auditory and prefrontal cortex. These results elucidate critical roles for the auditory mnemonic system in transforming sensory events into mental structures, providing insights into the mechanistic contributions of medial temporal, prefrontal and superior temporal regions in the maintenance, prediction and ordinal manipulation of sequential information.

M6. Comparison of Speech in Noise Processing in Hearing Impaired Populations Using O-15 Water Pet

Phillip Gander*[1], Laura Kiskunas[2], Joel Berger[1], Bob McMurray[2], Inyong Choi[2], Laura Ponto[1], Timothy Griffiths[3]

[1]*University of Iowa Hospitals and Clinics,* [2]*University of Iowa,* [3]*Biosciences Institute, Newcastle University Medical School*

Category: Auditory Cortex and Thalamus: Human Studies
Background: One of the most important issues in hearing impairment (HI) is difficulty with speech in noisy real-world environments. Research in normal hearing listeners indicates that auditory cortex is active while abstracting speech objects from noise and provides input to fronto-temporal networks for further perceptual, attentional, and semantic analysis. We want to understand whether these are the same neural mechanisms across hearing impaired listeners with different devices (CI and hearing aid) and configurations, and how these mechanisms relate to successful hearing outcomes.
Previously we demonstrated in a proof-of-concept study that we could robustly and reliably measure brain [15O]Water positron emission tomography (PET) blood flow activity to speech in noise in cochlear implant users at a single subject level. We observed activity in a fronto-temporal network to speech in noise in each single subject. Here we expand our investigation to include a broad range of hearing impairment (CI and hearing aid users) and device configurations to capture variability that may be different to that of a matched control population.

Methods: We measured PET blood flow in a group of 38 cochlear implant subjects, 11 hearing aid users, and 20 age-matched normal hearing controls while they performed a word-in-noise identification or noise-control

task (matched on RMS sound level). Six runs were performed for each condition. On a given 3-min run for speech in noise (+15 dB SNR), word tokens were presented in multi-talker babble, and at random intervals subjects were prompted to perform a 4-alternative forced choice task. The control condition was a noise level detection task matched for response demands. PET data were analyzed in SPM12 using a flexible factorial model.
Results: We found robust activations in single subjects for the contrast speech in noise vs control noise in auditory cortex and inferior frontal cortex. Group level activation regions of interest in auditory cortex and inferior frontal cortex were significant, along with activations across a network of areas involved in language processing (p less than 0.05, corrected). Group comparisons highlighted differences in the involvement of frontal cortex which may relate to the existence of acoustic hearing. A regression analysis including speech in noise task performance indicated the involvement of brain regions for attention and object analysis.
Conclusions: Our results show that speech in noise processing in different hearing impaired populations may depend on the availability of residual acoustic hearing, and the use of brain networks for language more similar to that of the normal hearing population.

M7. Hearing Impairment Exacerbates Cognitive Dysfunction and Accelerates the Progression of Alzheimer's Disease by Increasing Inflammation in the Brain

Yoo-Seung Ko[1], Young Mi Choi[1], Hyong-Ho Cho[1], Sungsu Lee[2], Yoo-Seung Ko*[1]

[1]*Chonnam National University Medical School, Chonnam National University Hospital,* [2]*Chonnam National University Hospital, Gwangju*

Category: Auditory Cortex and Thalamus: Structure and Function
Background: Recent studies have identified hearing loss as the most significant risk factor for developing Alzheimer's disease. However, the precise mechanisms that connect hearing impairment and Alzheimer's disease are still unclear. The study aimed to investigate the impact of drug-induced hearing loss (DIHL) on cognitive function, memory retention, and protein expression closely related to the progression of Alzheimer's disease in 5XFAD and Tg2576 mice.
Methods: The DIHL animal models were established by injecting kanamycin (700 mg/kg/s.c.) and furosemide (600 mg/kg/i.p.) into mice aged 3.5 to 4 weeks. Cognitive function and long-term potentiation (LTP) were measured to investigate the potential correlation between hearing loss and Alzheimer's disease. Immunohistochemistry and immunoblotting were utilized to assess the accumulation and expression of beta-amyloid, p-tau, Iba-1, and GFAP. The protein expression levels of the mammalian target of rapamycin (mTOR) signaling pathway and its downstream pathways, as well as pro-inflammatory cytokines, were analyzed. The reversible hearing loss model was established to investigate whether restoring hearing can reverse impaired LTP.
Results: DIHL exacerbated cognitive dysfunction and resulted in a significant increase in the accumulation of beta-amyloid and hyperphosphorylated tau in the hippocampus and cortex of mice with Alzheimer's disease, when compared to the control group. The levels of protein expression for neuroinflammatory markers, such as Iba1 and GFAP, as well as pro-inflammatory cytokines, including interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α), were found to be elevated. Additionally, DIHL increased the protein expression of phosphorylated mTOR and the phosphorylation of p70 ribosomal S6 protein kinase 1 (S6K1) and S6 pathways. Furthermore, restoring hearing loss reversed the impaired LTP in both wild-type and Alzheimer's disease mice.
Conclusions: Hearing loss exacerbates cognitive dysfunction, impairs memory retention, and significantly increases the accumulation of beta-amyloid and hyperphosphorylated tau protein aggregates in the brain. Activation of the mTOR pathway, astrocytes, and microglia in the brain can also occur, leading to the release of pro-inflammatory cytokines that exacerbate the progression of Alzheimer's disease. These results suggest that hearing loss alone can cause an increase in neuroinflammation in the brain. Therefore, early intervention for hearing loss may reduce the risk of developing Alzheimer's disease.

M8. Similar Abstract Coding Properties of Frontal Cortex Neurons in Appetitive and Aversive Auditory Discrimination Tasks in Ferrets

Pingbo Yin*[1], Jonathan Fritz[2], Shihab, A Shamma[3]

[1]*University of Maryland,* [2]*New York University,* [3]*University of Maryland, École Normale Supérieure*

Category: Other, Frontal Cortex and Behavioral Physiology

Background: Frontal cortex (FC) has been implicated in many of the cognitive and executive control functions necessary for goal directed behavior, including working memory, attentional control, reward-based decision-making and response inhibition. In earlier work (David et al. 2012) we explored how task reward structures shaped A1 rapid plasticity. Here we focus on how the FC encodes execution and inhibition of opposite forms of action in Go/NoGo tasks.

Methods: We trained five ferrets on two opposite variants of a Go/NoGo task in which the stimulus-reward-action behavioral contingencies in one task were reversed relative to the other. Three ferrets were trained on an appetitive version of the task, in which the animals learned to refrain from licking a waterspout (default behavior) to initiate the trials, and to continue refraining licking (NoGo) while one type of sound (Reference) was presented, but to lick the waterspout (Go) when another type of sound (Target) was presented. By contrast, two other animals were trained on the aversive version of the task, in which the animals' default behavior was to freely lick from the waterspout, and continue licking (Go) during Reference sounds, but to withhold licking (NoGo) for Target sounds. All animals learned these tasks with two types of stimuli: tones and AM-noise with different frequencies and rates, where each type was subdivided into two categories of Reference and Target sounds. FC neuronal recordings were made during passive listening and active, task-engage conditions.

Results: We measured single-unit FC responses to various stimuli from 994 neurons in the appetitive task, and 680 neurons in the aversive task. This allowed us to compare FC responses across stimulus types (Tone and AM-noise), behavioral conditions (passive vs active, appetitive vs aversive), and to identify common FC response properties in all conditions. The main findings were noteworthy, revealing a striking consistency of FC neuronal response patterns, independent of stimulus-reward-action associations. All Reference sounds associated with animals continuing their default behavior (either licking or non-licking depending on whether it was an aversive or appetitive task) produced relatively short (~250ms) phasic responses after stimulus onset. In contrast, Target sounds that signaled change in behavior, induced sustained long-lasting responses (greater than 500ms). Thus, the sounds associated with different actions (Go or NoGo) evoked similar response profiles in the two task variants. Furthermore, Target and Reference stimuli, as well as different types of sensory stimuli, evoked segregated responses in neuronal populations in FC.

Conclusions: Irrespective of task reward structure (appetitive or aversive), and irrespective of the actions (Go or NoGo), FC neurons selectively encoded stimulus categories and encoded abstract sound-action meaning relative to the default behavior in each task variant. The dynamics of these response profiles likely reflect the inhibitory control of FC on task-demand behaviors through the associated circuitries.

M9. Auditory Cortex Subplate Neurons Form Distinct Molecular and Functional Classes During Early Postnatal Development

Minzi Chang*[1], Zheng Xu[1], Patrick O. Kanold[1]

[1]*Johns Hopkins University*

Category: Auditory Cortex and Thalamus: Structure and Function

Background: Subplate neurons (SPNs) are one of the earliest generated cortical neurons responding to sound, even before the ear canal opens in the mice (around postnatal day (P) 10). SPNs are embedded in the ascending thalamocortical and intracortical circuits during early postnatal development. SPNs are not a uniform group but contain distinct molecular subpopulations expressing SPN-specific markers such as CTGF and Drd1a. Here we investigate if these molecularly defined subpopulations also reflect different functional properties and circuits.

Methods: To understand if the distinct subpopulation of SPNs differs in their associated functional circuits during the first three postnatal weeks (PNWs), we performed thalamocortical slice recording and laser-scanning photostimulation (LSPS) of SPNs in the primary auditory cortex during the first 3 PNWs. To selectively record from the CTGF or Drd1a expressing neurons, we crossed each transgenic mouse line expressing CTGF or Drd1a with a tdTomato reporter mouse line. CTGF and Drd1a SPNs were recorded before and after ear opening (P7-P9 and P14-P20).

Results: Both groups of SPNs receive more intra-cortical inputs from subplate and layers 5/6 than the upper layers. Developmentally, the probability of CTGF and Drd1a SPNs receiving cortical inputs from other cortical layers decreased with age. Similarly, the synaptic strength for both excitatory and inhibitory inputs declines with age. These results suggest that the intracortical connection between the upper cortical layers and SPNs is pruned between the 2nd to 3rd postnatal week. In addition, there were differences in the pattern of intracortical input between the groups of SPNs. At P7-P9, Drd1a SPNs received more excitatory and inhibitory inputs from within the subplate layer than CTGF SPNs. However, at P14-P20, the pattern was reversed with CTGF SPNs receiving more inputs from within the subplate layer than Drd1a SPNs. These results suggest that despite the developmental decline of the intracortical connection to both subtypes, CTGF SPNs retain most of their connectivity at the 3rd PNW. Moreover, at P7-P9, the intracortical excitatory inputs to Drd1a SPNs form a vertical "columnar" pattern, while for CTGF SPNs most of the excitatory inputs is spread within the layer 6 and subplate, forming a horizontal "trans-columnar" pattern. Biocytin staining showed that Drd1a SPNs had longer apical dendrite with less complex basal dendritic branching compared to those CTGF SPNs, consistent with the LSPS data.
Conclusions: Overall, our study demonstrates that SPNs for molecularly and functionally distinct populations with different developmental trajectories likely play different roles in development.

M10. Role of Oxidative Stress in Excitotoxicity Induced Auditory Synaptopathy: Implication for Molecule Screening

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Category: Auditory Nerve
Background: The type I spiral ganglion neurons (SGN) contact the sensory inner hair cells (IHC) via their peripheral dendrites and relay auditory information to the brainstem via their central axon fibers. The disruption of the synaptic connection between IHCs and SGNs have been shown to occur early in many cochlear pathology conditions such as noise- or ototoxic drug-exposures or cochlear aging. It has been proposed that the excitotoxic process may be a primary initial event in the degenerative cascade observed after noise exposure or during cochlear aging.
Methods: To investigate the underlying molecular mechanisms involving in cochlear synaptopathy after noise exposure and to develop effective therapies, we examined the molecular basis responsible for kainate-induced loss of IHC–SGN synapses and degeneration of the distal type 1 SGN peripheral axons using a cochlear explant culture from P3 mouse pups. In addition, we evaluated the efficiency of synapse regeneration using several novel BDNF mimetics and antioxidants.
Results: Our results revealed that increased levels of oxidative stress and upregulated some proinflammatory factors involved in kainate-induced the disruption of the synaptic connection between IHCs and SGNs. Addition of antioxidants and BDNF mimetics increases axon growth and synaptogenesis
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

M11. Optogenetic Activation of Single Spiral Ganglion Neurons Using ChReef

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Category: Auditory Nerve
Background: In cases of severe hearing loss, an electrical cochlear implant (eCI) can partially restore hearing sensation by electrically stimulating the auditory nerve. The eCI is the most successful neuroprosthesis, with more than 1 million users worldwide (WHO, 2021). However, many eCI users have difficulty comprehending speech in noisy environments, likely due to the broad electrical signal spread exciting a large number of auditory nerve neurons – (spiral ganglion neurons - SGNs), limiting the number of individual perceptual

channels. Using optogenetics, it is possible to stimulate the auditory nerve using an optical cochlear implant (oCI). This can increase the number of perceptually distinct frequency channels, as light spread can be better confined in space. The optogenetic activation of the auditory pathway is achieved by transducing the SGNs with adeno-associated viruses (AAVs) that harbor the genetic information for light sensitive ion channels (Channelrhodopsins, ChR). The properties of the chosen ChR strongly define the optogenetic activation profiles of the transduced SGNs.
Methods: In the current study, we used AAV-mediated transfer of ChReef – a novel green-light activated ChRmine variant that is characterized by large photocurrents, slow kinetics and low desensitization. We have investigated the electrophysiological responses of the auditory pathway and of single SGNs to ChReef-mediated optogenetic activation in mice. The virus was delivered early postnatally via round window injection. Auditory Brainstem Responses (ABR) and juxtacellular recordings of SGNs have been combined with optical stimulation by a laser (522 nm)-coupled fiber that was placed in the round window niche at the base of the cochlea weeks after AAV-administration.
Results: The mean radiant energy activation threshold for optical ABRs (oABR) was 140nJ. The SGN thresholds were slightly elevated compared to the oABR threshold but remained below 2 μJ, lower than values reported previously using different opsins. Furthermore, the measurements revealed two distinct neural populations 1) those that produced a single spike; and 2) those that produced multiple spikes per light pulse. Neurons with single spikes displayed reduced temporal fidelity, with most failing to respond above 50Hz. In contrast, over half of the neurons with multiple spikes per pulse could follow rates exceeding 100Hz.
Conclusions: ChReef enables robust neuronal activation for low-light intensities, thereby reducing power requirements, which is important for feasible and safe implementation of optogenetic hearing restoration. SGN measurements reveal the effectiveness of certain neurons in responding to higher stimulation rates (greater than 100Hz). Further exploration is warranted to understand the diversity in neuronal response profiles, with the goal of optimizing the use of fast-following neurons. Despite its relatively slow kinetics compared to the temporal requirements of the auditory periphery, ChReef's favorable low thresholds make it a valuable tool for advancing the development of sensory restoration applications, encompassing not only hearing but also other sensory modalities.

M12. Comparing Spiral Ganglion Neuron Subtypes: Synaptic Properties and in Vivo Responses at Targets in the Anteroventral Cochlear Nucleus

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Category: Auditory Nerve
Background: Information about sounds is conveyed from the cochlea to the brain primarily through type I spiral ganglion neurons (SGNs) that differ in spontaneous activity, sound sensitivity, and molecular markers. Type 1a SGNs appear to correspond to units with higher spontaneous rate and level sensitivity, and express calretinin (Calb2). Type 1c SGNs appear to correspond to units with low spontaneous rate and level sensitivity, and express Lypd1. It is not understood how the different SGN subtypes sort onto targets in the anteroventral cochlear nucleus (AVCN). Furthermore, SGN synapses formed onto bushy cells in the AVCN show deeper short-term depression when activity is low, raising the possibility that type 1c synapses depress more than type 1a.
Methods: We examined these issues in transgenic mice expressing channelrhodopsin in Calb2+ or Lypd1+ SGNs. We made voltage-clamp recordings from bushy cells in slices of the AVCN and stimulated SGN axons optogenetically.
Results: Calb2+ and Lypd1+ synapses showed similar levels of synaptic depression. Additionally, we classified AVCN units in vivo as 1a- or 1c-receiving using optogenetic stimulation. There were no significant differences in spontaneous rate or level sensitivity between 1a- or 1c-receiving units. Anatomical analysis indicated that bushy cell somata receive a mix of 1a, 1b, and 1c synapses.
Conclusions: These results suggest that SGNs do not segregate by subtype in the AVCN. Rather, principal cells in the AVCN integrate information from all SGN subtypes. This work gives new insights into how AVCN units retain diverse intensity sensitivity.

M13. Aging Effects on Human Auditory Nerve Myelination: Morphological and Clinical Associations

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Category: Auditory Nerve

Background: Age-related disruptions in myelin have been reported using low-resolution MRI approaches in humans. Neural presbyacusis is a highly prevalent cause of age-related hearing loss that is characterized by reduced suprathreshold auditory nerve (AN) dysfunction and poorer speech recognition in noise. AN dysfunction, assessed as smaller neural response amplitudes, declines precipitously starting around 50 y/o, however the role of myelin in neural presbyacusis and auditory processing has yet to be established. This study examines the changes in myelin integrity of the vestibulocochlear nerve (CNVIII) in aging adults using non-invasive clinical magnetic resonance imaging (MRI) and post-mortem human temporal bone morphologic analyses to determine the role of myelin in the pathology of neural presbyacusis.

Methods: As a clinically-relevant measure of myelin integrity, we performed ex vivo diffusion tensor imaging (DTI), a type of MRI used to evaluate myelin. We performed DTI on CNVIII of four human temporal bones from two young-old individuals (57 y/o, 57.8 y/o; young-old) and two old-old individuals (88.3 y/o, 93.4 y/o; old-old). Images were segmented manually to determine the diffusion metrics of each nerve to measure of myelin integrity.

For a morphological assessment of the AN in post-mortem human temporal bones, we developed a novel procedure to remove CNVIII from the bone. This technique is an efficient method accomplished in approximately 1 hour per human temporal bone without a decalcification process. After excision, each AN is cryo-sectioned at 12 microns in two different orientations: (1) cross-sections of the nerve bundle and (2) parallel to the nerve bundle. We stained each section with fluoromyelin, performed confocal imaging, and quantified myelin as a function of fluorescence intensity in the young-old and old-old age groups.

Results: We compare the age-related changes in myelin between young-old and old-old individuals using noninvasive clinical DTI metrics and a morphological assessment of myelin integrity in human temporal bones. Qualitatively, the vestibulocochlear nerve appears smaller in diameter in old-old human temporal bones compared to those in the young-old age group. Comparing measures of myelin integrity in DTI with the pathology across ages is ongoing. These analyses will contribute to our understanding of the role of demyelination in neural presbyacusis.

Conclusions: Evaluating the relationship between readily accessible clinical imaging of the AN with histologic data is important in validating the use of DTI as a clinical measure of neural presbyacusis, specifically myelin defects. Understanding the role of myelin in neural presbyacusis is important in identifying new therapeutic targets to treat age-related AN degeneration and functional declines.

M14. Open Board

M15. The Impact of Small Arms Fire-Like Noise on Temporal Processing of Simple and Complex Stimuli

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Category: Brainstem: Structure and Function

Background: Exposure to acute, high-intensity sounds, as encountered in military firearms usage, is a common precursor to noise-induced hearing loss (NIHL). This prevalent subtype of NIHL exhibits potential disparities in physiological profile, cochlear pathology, and recovery dynamics compared to auditory exposures commonly encountered in laboratory or everyday settings (e.g. narrowband exposures). Improved understanding of the effects of firearms-related NIHL on auditory processing could aid in the optimization of patient treatment. Therefore, we investigated the effects of firearms-related NIHL on audiometric thresholds and subcortical temporal processing of both simple and complex sounds.

Methods: Male and female F-344 rats were sedated using ketamine and dexdomitor (IM) before exposure to small arms fire-like (SAF) noise (50 repetitions of 120 dB peak SPL biphasic pulses over 2.5 min). Auditory

evoked potentials (AEPs) were conducted at baseline (pre-exposure) and on days 7, 14, 28, and 56 post-exposure. Two electrode channels (s.c.) were used, positioned along the nasal midline and interaural bregma. Auditory brainstem responses (ABRs) were recorded in response to clicks and 8 kHz pure tones. Middle latency responses (MLRs) were measured using click trains featuring groups of 1, 2, and 8 clicks presented at progressively decreasing inter-stimulus-intervals. Envelope following responses (EFRs) were recorded in response to dynamic amplitude modulated-frequency modulated sweeps with 16 - 1500 Hz amplitude modulation, and speech sounds designed using the envelope of an adult male voiced "Purdue"
Results: Click and 8 kHz ABR thresholds exhibited lasting increases in thresholds across channels following SAF noise. Notably, females sustained a significantly increased ABR threshold shift compared to males. At suprathreshold levels, ABR, MLR, and EFR waveforms exhibited amplitude reductions and morphological changes characterized primarily by a decrease in peak salience. MLR wave components demonstrated reduced amplitudes and peak salience, particularly during the decreased inter-stimulus interval. In the speech sounds, a smoothing of the onset response and loss of distinction between each syllable significantly increased. Recovery of threshold and morphological changes over time were non-monotonic, indicated by a cyclic period of initial recovery followed by subsequent degradation of neural responses.
Conclusions: The progression of SAF-induced NIHL broadly affected ABR, MLR, and EFR waveform responses and resolved in a non-monotonic fashion. These results suggest complex, multi-phase mechanisms of recovery and potential windows for treatment.

M16. Discrimination Between Natural Vocalizations in Challenging Conditions: Relationships Between Behavioral Performance of CBA Mice and the Responses of Inferior Colliculus Neurons

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Category: Brainstem: Structure and Function
Background: Over the last decades, a large number of studies have described the robustness of auditory cortex responses when target stimuli are presented in situations of acoustic degradations such as noise addition, reverberations, vocoding etc… In very few experiments comparisons have been made with the resistance to noise observed from recordings obtained from subcortical neurons. In previous experiments performed in anaesthetized guinea pigs (Souffi et al 2020, 2023), we reported that the responses of inferior colliculus neurons were those that showed the highest resistance to noise compared to the other auditory structures.
Methods: To assess whether this resistance to noise is potentially related with the behavioral performance of the animals, we trained awake, water deprived, mice in a discrimination task between two guinea pig vocalizations (two whistles; 300ms) in a Go/No Go protocol: One vocalization was used as CS+ the other as CS-. The CS+ allowed the mouse to obtain a drop of water if the animal licked a spout during the 5 seconds after the CS+ presentation. Initially, the mice were trained in silence, and when they reached 80% of correct performance to both the CS+ and the CS-, they were trained in increasing levels of stationary noise (at SNR of +10dB, 0dB and -10dB), or of cocktail noise made of the simultaneous vocalizations of a group of guinea pigs.
Results: All mice performed above 80% in silence and in the +10dB SNR, but their performances were decreased at a 0dB SNR and even more at -10dB SNR. Neuronal recordings were collected in these mice, both in anesthetized conditions and in awake, passively listening conditions. Neuronal recordings were also obtained while the animals were also engaged in the task. As control, neuronal recordings were obtained from passively exposed mice, which were submitted to exactly the same number of vocalizations as the trained mice.
Conclusions: These results should allow determining to what extent the inferior colliculus neurons contribute to the behavioral performance in challenging conditions.

M17. Molecular Profiling of Tonotopy in the Developing Auditory Brainstem

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Category: Development: Cellular/Systems
Background: Auditory perception is critical for communication through speech and music. The pitch or frequency of a sound is processed by the brain using tonotopic maps, which are the spatial organization of how sounds with different physical frequencies are represented in all auditory regions. Tonotopic connections are formed during development and further refined by sound-evoked activity after the onset of hearing. It is unclear what molecules and mechanisms guide the assembly of tonotopic maps before the onset of hearing and how these are disrupted in neurodevelopmental disorders.
Methods: Using Smart-seq2 we profiled excitatory neurons from embryonic day 17 cochlear nucleus and inhibitory neurons from embryonic day 18 superior olivary complex using Slc17a6-Cre;Ai14 and En1-Cre;Ai14 mice to gain genetic access, respectively.
Results: Recently, we identified that the cell surface molecules Teneurin-3 (Ten3) and Latrophilin-2 (Lphn2) display tonotopic expression gradients in multiple regions of the developing auditory brainstem. Ten3 is expressed in low frequency responding regions and Lphn2 is expressed in high frequency responding regions. We leveraged Ten3 and Lphn2 expression levels to assign cells to distinct positions across the tonotopic axis and identified genes that displayed specific tonotopic expression patterns. We identified multiple molecules that show tonotopic expression patterns in the cochlear nucleus, superior olivary complex or both. This included cell surface molecules, transcription factors and RNA-binding molecules with a subset being associated with neurodevelopmental disorders. This dataset provides a resource to understand how these connections are assembled by cell surface molecules and how those cell surface molecules are regulated by transcription factors.
Conclusions: Here, we present the first molecular map of tonotopy in the developing auditory brainstem. We have identified a number of molecules with novel tonotopic expression patterns. This will provide the foundation for future studies investigating how tonotopy is established during development and how these connections are disrupted in neurodevelopmental disorders.

M18. Acoustic Trauma Reduces Sound-Offset Responses in Superior Paraolivary Nucleus Neurons

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Category: Brainstem: Structure and Function
Background: Acoustic trauma leads to debilitating conditions such as hyperacusis, tinnitus and deficits in detecting silent gaps in noise and recognizing speech. On a cellular level, acoustic trauma mostly causes increased excitability and decreased inhibition in neurons of the cochlear nucleus, the lateral superior olive or the inferior colliculus. Here we investigate the effect of noise exposure on neurons of the Superior Paraolivary Nucleus (SPN). SPN neurons receive almost exclusively inhibitory input and have been shown to encode sound offsets which are crucial for gap-in-noise detection and processing of sound rhythms such as speech. The SPN is therefore a key candidate to uncover cellular and circuit adaptations following loud noise exposure.
Methods: Young (P18-P22) C57Bl6 mice were bilaterally exposed for 2 hours to 107dB SPL bandpass filtered noise between 8-16kHz. The effect of noise exposure was monitored by ABR recordings before and immediately after the exposure. After 24 hours, mice were subjected to either in vivo single-cell recordings or in vitro patch-clamp recordings of SPN neurons. For anatomical assessment of changes in synaptic inputs, we took advantage of the monaural nature of SPN neurons and performed monaural noise exposure on another group of mice before subjecting them to immunohistochemical staining of GlyT2 to quantify inhibitory synaptic terminals against a within-subject control.
Results: Our data show that after noise exposure, SPN neurons indeed become hyperexcitable based on significantly increased numbers of spontaneously active neurons in vitro (!), an increase in input resistance, a decrease in rheobase and an increase in the number of action potentials following current injections directly into the cells. Interestingly, this increase in intrinsic excitability is accompanied by an increase, rather than a decrease in inhibitory inputs based on higher frequencies of miniature IPSCs, larger amplitudes of evoked IPSCs and more glycinergic synaptic boutons per SPN neuron. To understand how increased excitability on one hand and increased inhibition on the other hand act together during sound processing in the SPN, in vivo recordings were performed before and after noise exposure. These data show that the number of SPN neurons

with offset responses is significantly reduced after noise exposure. After noise exposure, the remaining offset responses were less pronounced with smaller signal-to-noise ratios for the transitions from sound to silence.
Conclusions: With these recent data showing a significant impact of noise exposure on SPN offset responses, we are confident that SPN neurons contribute to the failure to detect silence following acoustic trauma such as tinnitus-inducing noise and should be part of the equation in studying cellular mechanisms of tinnitus induction.

M19. Descending Projections From the Inferior Colliculus to the Superior Olivary Complex in Mice

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Category: Brainstem: Structure and Function
Background: Projections from the inferior colliculus (IC) to the superior olivary complex (SOC) have been characterized in several species and are thought to play a role in modulating early auditory processing. The main targets of the IC projection are the ventral nucleus of the trapezoid body (VNTB) and the superior paraolivary nucleus (SPN). A projection from the IC to the VNTB has been shown in mice but projections to other SOC nuclei are uncharacterized, an important issue given the growing prominence of mice in auditory research.
Methods: We injected an adeno-associated virus into the IC in 6 female and 4 male adult mice (mixed CBA/CaJ/C57Bl6J background) to induce expression of a red or green fluorescent protein by IC neurons. The brain was subsequently fixed by perfusion, frozen and cut into 40 μm thick coronal sections that were stained with Neurotrace for Nissl substance, anti-VAChT or anti-ChAT to identify cholinergic neurons, or anti-GAD to identify GABAergic neurons. Alexa-Fluor dyes (AF546, AF647, AF750) were used to visualize the GABAergic and cholinergic markers separately from the Neurotrace and fluorescent protein labels.
Results: We observed bilateral projections from the IC to the SOC, with ipsilateral terminations typically more numerous than contralateral ones. On both sides, labeled axons terminated heavily in the VNTB and moderately in the SPN. In addition, there was a bilateral termination in the lateral superior olivary nucleus (LSO). Across nuclei, labeled axonal boutons were located in the neuropil or in close apposition to neuronal cell bodies. In each of the nuclei, the apparent targets included neurons labeled with GABAergic or cholinergic markers.
Conclusions: Our results demonstrate a robust projection from the IC to the SOC in mice, more widespread than previously described in other species. A strong ipsilateral projection to the VNTB and smaller projection to the SPN is common across species; the mouse has a more substantial projection to the contralateral side. We also observed a projection to the ipsilateral and contralateral LSO; such projections have not been described in other species. Regarding the identify of neurons targeted by the IC projections, we identified presumptive cholinergic neurons and GABAergic neurons as likely targets of the collicular axons. We conclude that the descending projection from the IC is likely to modulate auditory brainstem processing bilaterally through direct actions on inhibitory (GABAergic) and modulatory (cholinergic) brainstem circuits. These circuits likely include both ascending (e.g., olivocollicular) and descending (e.g. olivocochlear and olivo-cochlear nucleus) pathways from the SOC.
Supported by NIH DC004391.

M20. Effect of Precursor Level Statistics on Adaptation to Noise Measured With Electroencephalography

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Category: Brainstem: Structure and Function
Background: In noisy backgrounds, the recognition of isolated words, the detection of pure tones, and the detection of amplitude modulation (AM) all improve as the target sounds are delayed in the noise. It has been hypothesized that this 'adaptation to noise' occurs because auditory neurons shift (adapt) their dynamic range towards the most common level in the noise preceding the target sound. This hypothesis is supported by

behavioural experiments that show that adaptation occurs when the level of the noise preceding the target is steady but not when it is very fluctuating. However, physiological evidence is still lacking in support of this hypothesis. Here, we investigate whether adaptation as measured with electroencephalography depends on the precursor level statistics.
Methods: Envelope- (EFR) and frequency-following responses (FFR) were obtained for normal-hearing listeners using a 64-channel Biosemi system (16 kHz sampling). Participants were presented monaurally with AM tones (70 dB SPL, 250 ms, 576 Hz carrier, 84 Hz modulation frequency, 100% modulation depth) in steady noise (75 dB SPL; 0.1-10 kHz) that started and finished 50 ms before and after the tones. The stimulus could be preceded by a noise precursor (350 ms in duration) with the same spectrum and average level as the simultaneous masking noise. The precursor was steady or fluctuating in level (from 30 to 83 dB SPL every 50 ms). Adaptation was estimated as the difference in the EFR and FFR with and without precursor. Participants were asked to remain awake and with their eyes closed while recording. 2400 tones in alternating polarity (1200 for each polarity) were presented in each measure. Recordings were added to obtain the response to the modulating frequency (EFR) or subtracted to obtain the response to the carrier frequency (FFR). The inter-stimulus interval was 500 ms.
Results: Preliminary results for four participants suggest that the EFR and FFR are larger with than without the steady precursor (~2 dB on average).
Conclusions: This provides physiological evidence for adaptation to noise, i.e., for an objectively improved neural representation of the AM tones in continuous versus gated noise. More data are necessary to shed light on the effect of precursor level statistics on noise adaptation. [Supported by the Spanish Ministry of Universities, Unión Europea NextGenerationEU/PRTR, and University of Salamanca to MIMP, Spanish Ministry of Science and Innovation (grant PID2019-108985GB-I00) and the European Regional Development Fund to EALP, and the MRC Senior Fellowship in Hearing Research (MR/S002537/1) to DAV].

M21. D-Stellate Neurons of the Ventral Cochlear Nucleus Are Predominantly Innervated by Calretinin-Deficient Auditory Nerve Synpases

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Category: Brainstem: Structure and Function
Background: D-stellate neurons are one of the major inhibitory neurons of the ventral cochlear nucleus that provide wide-band inhibition to other principal neurons. They have extensive dendrites that span across wide frequency bands and are broadly tuned. However, it remains unclear how auditory nerve (AN) synapses innervate these neurons, including the profile of innervation over the entire neuronal structure and the subtype specificity of the AN synapses. In this study, we combined electrophysiology and immunohistochemistry to characterize the physiological and morphological properties of AN synapses onto D-stellate neurons, using CBA/CaJ mice of either sex.
Methods: Whole-cell patch clamp recording was performed from D-stellate neurons of the VCN in acute brain slices to characterize the synaptic properties of the AN input. Target neurons were filled with fluorescent dye included in the electrode solution and preserved upon the completion of the recording. Brain slices were then immediately fixed and immunostained using antibodies against VGluT1 and calretinin, which labels glutamate vesicles in all AN synapses and only type Ia AN synapses, respectively. Confocal images of the filled neurons and innervating synapses were acquired and reconstructed in 3D using Imaris software. VGluT1-labeled AN synapses were classified as either type Ia, which express calretinin and are presumably from spiral ganglion neurons (SGNs) with high spontaneous rate spikes and low sound threshold, or non-type Ia, which do not express calretinin and presumably from SGNs with medial/low spontaneous rate spikes and medium/high sound threshold.
Results: We found that D-stellate neurons receive small bouton synapses from the AN, and most of the synapses are on the dendrites. Particularly, more than 80% (by VGluT1-labeled puncta volume) of the AN synapses were not labeled by calretinin and therefore were non-type Ia. Consistent with our previous study, AN-stimulation evoked EPSCs showed slow rise and decay kinetics, which is likely due to the low-pass filtering of evoked EPSCs from remotely located synapses on dendrites. Train stimulation at high rates evoked sustained EPSCs that are capable of driving prolonged firing throughout the duration of the stimulus train, hence long-lasting inhibition onto postsynaptic neurons.

Conclusions: Our results suggest that D-stellate neurons receive numerous bouton synapses from predominantly non-type Ia AN, and thus are mostly driven by medium/low spontaneous rate SGNs. These synapses are located primarily on dendrites and are abundant in number, which contributes to the slow but sustained excitatory drive upon high rate AN activity and last lasting inhibition to target neurons. These features may play significant roles in improving signal detection in noisy environments under physiological conditions.

M22. Mechanisms Underlying Frequency Sweep Selectivity in Inferior Colliculus Neurons

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Category: Midbrain: Structure and Function

Background: Rapid changes in frequency known as frequency-modulated (FM) sweeps are common components of many complex sounds including conspecific vocalizations. FM sweeps are encoded in the auditory system through neuron selectivity for upward or downward sweeps. In particular, the inferior colliculus (IC) is a midbrain hub of auditory integration and has been implicated as the site where FM sweep selectivity is generated via the convergence of excitatory and inhibitory inputs tuned to different sound frequencies. However, the specific mechanisms underlying FM sweep selectivity remain poorly understood, along with whether and how this selectivity is influenced by altering different properties of FM sweep stimuli.

Methods: To answer these questions, we performed in vivo juxtacellular recordings from neurons in the IC of awake, head-fixed mice while presenting FM sweeps of different speeds (10 – 200 octaves/second), directions (up, down), and frequency ranges (4 – 64 kHz, 4 – 16 kHz, 16 – 64 kHz) to examine the factors that shape direction selectivity in IC neurons. To determine neuron best frequency, we played pure tone pips ranging from 4 – 64 kHz at 0, 30, 50, and 70 dB, and best frequency was calculated as the frequency at which the neuron produced the greatest number of spikes.

Results: We find that the direction selectivity of an individual IC neuron is dynamic and flexible, where the direction selectivity index (DSI) of a cell can change depending on both the size (in octaves) and speed of the sweep. In line with previous literature, we find that direction selectivity indexes (DSIs) for 4 – 64 kHz sweeps are correlated with a neuron's best frequency, but this is not always true of sweeps covering smaller frequency ranges. Interestingly, we also find that a large proportion of IC neurons were strongly inhibited by FM sweeps and that direction selectivity in some cells was generated after the sound via direction-specific offset spiking.

Conclusions: Overall, these results highlight diverse mechanisms underlying FM selectivity in the awake mouse IC, which are likely important for understanding how IC neurons process complex sounds, such as conspecific vocalizations, that contain FM sweeps. These results also demonstrate that IC neuron selectivity for complex sound features such as FM sweeps can be context dependent rather than stereotyped by neuron, illustrating flexibility in IC neuron complex sound encoding and providing further supporting evidence of the IC as a computational hub in the auditory system.

M23. The Influence of Spectrum and Modulation Cues on the Neural Representation of Vocalizations in Natural Background Sounds

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Category: Midbrain: Structure and Function

Background: Real-world listening poses significant challenges for humans and other animals when communicative sounds occur in competing background noise. These same acoustic scenarios are often the most challenging for the hearing impaired and artificial speech recognition systems. Although perceptual studies have shown that both the spectrum and modulation statistics of a background sound can influence the perception of a foreground target, how the brain separates sound mixtures and solves this computational problem is poorly understood.

Methods: Here, we recorded neural activity from populations in the auditory midbrain in order to assess how the statistics of natural background sounds alter the neural representation of a foreground vocalization. Multi-

unit population activity was obtained from the inferior colliculus of head-fixed unanesthetized rabbits listening to natural sound mixtures using linear 64-channel recording arrays (Neuronexus). Speech sentences or zebra finch song motifs were presented as foregrounds in the presence of seven competing natural background sounds and perturbed variants at multiple signal-to-noise ratios. These backgrounds encompassed a wide range of modulation statistics and included speech babble, bird babble, running water, and construction noise. The backgrounds were delivered in the original unmodified (ORIG) or the perturbed phase randomized (PR) or spectrum equalized (SE) configurations. The PR perturbation preserves the original sound spectrum but distorts (whitens) the original sound modulations; whereas the SE perturbation distorts the spectrum (whitens) and preserves the original sound modulations. Using shuffled correlation methods, we separated the foreground- and background-driven neural response components for each of the sound mixtures and conditions (ORIG, PR and SE).

Results: Preliminary results show that the distance between the foreground-driven population activity with noise and without noise strongly depends on the background sound statistics. For some background sounds, the spectrum dominates and distorts the foreground sound encoding. While for other backgrounds, the modulation statistics more strongly interfere with the encoding of the foreground.

Conclusions: Collectively, the findings demonstrate how both the spectrum and modulation statistics of natural backgrounds influence and interfere with the representation of vocalization foreground sounds suggesting that these are both critical features underlying masking of real-world natural sounds.

M24. How Speech is Coded in the IC of Gerbils and Affected by Hidden Hearing Loss

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Category: Midbrain: Structure and Function

Background: Previous studies show that noise exposure can cause permanent cochlear synaptopathy (CS), or damage to the connections between auditory nerves and inner hair cells, even at noise exposure levels that cause only a temporary threshold shift in auditory thresholds (Kujawa and Liberman, 2009). Related work showed CS may be visible in humans (Schaette and McAlpine, 2011). This indicated that our clinical measures of hearing function were inadequate to detail all kinds of auditory damage present and how that damage could affect auditory processing.

These papers focused primarily on cochlear histology and non-invasive electrophysiology and did not investigate any effects on downstream auditory coding. Follow up work showed that central gain in the auditory midbrain is modified by noise-induced hidden hearing loss (Bakay et al., 2018). However, this study, and most follow-up research, has focused on the effects of cochlear synaptopathy on simple auditory stimuli. In order to develop treatments and noise exposure recommendations, it is critical to understand how these central gain changes, and cochlear synaptopathy, affect speech coding, both in silence and in masking noise.

Methods: Awake unrestrained wildtype Mongolian gerbils (age 6 through 16 weeks) were placed in wire cages and exposed to octave-band noise (2.8-5.6kHz) at 100 dB SPL for 2 hours in an anti-parallel chamber. Auditory Brainstem Responses (ABR), Distortion Product Otoacoustic Emissions (DPOAE) and Cortical Auditory Evoked Potentials (CAEP) were recorded pre-exposure, 1-day post-exposure, and 4-weeks post-exposure. Extracellular single-unit recordings from the IC were obtained using coated tungsten electrodes, 4-weeks post-exposure. ABR, DPOAE, CAEP were performed under Ketamine/Medetomidine anesthesia, while single-unit experiments were performed under Urethane anesthesia.

Individual neurons were found using broadband stimuli and categorized using Frequency Response Area (FRA) stimuli. Speech stimuli were then presented, identical to those used in our collaborative project on humans. They consist of pairs of synthetically generated speech tokens (e.g. /di/, /bi/) plus a series of steps between the endpoints. Four different pairs, with 8 steps across each pair, for 32 total stimuli. All stimuli were presented in silence, ipsi-, contra-, and bi-lateral OLSA masking noise (Wagener and Brand, 2005).

Results: DPOAE and ABR thresholds showed temporary threshold shifts at 1-day that recovered after 4-weeks. CAEPs also showed significant differences after noise exposure. Phase locking to the sustained frequency components of the speech stimuli occurs. Some IC neurons respond uniquely to one half of the

stimuli, not the other, even with identical sustained components. Adaptation to the stimuli has also been examined.
Conclusions: Speech tokens appear to be encoded at a token level, outside of purely frequency or intensity, at the level of the midbrain, and this coding is affected by noise exposure.

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M25. Changes in Spatial Response Properties of Inferior Colliculus Neurons During Behavioural Adaption to Unilateral Conductive Hearing Loss

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Category: Midbrain: Structure and Function
Background: The ability, through training, to overcome the impairment in sound localisation caused by asymmetric conductive hearing loss is well documented. Although the characteristics of such adaptation have been characterised behaviourally, its neural underpinnings are still unknown. Previous research in ferrets wearing an earplug has established that such experience-dependent plasticity requires a functioning auditory cortex and the integrity of its descending circuits to the inferior colliculus (IC). The IC is a hub for the ascending brainstem pathways that encode different spatial acoustic cues whose integration may be modulated by cortical activity through the corticocollicular projection. Therefore, it is likely that the behavioural adaptation is associated with changes in neural responses in the IC.
Methods: We recorded neural activity in the IC bilaterally using high-density probes (Neuropixels) over several weeks in three ferrets while performing a sound localisation task. The spatial tuning properties of IC neurons were explored in response to broadband sounds presented from each of 12 loudspeakers in the horizontal plane. Ferrets had to approach the target loudspeaker to indicate the stimulus location on each trial and receive a water reward. The location of the probes inserted dorsoventrally into the IC was estimated physiologically by recording neuronal frequency response areas under sedation and anatomically by reconstructing the probe tracts from histological sections at the end of the experiments. Most of the recordings were in the central nucleus of the IC, with some recording sites in its dorsal and lateral cortices.
Results: Under normal hearing conditions and across the tonotopic axis, the most commonly observed responses were primary-like and sustained. Most neurons (77.12% ± 14.89) had a contralateral preference (mean centroid 54.08° ± 12.84° for left IC neurons and -42.99° ± 45.48° for the right IC), with a broad spatial equivalent rectangle receptive field (145.77° ± 7.83°). No differences between left and right IC were observed. Moreover, population decoding models were able to decode the stimulus location from IC activity highly accurately.
The initial disruption of localisation behaviour caused by plugging one ear was accompanied by a marked change in the response properties of IC neurons. Neurons in the IC ipsilateral to the earplug exhibited increased excitatory responses and a diminished contralateral preference, whereas neurons contralateral to the earplug exhibited a profound suppression of their activity. During behavioural adaptation to unilateral hearing loss, we observed some changes in IC activity but no systematic recovery of normal spatial encoding.

Conclusions: Together, the contralateral preference and broad spatial tuning of IC neurons are consistent with opponent two-channel coding of sound location. The neural changes observed after plugging one ear could be explained by the input imbalance introduced by the earplug. The performance of the population decoding models seemed to improve as the animals' localisation accuracy recovered.

M26. Monaural and Binaural Level Tolerance of Duration Tuning in the Inferior Colliculus

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Category: Midbrain: Structure and Function

Background: Neurons selective for sound durations, known as duration-tuned neurons (DTNs), have been reported from the auditory midbrain of anurans and mammals. In species that have been examined, neuronal best durations (BDs) of DTNs closely match the durations of species-specific vocalizations, suggesting DTNs may contribute to temporal processing of behaviorally relevant sounds. In mammals, duration tuning is formed de novo at the inferior colliculus (IC) through the temporal interplay of excitatory and inhibitory synaptic inputs offset in time. Neural inhibition plays a pivotal role in creating DTNs because blocking inhibition abolishes duration tuning. Previous electrophysiological studies have shown that the excitatory and inhibitory synaptic inputs that create DTNs are primarily evoked by monaural, contralateral stimulation whereas ipsilaterally evoked inhibition plays a lesser role. This finding is consistent with other studies that suggest spiking activity of some DTNs can be modulated in response to interaural level differences (ILDs), indicating that ipsilaterally evoked inhibition is similar to other non-duration-tuned midbrain neurons. Although inhibitory inputs from the ipsilateral ear are crucial in binaural processing, we lack basic understanding of how binaural stimulation affects duration tuning.
Methods: To examine this, we conducted single-unit extracellular recordings from 38 DTNs in the IC of 13 big brown bats (Eptesicus fuscus) in response to monaural and binaural stimulation. We measured the level tolerance of duration tuning by presenting cells with BD, best excitatory frequency (BEF) tones at 10, 20, 30, and 40 dB above threshold first to the contralateral ear (monaural) and then to both ears (binaural). A subset of DTNs (n = 15) were tested for level tolerance of duration selectivity by varying the ILD, with the contralateral SPL set to 10 or 20 dB (re threshold) and the ipsilateral SPL roving from -20 to +20 dB (re contra).
Results: We found that neural response characteristics of DTN—BD, 50% width of duration tuning, and first or last spike latency—remained stable across varying SPLs for both monoaural and binaural stimulation. In some DTNs, the number of spikes evoked binaurally at 30 and 40 dB (re threshold) decreased in comparison to monoaural stimulation. Neuronal BDs, 50% width of duration tuning, and spike latencies remained relatively stable across various ILDs, although overall spike counts decreased with increasing ILDs. Our results agree with previous findings, showing that the monaural responses of DTNs were level tolerant.
Conclusions: Altogether, these results suggest that DTNs could contribute to robust perception of sound duration regardless of sound levels while coding for spatial locations. In echolocating bats, this feature may help to preserve responsivity to returning echoes in the presence of acoustic interference, including the sounds of other bats. Research supported by a Discovery Grant from the Natural Sciences and Engineering Research Council (NSERC) of Canada.

M27.Synthetic Vowel Encoding in the Inferior Colliculus of Awake Rabbits: Effect of Formant Bandwidth

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Category: Midbrain: Structure and Function
Background: Vowels are harmonic complexes with spectral peaks, or formants, produced by vocal-tract resonances. A neural cue proposed for coding of vowel formants can be manipulated by varying formant bandwidth: broadened formants reduce the contrast in neural fluctuations along the tonotopic axis. A previous psychoacoustic study reported that formant-frequency discrimination thresholds in human listeners are elevated as formant bandwidth is increased (Carney et al., 2023, Hear Res, 435:108788). The current work focused on neural mechanisms underlying responses to formants in the Inferior Colliculus (IC), a critical integration hub in the auditory pathway. We hypothesized that discharge-rate profiles of fluctuation-sensitive IC neurons would reflect the neural-fluctuation profiles elicited by formants with different bandwidths. We tested the hypothesis with recordings in the IC and a phenomenological model.
Methods: Extracellular recordings were made from the IC of awake Dutch-belted rabbits using tetrodes. Each neuron's characteristic frequency (CF) was determined using pure tones (250 Hz – 16 kHz), presented diotically. The sensitivity to amplitude modulation was characterized using broadband noise (10 Hz-10 kHz) that was sinusoidally amplitude modulated (2-600 Hz, 3 steps per octave). Modulation transfer functions (MTFs) were categorized as band-suppressed (BS) or band-enhanced (BE) based on whether discharge rates were reduced or increased, with respect to the unmodulated response, over bands of modulation frequencies. Single-formant stimuli had a fundamental frequency (F0) of 100 Hz, duration of 300 ms, 25 ms cos2 on/off

ramps, and were presented diotically at 70 dB SPL. To infer population rate profiles, the formant frequency was shifted across a frequency range around CF, in steps of 50 Hz, with bandwidths of 50, 100, and 200 Hz. The Same-Frequency Inhibition-Excitation model for IC neurons was tested against actual IC responses (Carney and McDonough, 2019, 81:1034).
Results: Independent of MTF shape, most neurons exhibited a peak response rate for formant frequencies near CF. The peak in the response profile for formant frequencies near CF was narrowest and highest for the narrowest formant bandwidth tested, and was broadest and lowest for the widest formant bandwidth. This result was consistent with reduced neural-fluctuation contrast for broader formant peaks. Formant-frequency discrimination thresholds computed from the neural responses exhibited similar trends as behavioral thresholds, increasing as formant bandwidth increased. As expected, the computational model illustrated broad, flat response profiles in rate-saturated model auditory-nerve fibers; however, model IC responses explained the trends in BS IC discharge rates, but not BE IC neurons.
Conclusions: IC responses were consistent with trends in formant-frequency discrimination thresholds in human listeners, which increased as formant bandwidth increased. Future directions include explore other mechanisms that contribute to neural coding of speech for different subpopulations of IC neurons, such as the impacts of efferent gain control and off-CF inhibition on model responses.
Supported by NIHR01-DC001641

M28. Synapse-Specific Plasticity Mechanisms in Cortical Excitatory Synapses After Noise Trauma

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Category: Primary Auditory Cortex
Background: Neurons in the auditory cortex (ACx) exhibit a remarkable capacity to maintain or even increase their firing rates in response to peripheral deafferentation caused by noise trauma. This adaptive response can compensate peripheral damage modifying sound processing. Overall, cortical adaptation after noise trauma is associated with: the reorganization of sensory processing in deafferented areas; a reduction of GABAergic inhibition; the increase of cortical gain; and the raise of spontaneous neuronal firing. Although significant progress has been made in understanding the mechanisms underlying the adaptive plasticity of ACx after acoustic trauma, the precise synaptic mechanisms, especially in excitatory neurotransmission, remain poorly understood. Here we evaluate the effect of noise trauma on the synaptic transmission of the excitatory connections formed by excitatory principal cells (PN) to PN, somatostatin (SOM), and parvalbumin (PV) cells.
Methods: We evaluated changes in excitatory synaptic transmission in L2/3 ACx synapses after noise trauma. We used a mouse model of noise-induced hearing loss (NIHL) and transgenic mice together with optogenetic approaches and whole-cell patch clamp recordings to assess the L2/3 ACx excitatory synaptic connections between PN→PN, PN→SOM, and PN→PV after noise trauma. We employed paired-pulse ratio (PPR) assay and miniature Excitatory Postsynaptic Currents (mEPSCs) analysis to evaluate changes in the three quantal parameters of synaptic transmission after noise trauma. Also, we perform in vitro dual patch-clamp recordings between synapse pairs to evaluate their relative synaptic strength after noise trauma.
Results: Our findings revealed synapse-specific changes in synaptic transmission after noise trauma. First, we evaluated changes in the three quantal parameters of synaptic transmission: the probability of neurotransmitter release (Pr), the number of functional release sites (n) to track presynaptic activity, and the quantal size (q), which follow the response of postsynaptic receptors to a quantum of neurotransmitter. We found that noise trauma increases q and n in PN→PV synapses from Day 1 to Day 10 after NE and increases q in PN→SOM and n in PN→PN synapses 3 and 10 Days after NE. Next, we further examined specific mechanisms that may underlie changes in n. We found that in PN→PV synapses the n increment is due to a rise in the number of synaptic contacts between individual PN and PV cells, while in PN→PN synapses is due to a rise in the number of PN cells targeting other PN cells. Finally, when evaluating the relative synaptic strength between the excitatory connections by comparing the EPSCs amplitude ratio between SE and NE groups, we found that noise trauma enhances the relative strength of the excitatory connections, where PN→PV are stronger than PN→SOM and PN→PN synapses, with PN→SOM synapses being stronger than PN→PN synapses.

Conclusions: Our results show synapse-specific changes, and overall strengthening of synaptic transmission between L2/3 excitatory connections after noise trauma.

M29. LHFPL5 Transmits Force From the Tip Link to the Hair Cell Mechanotransducer Channel

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Category: Hair Cells: Anatomy and Physiology

Background: During auditory transduction, sound-evoked vibrations of the hair cell stereociliary bundles open mechanotransducer (MET) ion channels via tip links extending from one stereocilium to the top of its neighbor where the MET channel is located. How tension in the tip link is delivered to the channel is not fully understood. The MET channel is thought to comprise a pore-forming subunit, transmembrane channel-like protein (TMC1 or TMC2), aided by several accessory proteins, including LHFPL5 (lipoma HMGIC fusion partner 5). LHFPL5 interacts with both TMC1 and PCDH15 at the lower end of the tip link.

Methods: We investigated the role of LHFPL5 in transduction by comparing MET channel activation curves in outer hair cells of Lhfpl5-/- knockout mice with those of Lhfpl5+/- heterozygotes. We recorded MET currents by patch-clamping apical outer hair cells of P4 to P7 mice and deflecting hair bundles with a fluid jet. The driving force on the hair bundle, generated by the fluid jet, was calibrated against a flexible glass fiber of known stiffness (1 mN/m). The single-channel gating force, Z, was derived from the 10-90 percent working range (WR) of MET current activation curves (Z = 4.4kBT/WR).

Results: The maximum MET currents were 1.0 ± 0.14 nA in Tmc1+/+; Lhfpl5+/- and 0.24 ± 0.16 nA in Tmc1+/+; Lhfpl5-/-. The working range (WR) in Lhfpl5+/- was 53 ±11 nm (N=19), giving Z=0.34 pN, but in Lhfpl5-/- mice, the WR was 123 ± 12 nm (N=10) and Z was more than halved to 0.13 pN. In addition, the half-saturation displacement of the I-X relation increased from 39 ± 10 nm in Lhfpl5+/- to 112 ± 28 nm in Lhfpl5-/-. Tension in the tip link is thought to activate the channel via a gating spring; the gating spring stiffness was inferred from the change in bundle stiffness on severing the tip links with BAPTA. The gating stiffness was about a third of the total bundle stiffness in wild-type (total = 5.5 mN/m) but was much smaller in Lhfpl5 /-. Coupling between LHFPL5 and TMC1 may partly involve TMC1 residues 559 – 578; the mutation Tmc1 p.D569N (the site of a dominant human deafness mutation) reduced the LHFPL5 immunolabeling in the stereocilia, and like Lhfpl5 knockout doubled the WR of the MET activation curve. Other missense deafness mutations, Tmc1 p.D528N and Tmc1 p.M412K, had no effect on the working range.

Conclusions: We conclude that tip-link tension is transmitted to the channel primarily via LHFPL5; residual activation without LHFPL5 may occur by interaction between PCDH15 and TMC1.

M30. In Vivo Calcium Imaging in the Developing Mouse Cochlea

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Category: Hair Cells: Anatomy and Physiology

Background: Sensory-independent calcium activity regulates the development of mammalian sensory systems. In the auditory system, calcium action potentials from the pre-hearing inner hair cells (IHCs) propagate along the auditory pathway and promote not only the survival of the auditory fibres but also the refinement of the tonotopic maps in the brainstem and auditory cortex. Our current understanding of the origin and modulation of the action potential activity in IHCs comes from ex vivo experimental work, which although useful cannot replicate the sophisticated anatomy, innervation and physiology of the intact mammalian cochlea. We have developed surgical and microscopy approaches that, combined with transgenic animals expressing fluorescent indicators, allow us to study how mammalian sensory hair cells operate in vivo. Using this approach, we investigated the dynamics of spontaneous calcium activity in the prehearing cochlea of live mice at the cellular level.

Methods: Transgenic mice (P3-P10) expressing the genetically encoded calcium indicator GCaMP6f in either the hair cells or the supporting cells were anaesthetised using isoflurane and their body temperature maintained with a heat mat. The surgical procedure only led to a very small opening in the apical coil of the cochlear

bone, leaving the cochlear canals intact and unopened. The mouse was then transferred on the stage of a two-photon microscope equipped with long working distance water immersion objectives. The middle ear cavity was flooded with saline solution until it was possible to maintain a column of fluid under the objective lens, and the fluorescence was visualised under two-photon excitation conditions.
Results: This approach allowed us to record from the same cochlear region spanning 15-30 IHCs. Images were post-processed to remove out-of-focus frames and correct motion and drift artifacts, including those caused by the breathing of the anaesthetised animals. We found that IHCs and supporting cells displayed spontaneous calcium activity in vivo throughout the age-range investigated. IHC activity mostly appeared in bursts and some IHCs appeared to transition between quiescent periods and periods of prolonged spontaneous activity. Nearby IHCs displayed both independent and coordinated activity, which was compatible with the modulation on IHC excitability by calcium waves from the supporting cells.
Conclusions: In conclusion, our approach provides significant insights into the nature of spontaneous cochlear activity in prehearing mice, emphasising its importance in shaping the auditory system's architecture. These findings provide the first in vivo physiological recordings of spontaneous calcium activity occurring in the mouse pre-hearing cochlea. The application of two-photon imaging to study cochlear activity in vivo offers a promising avenue for future research.

M31. Elucidating the Role of the Potential Mechanosensor Protein CRIP3 in Hair Cell Repair

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Category: Hair Cells: Anatomy and Physiology
Background: As the primary mechanoreceptors of the vestibular and auditory system, sensory hair cells are under constant mechanical stress. The stereocilia portion of the hair cell which receives the mechanical stimuli is comprised of a filamentous (F-) actin core. Multiple studies have shown that the stereocilia F-actin turns over very slowly, thus necessitating an active repair mechanism to ensure long term function. It is known that noise exposure can cause gaps in this actin core. Our lab interestingly found that these lesions were being repaired over time. It is essential to investigate how these lesions in the F-actin cores are being sensed and repaired. With the emerging idea of F-actin itself being mechanoresponsive we began to look for ways it could be interacting with repair proteins. It is known that mechanical strain on actin produces binding sites for a subset of LIM domain containing proteins, allowing for the generation of a downstream response. We hypothesize that a similar mechanism could be occurring at the stereocilia core after damage. When looking for potential candidate proteins we examined those that were both enriched in the hair cell and contain a mechanosensitive domain. Thus, based on our preliminary data, we hypothesize that CRIP3 (Cysteine Rich Protein 3, aka Thymus LIM Protein) is a mechanosensitive protein capable of sensing actin damage as well as generating a downstream response to invoke stereocilia repair, essential for maintenance of the functional unit of the cochlea.
Methods: Multiple mouse models were generated: For loss-of-function studies, we generated a CRIP3 knockout mouse line. Hearing function was tested using ABR measurement at different ages and after noise exposure. We also generated a HA-CRIP3 knock-in (KI) mouse and determined CRIP3's localization in hair cells using confocal fluorescence microscopy. For live imaging of CRIP3 in hair cells, we generated a GFP-CRIP3 KI mouse line using the split-GFP approach. Lastly, we are testing CRIP3's interaction with actin stress-fiber strain sites (SFSS) via cell stretch and laser ablation in transfected fibroblasts and cochlear explants.
Results: Immunolocalization experiments on the CRIP3-HA KI mouse showed CRIP3 to be enriched at the taper region of the stereocilia in outer hair cells, consistent with previous RNAseq data. Furthermore, we have preliminary evidence that CRIP3 KO mice are more sensitive to noise-induced hearing loss.
Conclusions: Based on our data thus far, CRIP3 could be important for responding to increased mechanical stress, and possibly for initiating a force-dependent repair process in the taper region of the hair bundle. Its absence could alter the structural integrity of the hair cell leading to elevated ABR thresholds after noise exposure.

M32. Multiple Modes of Motion and the Effectiveness of Outer Hair Cells

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Category: Hair Cells: Anatomy and Physiology

Background: The effectiveness of outer hair cells (OHCs) as the feedback motor in the cochlea is limited by the mismatch between the stiff basilar membrane and soft OHCs, particularly in the high frequency region near the base. With a single mode of motion this limitation is inevitable. However, motion in the cochlea is rather complex as revealed by recent experiments, particularly with OCT method. Can complex modes of motion facilitate energy transfer by reducing the mismatch? Here, simple coupled oscillator models are used to examine this possibility.

Methods: Consider two oscillators with matched resonance frequencies. In one of them, the heavier oscillator (HO), the stiff basilar membrane serves as its elastic element and has viscous damping. In the lighter oscillator (LO), OHCs serve as an amplifier. External force is applied to HO and these two oscillators coupled with either elastic or viscous means. The applicability of these models could be limited to the very basal end, where traveling waves originate, because lateral energy flow is ignored.

The OHC could be stimulated by a combination of the displacement of HO as well as of LO. However, we assume either of the two, i.e. HO-driven or LO-driven, for simplicity. The efficiency of energy transfer can be evaluated by comparing the amplitudes of these coupled oscillators with that of a single mode oscillator, consisting of the basilar membrane, viscous drag and an OHC.

Results: For LO-driven modes, elastic coupling lead to significant amplitude gain (greater than 10 fold) for both LO and HO by compensating the additional stiffness from the coupling element with an increase of the mass. With viscous coupling, HO obtained much less gain than LO did.

For HO-driven mode with elastic coupling, amplitude gain was where coupling is weak and limited to LO. That was also the case for viscous coupling, but the gain was smaller.

It was noticed that OHC stiffness, which increases with OHC activity, had a significant effect on the performance of OHC.

Conclusions: The present proof of concept study shows that there are conditions, where multiple mode of motion in the cochlea can enhance the effectiveness of OHCs as the cochlear amplifier.

M33. A Revised Biophysical Inner Hair Cell Model for the Meddis Model of the Auditory Periphery

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Category: Hair Cells: Anatomy and Physiology

Background: The Meddis model of the auditory periphery (Version MAP1_14j) uses a biophysical inner hair cell (IHC) model with a single passive (linear) basolateral K+ current and shows an unphysiologically high AC component in response to high-frequency sounds (greater than 3 kHz). Recent physiological measurements identified fast, slow, and negatively activating basolateral K+ currents and found implications of their voltage-dependency for the IHC physiology. In vitro studies have shown that these currents cause a drop in membrane potential at the on- and offset of a stimulus, which modeling studies have proposed to be an additional mechanism for spike-rate adaptation of auditory nerve fibers (ANFs). In addition, whole-cell voltage-clamp measurements by Johnson (2015, eLife) revealed that low- and high-frequency IHCs have different compositions of K+ currents and different sizes of the resting mechanostransducer (MT) current.

Methods: In the present study, we adjusted the IHC model to simulate the decline in AC component for high sound frequencies observed in physiological data. We revised the model by implementing the above-mentioned voltage-dependent basolateral K+ currents to improve the adaptation in ANF spike rates. We included tonotopic differences in IHCs by developing two model configurations, a low- and high-frequency IHC, which differ in MT and K+ currents. The MT current activation function was chosen to produce the resting MT current reported by Johnson (2015) that is larger in low- than in high-frequency IHCs. The basolateral K+ currents were fitted to Johnson's steady-state and instantaneous tail current of the total K+

currents by adjusting the maximum conductance, activation curve, and reversal potential of the identified K+ currents. The pronounced outward rectification of the slow and fast K+ current visible in Johnson's data was addressed with the nonlinear Goldman-Hodgkin-Katz (GHK) equation for the I-V relationship. We considered several model parameter combinations for low- and high-frequency IHCs with different reversal potentials, activation and rectification properties of the single currents to reproduce Johnson's data, and investigated their impact on the AC and DC components of the IHC membrane potential and ANF spike-rate adaptation.
Results: The simulated responses of the low- and high-frequency IHC model matched the in-vivo-like current clamp recordings by Johnson (2015), and their AC and DC components were found to be determined mainly by the MT current activation function. In addition, the IHC membrane potential showed a pronounced decay at stimulation onset and offset caused by the voltage-dependent activation of K+ currents, leading to stronger adaptative responses in ANF spiking. The different parameter combinations of the K+ currents that were adopted to model the differences in low- and high-frequency IHCs did not have a physiologically relevant impact on the IHC responses.
Conclusions: Our study confirms the influence of voltage-dependent K+ currents on ANF adaptation and simulates proposed differences between low- and high-frequency IHCs.

M34. Localization of PIEZO2 in Vestibular Hair Cells in Mice

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Category: Hair Cells: Anatomy and Physiology
Background: Previous studies showed that PIEZO2 protein is localized at the apical surface of auditory outer hair cells and provides a novel mechanoelectrical transduction (MET) mechanism (Wu et al., 2017) that is complementary to the well-established MET mechanism mediated by transmembrane channel-like protein isoforms 1 and 2 (TMC1 and TMC2) (Pan et al., 2013; 2018; Kurima et al. 2015;). However, little is known about expression, localization and function of PIEZO2 in the MET of vestibular hair cells. The goal of this study is to investigate where PIEZO2 protein is localized in hair cells of cristae and maculae.
Methods: Piezo2-GFP-IRES-Cre mice (P7 and P30) were used in the study. Temporal bones were harvested and fixed in 4% paraformaldehyde solution. Following decalcification in EDTA, temporal bones were cryosectioned at 20 um. Tissues were then stained with phalloidin to label F-actin in the stereocilia and cuticular plates of hair cells and immunostained with antibodies against MYOSIN VIIa, SOX2 and GFP to identify Type I and II vestibular hair cells and localize PIEZO2-GFP expression in cristae and maculae. The cross-sectioned cristae and maculae were mounted on slides and examined using a confocal laser scanning microscope (Zeiss LSM880).
Results: PIEZO2-GFP fusion protein was observed in Type I (MYOSIN VIIa+SOX2-) and Type II (MYOSIN VIIa+SOX2+) hair cells in cristae and maculae. Preliminary analyses found that GFP+ hair cells in both the cristae and maculae were primarily Type II hair cells. Similar to auditory hair cells, PIEZO2-GFP was most prominent in the cuticular plate, but unlike auditory hair cells, vestibular hair cells also demonstrated PIEZO2-GFP in the stereocilia.
Conclusions: Although preliminary, these results suggest that PIEZO2 may play a role in the MET of vestibular hair cells that is different from that in auditory hair cells. Ongoing studies will further examine 1) PIEZO2 protein distributions in cristae and maculae and 2) functional consequences of conditional knockout of Piezo2 in vestibular hair cells.

M35. ATP-Dependent Signaling Activity in Mouse Developing Inner Hair Cells

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Category: Hair Cells: Anatomy and Physiology
Background: Prior to the onset of hearing, the sensory cells of the cochlea, the inner hair cells (IHCs), fire calcium action potentials, which trigger glutamate exocytosis onto the fibers populating the auditory nerve.

Thus, the spiking activity in the IHC represents a critical mechanism to foster the synaptic maturation along the ascending auditory pathway. ATP secretion from the supporting cells within the immature cochlea has been identified as the first signaling step to eventually determine the electric behavior in the IHCs. However, conflicting data regarding the role of ATP on IHC excitability has been raised.
Methods: Here, we used calcium imaging in the immature neuro-sensory epithelium of the cochlea, the Kölliker´s organ, to probe the ATP-dependence of the IHCs activity.
Results: After loading the Kölliker´s organ with the calcium dye fura-2 AM, we observed spontaneous calcium waves across the supporting cells and calcium transients in the IHCs. Incubation with apyrase, which hydrolyzes extracellular ATP, slows down the calcium waves spreading across the supporting cells, but leaves the spontaneous calcium transients in IHCs unaffected.
Conclusions: These findings suggest that the spiking activity in IHCs might be independent from the ATP waves propagation within distal supporting cells.

M36. Determining the Mechanical Properties of Inner Hair Cell Hair Bundles by Measuring Their Motions

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Category: Hair Cells: Anatomy and Physiology
Background: Inner hair-cell hair bundles (IHB) are sensory organelles required for hearing. They convert sound induced forces within the hearing organ into receptor currents, which induce the transmission of sound signals to the brain. This process, called mechano-electrical transduction (MET), involves ion channels that open and close in response to IHB deflections, caused by sound-induced forces. In the mammalian cochlea, at the threshold of hearing, IHB displacements are on the order of nanometers and the conversion of forces into a receptor current occurs in less than one millisecond. MET is limited by thermal noise, which create fluctuations in the displacements of the stereocilia, affecting the timing of the receptor current. We show, for a model of a hair bundle with only two stereocilia, how the coherency of stereocilium displacements depends on the stiffness and damping of the stereocilia and their links.

M37. Importance of the Mitochondrial Protein ACO2 for Hearing and Sensory Hair Cells

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Category: Hair Cells: Anatomy and Physiology
Background: Sensory hair cells heavily depend on mitochondrial function to supply the high energy demand associated with constant detection and transmission of stimuli. Consequently, mitochondria dysfunction has been implicated in many types of hearing loss. ACO2 is a nucleus-encoded citric acid cycle enzyme associated with human disease, with variants causing infantile cerebellar-retinal degeneration and optic neuropathies. ACO2 variants have also been linked to hearing loss, however this role is poorly characterized. Understanding how ACO2 participates in hearing and balance, or disease at large, is complicated due to a lack of viable animal models.
Methods: We identified and studied both mouse and zebrafish Aco2 models. A spontaneous mutant strain exhibiting recessive circling behavior was isolated at The Jackson Laboratory, and exome sequencing uncovered an R56L substitution in Aco2. We assessed this strain and a strain carrying a constitutive null Aco2 allele for viability and recorded ABR to test hearing. We also examined a zebrafish strain with a null mutation in aco2 to study hair cells in the lateral-line sensory system. In both zebrafish and mice Aco2 mutants, we used transgenic lines, vital dyes and immunohistochemistry to examine hair cell survival, as well as mitochondrial morphology and health.
Results: We found that while Aco2 null mice did not survive past birth, Aco2R56L produced fertile homozygote adults, and may thus represents the first viable mouse strain to model disease in ACO2 patients. At 4 weeks of age, Aco2R56L homozygotes showed hearing deficits that degraded into complete hearing loss at all frequencies tested at 6 weeks. We are currently assessing ACO2 protein levels and mitochondrial profile

in hair cells and other cell types. In zebrafish, we found that larvae lacking Aco2 became paralyzed just prior to the onset of hearing. Homozygotes had no Aco2 immunolabeling, while heterozygotes showed reduced protein amount in hair cells compared to wild type siblings. Aco2 null zebrafish mutants had fewer hair cells and synapses compared to wild type. Remaining hair cells showed reduced FM 1-43 uptake, indicating a defect in mechanotransduction. Using live mitochondrial labels, we found that zebrafish hair cell mitochondria were functionally and structurally perturbed in aco2 null mutants but not in heterozygotes. From our zebrafish work we conclude that Aco2 haploinsufficiency may not impair hair cell mitochondria.
Conclusions: We identified two new animal models that can be leveraged to study ACO2 function in disease. Preliminary mouse data indicates that ACO2 is required to maintain auditory function two weeks after onset. Zebrafish data demonstrates that while Aco2 haploinsufficiency may not impact hair cells in the lateral-line system, Aco2 is essential for hair cell and mitochondrial health and function. We will next leverage these models to develop therapeutic approaches that bypass metabolic deficiency in the citric acid cycle.

M38. In Vivo Study of Truncated and Zero Layer Mutations of the C-Terminus of SNAP-25 Reveals Its Direct Role in Auditory Hair Cell Ribbon Synaptic Exocytosis

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Category: Hair Cells: Anatomy and Physiology
Background: SNAP-25, a component of the SNARES (soluble NSF attachment protein receptor) complex, is central to synaptic vesicle exocytosis in neurons and neuroendocrine cells. We recently untangled the long-standing issue surrounding the implication of SNAP-25 in IHC synaptic transmission by demonstrating that the targeted deletion of SNAP-25 exclusively in the IHC of a normally hearing mouse results in profound deafness. This deafness primarily stems from the disruption of IHC synaptic exocytosis and subsequent IHC degeneration (Calvet et al., 2022). However, one key question is whether the loss of SNAP-25 triggers synaptic dysfunction leading to degeneration, or if SNAP-25 is responsible for another form of dysfunction that indirectly leads to the exocytic defects. To answer this question, we implemented a strategy for inducing in vivo AAV-mediated overexpression of either the native or mutated forms of SNAP-25, fused with green fluorescent protein (GFP), within IHC.
Methods: We choose two AAV constructs: i) SNAP-25-Δ9, which lacks the C-terminal nine amino acids, thereby mimicking the cleavage of endogenous SNAP-25 by botulinum toxin A (BoNT/A), which has been demonstrated to be just as potent as the toxin itself in inhibiting transmitter release, ii) SNAP-25-Q174L, a point mutation which disrupts the protein's zero layer, resulted in only a moderate alteration in neurotransmission. The main objective was to create a competitive environment in vivo with endogenous SNAP-25, thereby allowing us to investigate its impact on IHC synaptic exocytosis, mirroring findings reported in chromaffin cells.
Results: After being virally delivered to the cochlea, all SNAP-25 constructs fused with GFP were mainly targeting the plasma membrane of IHC. The viral intracochlear delivery of the native SNAP-25 form fused to GFP demonstrated no significant effect on either IHC exocytosis or the hearing capacity of the injected ear. However, in mice that received cochlear delivery of GFP-SNAP-25-Δ9, a profound deafness occurred due to the disruption of the rapid and sustained phases of IHC synaptic exocytosis despite near normal calcium current. This finding is consistent with what has been reported in both chromaffin cells and neurons (Criado et al., 1999; Gutierrez et al., 1997; Weber et al., 2010).
Remarkably, the injection of GFP-SNAP-25-Q174L into the cochlea led to a moderate hearing loss that progressed to a severe level but did not reach the profound deafness observed in the case of GFP-SNAP-25-Δ9. While assessing IHC exocytosis via capacitance measurements, we detected a distinct reduction specifically in the sustained exocytotic phase. It is noteworthy that at the age when IHC synaptic exocytosis was investigated, the morphoanatomy of the organ of Corti appeared to be normal.

Conclusions: Taken together, these findings clearly uncouple the IHC exocytotic defect and the subsequent deafness from cellular degeneration, underscoring the critical role of SNAP-25 in IHC synaptic transmission.

M39. Exploring the Role of Exon-47 Encoded (Brain-Splicing Isoform) of Otoferlin in Cochlear and Central Sound Processing

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Category: Hair Cells: Anatomy and Physiology

Background: Mutations in OTOF represent one of the most frequent cause of auditory neuropathy (synaptopathy) spectrum disorder (DFNB9). Otoferlin is expressed in both vestibular and cochlear sensory hair cells but also in several neuronal brain regions. Remarkably, brain and inner ear tissues express preferentially two different alternatively spliced forms of the otoferlin C-terminal transmembrane domain (TMD), encoded by either exon-47 or exon-48, respectively. While the function of otoferlin in sensory hair cells (TMD-isoform exon 48) of the peripheral hearing and vestibular organ is clearly established as a calcium-sensor controlling synaptic vesicle exocytosis, the role of the brain TMD isoform of otoferlin encoded by exon-47 is 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 TMD-brain otoferlin isoform in brain and 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 (expressing Cre recombinase under the control of the phosphoglycerate kinase 1 promoter). We recorded ABRs and DPOAEs in P25-P40 mice using a TDT RZ6/BioSigRZ system (Tucker-Davis Technologies).

Results: In Otof exon47-mScarlet-flx/flx mice, we confirmed, both by RT-PCR and by direct fluorescence imaging of mscarlet, that the splicing exon-47 otoferlin (TMD brain isoform), is indeed expressed in cochlear hair cells, but at a much lower level as compared to the cochlear exon-48 main splicing isoform. Both direct imaging of the mscarlet-tagged otoferlin brain isoform in live organs of Corti, or in fixed tissue using anti-RFP immunolabeling, showed similar protein expression in IHCs, below the cuticular plate and at the synaptic ribbons, similar to the native otoferlin. To investigate the potential role of this exon-47 TMD brain isoform in IHCs we then generated a mouse model in which exon-47 was constitutively deleted.

Otof exon47-mScarlet-flx/flx-PGK-Cre+/- mice, in which exon-47 was ubiquitously excised at the diploid phase of genesis by using Cre-Lox recombination with the PGK- promoter, showed apparent normal hearing function similar to control non-floxed Otof exon47-PGK-Cre+/- mice, as indicated by ABR and DPOAEs thresholds and latencies. The morphological distribution of ribbon synapses in mutant IHCs as well as their exocytotic properties is currently under investigation.

Conclusions: These results indicated that alternative splicing generating the exon-47 encoded TMD-brain isoform of otoferlin is dispensable for normal cochlear hair cells neurotransmission. Further investigations are now needed to explore whether the splicing balance between exon-47 and exon-48 TMD isoforms can be modulated under certain patho-physiological conditions. We are also now currently exploring in our mouse models, the functional expression of the exon-47 encoded otoferlin in auditory brain structures, notably the ones involved in acoustic sensorimotor and memory function.

M40. On the Identity of Spectrin Subtypes in the Sub Plasma Membrane Space Responsible for eM-NLC Uncoupling in Outer Hair Cells

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Category: Hair Cells: Anatomy and Physiology

Background: Outer hair cells (OHC) evidence electromotility (eM), a voltage-dependent length change. The OHC lateral membrane that houses the motor protein prestin has an underlying cytoskeletal network of circumferential actin filaments and longitudinal spectrin filaments that are positioned between the plasma membrane and an underlying subsurface cisterna (SSC). The identity of the spectrin subtypes that constitute the cytoskeletal network is indeterminate. We recently established that beta 5 spectrin is not a constituent of the OHC cytoskeletal network.

Methods: Using immunofluorescence confocal microscopy we have determined the localization of spectrin subtypes in the OHC. Using cryo electron tomography of fixed OHCs that lack alpha 2, beta 1 or beta 2 spectrin, which all show an OHC deficient phenotype, we imaged and reconstructed the 3D structure of the OHC lateral membrane .and its underlying cytoskeletal network.
Using electron tomography (ET) of fixed OHCs from conditional knockouts (cKO) of alpha 2, beta 1 and beta 2 spectrin that also show an OHC deficient phenotype, we analyzed the cytoskeletal network beneath the plasma membrane of OHCs.

Results: We determine that beta 1 spectrin cKO mice show a deficient actin/cytoskeletal network beneath the plasma membrane and that the gap between the PM and SSC has widened significantly. These data comport with our electrophysiological measures from these cells that show decreased electromotility (eM) out of proportion to prestin's gating charge movement, pointing to eM-NLC uncoupling.
Conclusions: We establish that beta 1 spectrin forms a key component of the cortical lattice beneath the plasma membrane of OHCs and is responsible for coupling gating charge movement (NLC) in prestin with eM.

M41. A Unique Collection of Animal Temporal Bone Specimens: Otopathology Laboratory at the University of Minnesota

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Category: Other, Otopathology (Animal Collection)
Background: The historical use of animals in scientific research, dating back to ancient Greek physician-scientists, has been pivotal in advancing our knowledge of disease mechanisms. Animal experiments remain crucial in biomedical research, especially for evaluating in vivo techniques applicable to humans. Temporal bone studies have significantly enhanced our understanding of various ear-related pathological conditions and diseases, enriching the scientific literature on these topics.
Methods: This study catalogues the animal temporal bone specimens housed in the "University of Minnesota Otopathology Laboratory," established by Professor Michael Paparella in the late 1960s, currently one of the few active temporal bone laboratories. The inventory includes both sectioned samples and temporal bones preserved in alcohol following decalcification.
Results: The temporal bone laboratory collection comprises samples from 19 distinct species, including rodent models (chinchilla, mice, rat, etc.), larger mammals (dog, cat, monkey, etc.), domestic and wild birds (chicken, owl, etc.) and aquatic species such as dolphins. Detailed information regarding experimental procedures and chemical analyses, if conducted, is archived with the respective bone files.
Conclusions: Diverse animal models provide valuable insights into various ear diseases and audiology research. The University of Minnesota Animal Temporal Bone collection stands as a unique and invaluable resource, featuring a wide array of animal species with distinct anatomical and physiological characteristics that aid in studying various diseases. This collection contributes significantly to our understanding of conditions like otitis media, acoustic trauma, hearing loss (both syndromic and non-syndromic), peripheral vestibular system disorders, metabolic and endocrine diseases, and more. Despite financial challenges, active research centers continue to produce fundamental research annually. Increased support for and emphasis on animal studies in these centers can further our comprehension and treatment of otological diseases.

M42. Role of the RNA-Binding Protein FMRP in Spiral Ganglion Maturation

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Category: Inner Ear: Anatomy and Physiology
Background: Spiral ganglion neurons (SGNs) are classified into four subtypes, IA, IB, IC, and II, each with distinct connectivity, firing properties, and molecular signatures. This project aims to identify the role of

Fragile X messenger ribonucleoprotein (FMRP; encoded by Fmr1), an RNA-binding protein, as a molecular determinant driving spiral ganglion development. In the brain, FMRP regulates neuron maturation and circuit assembly, often through activity-dependent neuronal events. We recently discovered intense FMRP expression in developing and mature SGNs across vertebrates. How FMRP regulates SGN development and connectivity, however, is completely unknown.
Methods: We examine the hypothesis that FMRP regulates activity dependent cell type specification of SGNs and the patterning of their central projection to the ventral cochlear nucleus (VCN). In Fmr1 knockout mice in which FMRP is lost globally, we have identified altered transmission from the ear to the brainstem, showing altered amplitude and latency of wave I of the auditory brainstem responses (ABRs). Additionally, the tonotopic organization within the VCN is reduced, as demonstrated by more diffused distributions of c-fos-expressing neurons following pure tone sound exposure. To determine the contribution of cochlea FMRP in these deficits, we selectively knock out FMRP expression from the peripheral, but not central, auditory system, by injecting adeno-associated virus (AAV)-Cre into the inner ear of Fmr1LOXP mice at postnatal day 3 (P3). To develop this approach, we performed a pilot study using a control AAV (without Cre) tagged with eGFP and driven by the human synapsin promoter for preferential transduction of neurons.
Results: Closed field ABRs were conducted at P14 and P21 and verified no changes in basic hearing ability, showing comparable ABR thresholds between the injected and uninfected ears of the same animals. In the cochlea, a substantial portion of SGNs (20-60%) were transfected. Double labeling with calretinin (type IA SGN marker) further revealed that the transduction occurred in both calretinin-lack and calretinin-expressing neurons. We are currently quantifying how this manipulation affects the cell type composition of SGNs following inner ear delivery of AAV-Cre. In the brainstem, the central projection of transfected SGNs was identified in the VCN. As expected, eGFP-labeled axon terminals contain vGluT2, a marker of glutamatergic presynaptic terminals. Ongoing experiments determine how the arborization patterning as well as the molecular and functional maturation of the auditory nerve axonal terminals are affected by inner ear delivery of AAV-Cre. As a second approach, we have and will continue to generate conditional Fmr1 knockout mouse strains by crossing Fmr1LOXP mice with Cre lines driven by distinct SGN cell markers that do not affect the auditory brainstem.
Conclusions: Understanding the roles of FMRP in SGNs is expected to provide insights into potential therapeutic interventions for reducing spiral ganglion degeneration following hearing loss and elevating auditory dysfunctions in neurological disorders.

M43. Structural Basis of G-A Interacting Protein, C-Terminus 3 (GIPC3) Function as an Adaptor for Myosin-6-Dependent Transport

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Category: Inner Ear: Anatomy and Physiology
Background: The GIPC family of proteins function as key molecular scaffolds that are critical for integration of multiple steps of signaling from receptor activation to downstream effects. GIPC3 plays an essential role in the auditory system, with mutations in GIPC3 causing inherited nonsyndromic hearing loss (Rehman et al., 2011; Charizopoulou et al., 2011). GIPC3 has been demonstrated to be a pleotrophic signal adaptor protein (Chatterjee et. al., 2023). Therefore, understanding the competitive and cooperative molecular interactions involved in GIPC3 signaling is critical for understanding its mechanism of action in the auditory system. Further, a number of patient mutations have been identified that span the three structural domains of GIPC3, with varying severity of hearing loss leaving open the question on the exact molecular mechanism(s) underlying GIPC3 function in hair cells.
Methods: Integrated structural and biophysical studies have been used to define the biochemical basis for GIPC3 function. X-ray crystallography has been combined with Hydrogen Deuterium Exchange (HDX) to define the structural dynamics that define GIPC3 activation, including receptor and MYO6 binding. Size Exclusion Chromatography Multi-Angle Light Scattering (SEC-MALS) has been used to define the oligomeric state of GIPC3 at various steps of activation. Stability measurements, quantitative binding measurements, and HDX has been used to define the effect of patient mutations. Coupled to experimental structures, AlphaFold2-based modeling has been used to explore alternative binding modes of GIPC3 binding partners.

Results: Our data demonstrate the basis for receptor-activated engagement of GIPC3 with MYO6. Receptor-binding is found to regulate GIPC3 oligomerization and be directly coupled to MYO6. Detailed structural analysis with mutagenesis allows determination of key sequence details responsible for GIPC3 partner engagement. Intriguingly, patient mutations are shown to cause key deficits in GIPC3 function including both gain and loss of function. These distinct defects indicate that multiple structural mechanisms underlie the physiological deficits seen in GIPC3 patients. Assessment of hair cell abnormalities in newly generated Gipc3-/- mice support a role for GIPC3 in apex-to-base vesicular transport within hair cells (see companion poster by Dragich et al.). This physiological role is explored using structural modeling in the context of diverse potential GIPC3 binding partners.
Conclusions: We conclude that GIPC3 utilizes a sequential activation mechanism, with patient mutations demonstrating key defects in multiple steps of activation.

M44. Exploring the Role of Microfractures in Sensorineural Hearing Loss Among Otosclerosis Patients

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Category: Inner Ear: Anatomy and Physiology
Background: Otosclerosis or otospongiosis accounts for approximately 5-9% of all cases of hearing loss. The clinical presentation of the disease primarily includes hearing loss, vertigo, and tinnitus. There is a varying prevalence among different ethnic groups, being more common in Caucasians (0.04-1%), Blacks (1%), and Asians (5%). This disease is more common in women (2:1 ratio) and can worsen during pregnancy. Histopathological prevalence is higher than the clinical, ranging from 2.5% to 12%. Histological otosclerosis is the term used to describe the abnormal bone capsule when observed under a microscope, even in the absence of clinical symptoms. Although otosclerosis primarily presents with conductive hearing loss (CHL) caused by fixation of the stapes, some patients also present sensorineural hearing loss (SNHL). While pathophysiological mechanisms underlying SNHL in otosclerosis cases are not well understood. Studies have shown that SNHL can be the result of direct damage to the cochlea and spiral ligament caused by the lytic process or the release of proteolytic enzymes. Histological examination of otosclerosis cases frequently present with microfractures affecting the otic capsule, raising the question of whether those may also be involved with SNHL. Thus, our study aims to evaluate the presence of microfractures in cases with histological otosclerosis, and to compare their existence with clinical symptoms and their effects in the donor's audiograms.
Methods: Archived human temporal bones at the University of Minnesota were used in this study. We excluded specimens with cochlear otosclerosis (spiral ligament hyalinization and cochlear endosteum involvement), as well as ones from donors who underwent stapedectomy. Audiograms were used to determine the progression of hearing loss and to see if patients had initial CHL or SNHL. The samples were sectioned with a sliding microtome and every tenth section was saved for staining. Routine H and E staining was used. A light microscopy was used to visualize the sections. Past medical history was used to correlate any otological findings and clinical presentations.
Results: Our study included 12 TBs from 6 donors and 1 control case of otosclerosis without microfractures. Among the cases: 33% had conductive hearing loss, 50% had SNHL, and 17% had unknown-origin hearing loss.
Conclusions: Our study showed that patients with otosclerosis who had microfractures affecting the otic capsule also presented with SNHL even in the absence of involvement of the cochlear endosteum. Therefore, it seems that microfractures may potentially play a role in the presentation of SNHL in patients with otosclerosis.

M45. Peripheral Vestibular Dysfunction in Shank3 Mouse Model of Autism

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Category: Inner Ear: Anatomy and Physiology

Background: Autism spectrum disorder (ASD) is a neurodevelopmental condition. In addition to the core features, such as repetitive behaviors, many individuals with ASD experience various degrees of auditory and vestibular issues. These sensory challenges are particularly significant in children, as proper cognitive development relies on an accurate perception of the environment. Vestibular problems in ASD encompass issues like balance difficulties, abnormal walking patterns, and impaired focus. Additionally, some individuals with ASD exhibit hyposensitivity to vestibular stimuli, enabling them to engage in activities like prolonged swinging or spinning without feeling dizzy or nauseated. Traditionally, ASD was believed to originate solely from abnormalities in brain function. However, a recent study has shown that peripheral mutation of Shank3 gene (an ASD-related gene) in mice results in peripheral sensory dysfunction that could then results in disruption of central nervous system development and cause ASD-related phenotypes. To date, the function of the peripheral vestibular pathways has not been evaluated in ASD mouse models.

Methods: We investigated whether the peripheral vestibular pathway shows any abnormal responses. We quantified the response of the vestibular nerve using subcutaneous electrodes to measure the vestibular sensory evoked potentials (VsEP) in Shank3-/- mouse model of ASD. These field potential recordings signify synchronous activity of the most phasic group of afferents in response to fast 2 ms linear head movements. The threshold of the response, peak-to-peak amplitudes and latencies of the first wave were quantified for different stimulation jerks (0.5 – 2 g/ms). Responses were compared between KO, heterozygote, and WT mice.

Results: We found that the amplitude of VsEP responses in Shank3 KO and heterozygote mice (n = 9) were decreased by about 30% compared to WT mice. Despite the small number of current samples, responses of KO and heterozygote mice seemed to be grouped separately, with heterozygotes somewhere in between KO and WT mice.

Conclusions: The observed hypofunction in the vestibular periphery of mice with Shank3 mutation shows that the vestibular periphery could play a role in the observed vestibular dysfunction. The peripheral pathway in the inner ear could be specifically targeted by local drug applications to improve the vestibular function. Since untreated vestibular dysfunction in children can lead to delayed milestones such as sitting and walking and poor motor coordination later in life, the findings of this study could provide the foundations for new approaches for treating imbalance in ASD.

M46. Refractive Correction and Calibration of 3D OCT Vibrometry: Reconstruction of the Vector of Motion in the Mouse Cochlea

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Category: Inner Ear: Cochlear Mechanics

Background: Optical coherence tomography (OCT) is a valuable tool for investigating the mechanical motion of the cochlea. It can visualize cochlear soft tissues through the bony otic capsule and detect picometer scale motion, enabling precise measurements of intracochlear vibrations at micron scale resolution. However, this approach can only measure motion along the optical axis, hence only one component of the vector of motion can be measured. To address this, we developed an OCT system with three sample arms having different incident angles, allowing us to obtain a 3D measure of vibratory motion.

The change in refractive index at the air-bone interface causes light to be significantly refracted at the surface of the cochlea. Since we have three laser beams incident on the cochlea at different angles, the refraction experienced by each beam will be different. To address this issue, we first estimated the refractive error based on our typical measurement geometry, and then developed an algorithm to correct the vibrational data.

Methods: The workflow for the correction algorithm begins by fitting a plane to the surface of the registered data to calculate an orthogonal vector. An incidence vector is also derived from the geometry of each sample arm. Using Snell's law in vector form, we calculate transmitted vectors from these orthogonal and incident vectors. Converting the transmitted vector to spherical coordinates allows us to obtain the angles of refraction occurring in the XZ and YZ planes. These values can be applied to correct the registered data using a

transformation matrix. To test our algorithm, we used Zemax and Matlab simulations followed by further validation using a piezo with a refractive material on top that was vibrating at 9 kHz.
Results: Our initial estimate for refractive error for the three beams based on our typical imaging geometry was 4.2˚, 11.1˚, and 11.9˚. Simulations in Zemax and Matlab that modeled refraction along an arbitrary axis, permitted us to validate our approach using Snell's law. We are currently working to validate the entire workflow using the piezo before applying it to data from a mouse cochlea. Since this is entirely a post processing scheme, all the data we have collected so far can be corrected post hoc.
Conclusions: Our estimates show that refractive errors can be larger than the inherent measurement error of our system, necessitating refraction correction to improve the accuracy of cochlear vibrometry. Our algorithm assumes the bone directly over the organ of Corti can be approximated as a plane. That enables a single correction for each beam but is also a potential limitation. With this assumption, a single transformation matrix can be developed to convert the non-orthogonal experimental data into the orthogonal (x,y,z) components of the vector of motion, all in post processing.

M47. Improving Resolution of OCT Vibrometry in the Cochlea With Compressed Sensing

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Category: Inner Ear: Cochlear Mechanics
Background: Optical coherence tomography (OCT) has revolutionized experimental cochlear mechanics, facilitating vibrometry for structures within the organ of Corti complex (OCC). Areal vibration maps, where OCT-measured displacements are displayed across a cross-section of the OCC, are a common form of data presentation. However, these maps have limited resolution and suffer from noise due to time constraints imposed by sample drift and maintenance of the cochlear condition.
Methods: We present a method for compressed sensing vibrometry (CSVi) capable of reconstructing densely-sampled areal displacement maps from fewer samples. The method, based in total generalized variation (TGV) optimization, also acts as a simultaneous denoiser by penalizing aphysical high-spatial-frequency components of motion. Six optimization methods were tested: total variation, TGV, and wavelet-domain sparsity promotion, each with the use of either uniform or random subsampling schemes. TGV optimization with uniform subsampling was found to achieve the best results in reconstructing dense displacement maps.
Results: We find that using only 10% of samples, a densely sampled areal displacement map of the gerbil OCC can be reconstructed with less than 5% normalized mean squared error using CSVi. This performance was shown to generalize to maps measured at various stimulus frequencies and sound pressure levels, as well as beam axis orientations relative to sample anatomy. The resulting areal maps are also qualitatively less noisy, and free of aliasing artifacts that would be observed if a Nyquist-style upsampling were applied in place of a compressed sensing method.
Conclusions: CSVi can be used to accelerate the acquisition of displacement maps in the OCC, allowing for increased yield from in vivo experiments. Such acceleration could open the door for new experiments as well, such as measurements of densely sampled volumetric displacement maps, or areal displacement maps in perturbation studies where the timescales on which data can be acquired are smaller than in healthy cochleae.

M48. Effects of a Low-Side Suppressor on Vibrations in the Base of the Gerbil Cochlea

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Category: Inner Ear: Cochlear Mechanics
Background: Optical coherence tomography (OCT) has revealed that the outer hair cell (OHC) region, extending from the OHC/Deiters' cell junction to close to the reticular lamina (RL), vibrates with a higher amplitude and distinct tuning relative to the basilar membrane (BM). With broad-band stimuli, the OHC-region shows a pronounced low frequency nonlinearity extending well below the region's best frequency (BF). Unlike the BM, the OHC-region vibrations also exhibit hypercompression (near the 25 kHz place) or troughs (at the more basal Hook Region) at the highest sound pressure levels (SPLs).

Methods: Organ of Corti (OoC) vibrations were measured with a ThorLabs Telesto III OCT in the base of the gerbil cochlea through the round window membrane. In each experiment, vibrometry was performed at multiple longitudinal locations near either the 25 kHz place (with a primarily longitudinal/transverse view) or in the hook region where the BF was between 40 and 50 kHz (with a primarily transverse view). Vibrations were measured in a 2-dimensional, quasi-radial, pattern covering most of the width of the OoC in 10 or 20 micron steps in response to: (1) a pure tone frequency sweep, (2) the same sweep in the presence of an intense (3 kHz 100 dB SPL) low-side suppressor, and (3) a broad-band Zwuis complex. The analysis focused primarily on the tuning curves determined at each position in the radial sections or slices. Distortion product otoacoustic emissions (DPOAEs) were measured in response to swept two-tone stimuli throughout each experiment to monitor the general cochlear condition.
Results: The BM and OHC-region responses were affected differently by the suppressor tone. As has been previously shown, with the low-side suppressor, BM vibrations resembled a passive cochlea showing: (1) a reduced amplitude that is especially pronounced at lower SPLs, (2) a loss of the sharp BF-region peak, and (3) linear growth. The OHC-region vibrations also showed linear growth with the suppressor, but in contrast to the BM, the OHC-region was affected differently in roughly two frequency bands. Below the BF, the amplitude of the OHC-region vibrations fell to a level comparable to the BM vibrations under the same conditions while in the BF-region, the responses became low-pass in character, but with a very sharp cutoff. This high frequency roll-off may be more pronounced and slightly sharper at the 25 kHz location.
Conclusions: At both the 25 kHz location and in the hook region, the BM and OHC-region show a loss of amplitude and linearization in the presence of a low-side suppressor. At both locations, the OHC-region vibrations became low-pass with a cutoff that is too sharp to be explained by the passive mechanical properties of the OoC suggesting that the active process is still operational in spite of the saturating low frequency suppressor.

M49. Influence of the Head Boundary Conditions on the Skull Bone Motion Under Bone Conduction: A Hybrid Fem and Experimental Approach

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Category: Inner Ear: Cochlear Mechanics
Background: The aim of this work is to explore the effect of head support on the vibrational response of the human skull under bone conduction. This is relevant in both modeling and experimental work, where there is a great variation in boundary conditions and their effect is not well understood.
Methods: In a preliminary study, one Thiel embalmed whole-head cadaver specimen was measured, while the skull neck support was varied by adjusting the stiffness of a custom head support holder. The osseous pathways were activated percutaneously via stimulation of the mastoid, BAHA location, or forehead, sequentially. For each head support condition, the head stiffness, relative to its support, was evaluated by measuring the 3D deflection of the skull's vertex at several force levels (1-10N) in the anterior-posterior and left-right direction, sequentially. For each stimulus and neck support condition, the skull bone response was monitored by a three-dimensional laser Doppler vibrometer system, which measured the 3D motions of the ipsi-, top, and contra-lateral skull surface at approximately 40-50 points with a pitch of about 50 mm. Stimulation was provided from 0.1-2 kHz, with the goal of observing the low-frequency behavior of the skull and its transition from rigid-body motion to deformation. Some of the experimental conditions were also recreated numerically via a modified version of the LiUHead model.
Results: Moving the head support points from neck vertebrae to neck muscle attachment points changed the head stiffness 3-10 times. The low-frequency vibrational response of the skull changed in magnitude, spatial composition, and frequency dependence with changing the boundary conditions of the skull. Stimulation further away from the head support location resulted in a more pronounced directionality of the primarily rigid body motion of the skull at low frequencies.
Conclusions: In general, the motion of the skull bone is influenced by the neck support, but only at low frequencies, indicated by both FEM and experimental data.

M50. Comparing Kinematic Gains of the Mouse and Gerbil Cochlea

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Category: Inner Ear: Cochlear Mechanics

Background: One of the fundamental biophysical parameters to determine hearing sensitivity is the kinematic gain, defined as the ratio between the deflections of the hair bundle and the basilar membrane (BM). The kinematic gain, estimated before the finding of outer hair cell motility, was derived under the assumption that the organ of Corti (OoC) moves as a rigid body. However, the OoC deforms non-uniformly, especially when the cochlea is sensitive. The magnitude and timing of the kinematic gain have not been studied across species and at different CF locations.

Methods: We measured two-dimensional OoC vibrations in the excised cochleae of young gerbils and mice (P15-P30). The excised cochleae were placed in a custom-designed microfluidic chamber filled with artificial perilymph and either mechanically or electrically stimulated. Our excised preparation allowed us some advantages. The enhanced spatial resolution of vibrometry imaging enabled us to measure the relative motions between the tectorial membrane and the reticular lamina. Three-dimensional imaging of the cochlear coil allowed us to define radial sections accurately (i.e., sections perpendicular to the longitudinal direction). Measurements were conducted in the gerbil's middle turn (physiological CF locations of 1.5, 4, and 10 kHz) and in the mouse's upper turn (CF locations of 6, 10, and 20 kHz). Stimulating frequencies ranged between 2-3 octaves below and 1 octave above the CF at each location.

Results: Consistent with the literature, some geometrical characteristics of OoC remain similar between the two rodent species when compared at a similar CF location, such as the size of the outer hair cells and the angle of the TM relative to the BM at comparable CF locations. Our data indicate that in both species, the kinematic gain increases with the CF location, suggesting that a similar level of BM vibrations induces a larger stereocilia deflection toward the base of the cochlea. We observed minimal frequency dependence of shear gain at the investigated locations (less than 5 dB/oct). Albeit small sample numbers (n greater than 20 for the gerbil, n = 6 for the mouse), our data on the kinematic gain shows consistency between the two species. When represented as a function of CF, the kinematic gain of mice overlaps with that of gerbils.

Conclusions: Our kinematic gain measurement suggests that the hair cell stereocilia are agitated similarly across mammalian species. With further measurements, our findings may expand the existing knowledge of anatomical similarities across mammalian species to functional similarity.

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M51. Cubic Distortion Product Within the Cochlear Partition in the Basal Turn of Sensitive Gerbil Cochleae

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Category: Inner Ear: Cochlear Mechanics

Background: When two tones at frequencies f1 and f2 (f2 greater than f1) are presented to a sensitive cochlea, a sound at frequency 2f1-f2 can be detected in the external ear canal. This cochlea-generated sound, called the cubic distortion product otoacoustic emission (DPOAE), has been commonly measured in clinics and laboratories for assessing hearing and studying cochlear mechanisms. Two tone-induced distortion products (DPs) have been demonstrated in the receptor potential and the bundle movement of sensory hair cells and in the basilar membrane and reticular lamina vibrations. To understand how these cellular activities result in the cochlear partition vibration and otoacoustic emissions, we measured the vibration magnitude and phase at the primary tone and DP frequencies from the cross-section of the cochlear partition in the basal turn of living gerbil cochleae.

Methods: Young Mongolian gerbils of either sex with normal hearing were used in this experiment. The bulla on the left side was opened through a ventrolateral surgical approach. The cochlear partition vibration was measured through the round window or an opening in the cochlear lateral wall. For the lateral wall approach,

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, and the cochlear partition was positioned approximately in the horizontal plane. The magnitude and phase patterns of two tone-evoked vibrations were measured at frequencies f1, f2, and 2f1-f2 from the cross-section of the cochlear partition using a scanning heterodyne low-coherence interferometer.
Results: At low primary tone levels, the largest DP magnitude is in the reticular lamina-outer hair cell region. As the sound pressure level increases, the largest f1 and f2 responses move from the reticular lamina-outer hair cell region to the basilar membrane. Despite the level-dependent change of primary tone responses, the location of the largest DP response remains in the reticular lamina-outer hair cell region. The phase of DP vibration does not change across the cochlear partition or with the primary tone level.
Conclusions: The present results indicate that the mechanical cubic DP is generated by the outer hair cells and that the different structures of the organ of Corti vibrate approximately in the same direction on the cross-section of the cochlear partition. This finding is consistent with a previous observation that the DP at a given cochlear longitudinal location consists of a local and propagated component.

M52. Protein Profile of Endolymph in the Cochlea of Mouse Inner Ear

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Category: Inner Ear: Membranes and Fluids
Background: The mammalian cochlea contains two different extracellular fluids, perilymph in scala vestibuli and scala tympani and endolymph in scala media. The endolymph exhibits a positive potential of +100 mV, which sensitizes cochlear hair cells. In addition to hair cells, supporting cells and strial marginal cells are exposed to the endolymph. This characteristic suggests that the endolymph plays multiple roles. Previously, a group detected several proteins in the endolymph of guinea pigs (Thalmann et al., Hear Res 1992; Electrophoresis 2006). Nevertheless, in mouse, the most frequently used model for human deafness, the content remains elusive—in this animal, it is technically difficult to collect the endolymph, owing to narrow structure of the scala media and its minute volume of less than 1 μL. In this study, we therefore developed a technique to purely gather the endolymph from mouse cochleae and analyzed the protein profile.
Methods: A micropipette (tip diameter: ~10 μm) was fabricated from a glass capillary and the tip was filled with a conductive organic solvent, tetrahexylammonium tetrakis (4-chlorophenyl) borate (TPBCl). The barrel was connected via an Ag/AgTPBCl electrode to an electrometer. Male C57BL6/J mice were first deeply anesthetized with the mixture of medetomidine hydrochloride, midazolam, and butorfal tartrate. In the cochlea exposed by a ventrolateral approach, a small fenestra was made on the bony wall of the first cochlear turn. While the potential was continuously monitored, the micropipette was inserted through the hole. When the potential was elevated to ~+100 mV, the micropipette was held and negative pressure was applied to aspirate the fluid. Approximately 0.5 μL of the endolymph was collected from a cochlea. Aliquots from 8−10 cochleae were combined (~600 ng protein in total) and this endolymphatic sample was analyzed by SDS-PAGE together with a perilymphatic sample obtained from a cochlea by a glass micropipette. The gel was subjected to silver staining. Then, the fragments unique to the endolymphatic sample was extracted and underwent LC-MS/MS. Alternatively, the endolymphatic and perilymphatic samples were analyzed by immunoblotted with specific antibodies.
Results: From the LC-MS/MS data, we found that α2-macroglobulin, osteopontin, apolipoprotein D, apolipoprotein E, and apolipoprotein J/clusterin were much more abundant in the mouse endolymph than in the perilymph. Notably, apolipoprotein D and J exists in guinea pig endolymph. Immunoblot analyses with the specific antibodies ensured that these five proteins were enriched in the endolymph but negligibly present in the perilymph.
Conclusions: By our original procedure, we collected the endolymph from mouse cochleae with little contamination of the perilymph and identified the enrichment of α2-macroglobulin, osteopontin, and three different apolipoproteins. Because α2-macroglobulin interacts with apolipoprotein E and osteopontin associates with cholesterol metabolism, our result suggests that lipid metabolism contributes to maintenance of the endolymph. This work provides an approach to explore the pathophysiological mechanisms underlying deafness.

M53. HAP1 Regulates KCNQ4 Surface Expression and Channel Current

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Category: Inner Ear: Membranes and Fluids

Background: K+ recycling and homeostasis are necessary for auditory function, and voltage-gated channel subfamily Q member 4 (KCNQ4) plays a crucial role in K+ recycling. KCNQ4 possesses six transmembrane domains and a long cytoplasmic C-terminal tail. This C-terminal tail is known to interact with a number of proteins. Calmodulin (CaM) inhibits KCNQ4-mediated currents, for example, by binding to the C-terminal tail of KCNQ4. In this study, we aimed to identify and characterized KCNQ4 interactors.

Methods: We used yeast two-hybrid (Y2H) screening with a murine adult inner ear cDNA library as the prey and the C-terminal tail of KCNQ4 as the bait.

Results: Y2H identified HAP1 as a novel interactor of KCNQ4 in addition to CaM. GST pull-down assay with purified HAP1 interaction domain and KCNQ4 C-terminal tail confirmed the direct interaction of them. To examine the functional impact of the interaction, we performed electrophysiology and found that HAP1 overexpression decreased KCNQ4-mediated currents. Since it was previously reported that HAP1 was implicated in vesicular trafficking, we performed a surface biotinylation assay to determine whether HAP1 influences the KCNQ4 membrane expression. As a result, we determined that HAP1 overexpression decreased KCNQ4 surface expression. To further specify the interaction domain of KCNQ4, we made KCNQ4 constructs in which one of the four segments constituting KCNQ4 C-terminus was deleted. Co-immunoprecipitation and surface biotinylation assay of designed constructs revealed that the B segment of KCNQ4 is essential for the interaction between KCNQ4 and HAP1. Previous research indicated that the interaction site between CaM and KCNQ4 is also located in the B segment; therefore, we measured the surface expression KCNQ4 in response to HAP1 and CaM overexpression. When HAP1 was co-expressed with CaM, HAP1 could not decrease the surface expression of KCNQ4.

Conclusions: These findings suggest that HAP1 and CaM compete for the B segment of KCNQ4.

M54. The Tectorial Membrane Buffers Gentamicin in Vitro, in Situ, and in Vivo

George Burwood*[1], Pierre Hakizimana[2], Wajiha Zaidi[2], Rubing Xing[1], Teresa Wilson[1], Alfred Nuttall[1], Anders Fridberger[2]

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Category: Inner Ear: Membranes and Fluids

Background: The mechanical properties of the tectorial membrane (TM) are known to influence the longitudinal spread of acoustic energy, and the timing of mechanical feedback in the mammalian cochlea. The TM is also highly sensitive to its ionic environment. Prior studies have indicated that the TM has the capacity to buffer positive ions such as Ca2+ in a dynamic manner – TM Ca2+ can be depleted using moderate sound stimulation, implying that the buffered ion is taken up by the OHCs during mechanoelectrical transduction. Naturally, this observation leads to the hypothesis that other charged molecules – such as ototoxic aminoglycoside antibiotics – may also be selectively buffered by the TM in a manner consistent with its normal function. We also hypothesize whether the systemic administration of aminoglycosides results in a buildup of drug in the TM.

Methods: To test the first hypothesis, we isolated TMs from mice with and without the Y1870C mutation in the gene encoding alpha tectorin - a regulatory structural protein found in the TM - and bathed them in artificial endolymph. We performed fluorescence correlation spectroscopy, number and brightness and raster image correlation spectroscopy analysis on TMs in the presence of gentamicin Texas red (GTTR), a fluorescently conjugated aminoglycoside antibiotic.

To test the second hypothesis, adult guinea pigs were given an intraperitoneal injection of GTTR and their cochleae were harvested for in situ spectroscopy 30 minutes later.

Results: All spectroscopic analyses indicated that GTTR was taken up from the surrounding artificial endolymph and buffered in the TM. Tecta Y1870C/+ mutant TMs showed a difference in their buffering of GTTR.
We saw that GTTR collected in the TM following the intraperitoneal injection, confirming that GTTR gains entry to the scala media via the stria vascularis during aminoglycoside therapy, and showing that GTTR is buffered in the TM at concentrations higher than might be measured in the endolymph, and at concentrations highest in TM regions immediately adjacent to the mechanoelectrical transducer channels of the outer hair cells.

Conclusions: We found that GTTR is buffered by the TM, resulting in higher concentrations of aminoglycoside inside the extracellular matrix than in endolymph. The fact that the OHCs are closely attached to the TM may be another reason why OHCs are selectively vulnerable to aminoglycosides, and knowledge of the TM's role in aminoglycoside dynamics may provide an opportunity to lessen the toxicity burden that OHCs experience during aminoglycoside therapy.

M55. Aberrant Endocochlear Potential is Associated With Early-Onset but Not Late-Onset TMPRSS3 Hearing Loss

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Category: Inner Ear: Membranes and Fluids
Background: Pathogenic variants in the gene TMPRSS3 are a common causes of genetic hearing loss in humans and can lead to early onset severe to profound sensorineural hearing loss (DFNB10) or late-onset progressive sensorineural hearing loss (DFNB8). The mechanism by which mutations in the TMPRSS3 gene cause hearing loss is not known. A previously developed DFNB10 mouse model, Tmprss3-/-, demonstrates rapid cochlear inner and outer hair cell death at P10-P12 prior to the onset of hearing. We hypothesized that this massive cell death was due to abnormal endocochlear potential (EP) given that this time period corresponds to a rapid rise of EP to physiologic levels required for hearing in wildtype mice.
Methods: We performed cochlear explant cultures to assess the effects of intracochlear environment on hair cell survival in Tmprss3-/- mice. We created two new mouse lines to further evaluate the importance of EP to pathophysiology of Tmprss3 hearing loss: (1) a two-strain model, Tmprss3-/-; MitfMi-wh/+, which lacks any development of EP, and (2) a mouse model for the less severe form of human TMPRSS3 hearing loss (DFNB8), using CRISPR/Cas9, Tmprss3A447T/Y260X. We performed direct measurement of EP and fully characterized hearing and vestibular function of these mouse models. To pharmacologically reduce the EP, we injected furosemide systemically and assessed hair cell survival.
Results: Hair cells in cochlear explants from Tmprss3-/- mice survived in culture at a similar rate to WT control explants, implicating the intracochlear environment in hair cell death. Direct measurement of EP in Tmprss3-/- mice revealed an early supraphysiologic rise in EP prior to the onset of hair cell death followed by normalization of EP after cell death. The novel two-strain model Tmprss3-/-; MitfMi-wh/+ lacked development of EP and showed survival of inner and outer hair cells, however the mice, as expected, demonstrated hearing loss. The novel DFNB8 mouse model Tmprss3A447T/- showed normal EP but demonstrate late-onset hearing loss. Systemic administration of the loop diuretic furosemide was successful at reducing hair cell death in Tmprss3-/- mice.
Conclusions: Early-onset TMPRSS3 hearing loss (DFNB10) was associated with supraphysiologic early EP and cochlear hair cell death. Reduction of EP in vivo rescued hair cells but did not restore auditory function, as evidenced by our novel two-strain mouse model and lowering of EP using furosemide. The late-onset TMPRSS3 (DFNB8) mouse model developed here suggests a different pathologic mechanism for late-onset TMPRSS3 hearing loss distinct from altered EP.

M56. Computational Insights Into the Generation of Endocochlear Potential: Role of Potassium Channels and Tight Junctions

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Category: Inner Ear: Membranes and Fluids

Background: The endocochlear potential (EP) is an electric potential (~80 mV) that provides the driving force for the flow of K+ ions through hair cell mechanotransduction channels. Previous research has highlighted the role of the inward rectifier K+ channel, Kir4.1, present in the intermediate cells of the stria vascularis, in the generation of the EP. Early studies reported the EP is abolished in Kir4.1 knockout mice but later studies have called this result into question. The intrastrial space between the intermediate cells and the marginal cells that secrete K+ into the endolymph is electrically isolated by tight junctions composed of Claudin-11 beneath the basal cells of the stria vascularis. Claudin-11-null mice retain a high K+ concentration in the endolymph, but their EP is markedly reduced to ~30 mV, rendering the mice effectively deaf. This observation has not been mechanistically explained. The aim of this study is to understand the mechanisms underlying the generation of the EP and how it is affected by molecular perturbations. To do this, we build upon our previously developed computational model of cochlear ion transport and homeostasis.

Methods: The model calculates ion concentrations and potentials within seven cochlear compartments: the endolymph, hair cells, perilymph, fibrocytes, marginal cells, intrastrial space, and Na+ absorbing epithelia. This is mathematically described as a system of differential-algebraic equations that are solved at steady state. This approach enables us to modify and/or close channels and transporters to simulate the effects of ion channel blockade and gene knockout on the ion concentrations and voltages in all the cochlear compartments. We extended the model to incorporate and vary paracellular fluxes across the different tight junction barriers: endolymph-intrastrial space, endolymph-perilymph, and perilymph-intrastrial space.

Results: Our simulations show that the EP is maintained if the Kir4.1 channel is closed. However, if another voltage-dependent outward K+ current reported in intermediate cells (Kv) is concurrently closed, the EP is abolished. The experimentally observed decrease in the EP in Claudin-11-null mice was not accounted for by disruption of the intrastrial space-basal cell tight junction barrier (i.e., perilymph-intrastrial space). However, the observations were consistent with an increase in tight junction permeability between the endolymph and perilymph.

Conclusions: Our findings indicate that both Kir4.1 and Kv channels present in the intermediate cells collectively ensure the preservation of the endocochlear potential, suggesting a potential evolutionary safeguard mechanism. The extended model also predicts the loss of EP seen in Claudin-11-null mice occurs primarily because of the increased permeability of the endolymph-perilymph barrier. This prediction is consistent with immunohistochemistry results that demonstrate Claudin-11 expression between the epithelial cells in the spiral prominence that face the endolymph below the stria vascularis.

M57. Open Board

M58. AAV Variant ShH10 for Selective and Efficient Gene Delivery to Mammalian Inner Ear Supporting Cells

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Category: Gene Therapy

Background: Recent studies have found adeno-associated viruses (AAV) as promising vectors for targeting inner ear cell types. Some AAVs, such as Anc80L65 and PHP.eB, show excellent transduction efficiency and tropism towards hair cells and neurons, respectively. Others, such as AAV-KP1, AAV-ie, and its most recently studied variant, AAV-ie-K558R, transduce a broad variety of cells in the inner ear, and thus lack selectivity. Here we investigate ShH10, an AAV variant with high efficiency and tropism towards mammalian supporting cells, which are the putative target for hair cell regeneration and the cell type affected in many cases of congenital deafness.

Methods: Each vector of interest (ShH10, Anc80L65, DJ, AAV-ie-K558R and AAV2.7m8) was delivered to the cochleae of P1 wildtype mice through posterior semi-circular canal (PSCC) injections. The ubiquitous CAG promoter was used to drive reporter gene expression. Seventy-two hours post-operatively, the animals were euthanized and their cochleae and utricles were dissected and immunostained to quantitatively assess the prevalence and localization of reporter gene expression.

Results: Our analysis revealed that in the neonatal cochlea and utricle, the AAV serotype ShH10 transduces supporting cells with markedly higher selectivity and efficiency compared to the other tested AAVs. We observed an apex-to-base gradient in the rate of supporting cell transduction, with the greatest transduction occurring at the apex.
Conclusions: Our data shows that ShH10 is a promising novel AAV serotype for supporting cell-specific gene delivery in the neonatal mammalian inner ear.

M59. Peripheral Gene Augmentation Therapy Restores Hearing and Central Auditory Processing in a Preclinical Model of DFNB9 Auditory Synaptopathy

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Category: Gene Therapy
Background: Sound encoding relies on synaptic transmission between cochlear inner hair cells (IHC) and afferent fibers of the primary auditory neurons. This process requires otoferlin, a Ca2+ -binding transmembrane protein featuring six C2 (C2A-F) domains and acting as a calcium sensor in IHC for exocytosis. Indeed, upon Ca2+ binding otoferlin triggers the final steps of synaptic exocytosis by ensuring rapid release of vesicular neurotransmitter at IHC ribbon synapses. Several OTOF mutations were found to cause severe to profound deafness in human known as DFNB9. One particular mutation involving a deletion of a glutamic acid at position 1804 in the C2F domain of otoferlin, has been found to cause a temperature-sensitive (TS) auditory synaptopathy in patients. The patients harboring this mutation become deaf when the body temperature exceeds 38 °C but, recover their hearing several days after the end of the fever period.
Methods: We have generated and characterized a knock-in mouse model Otof TS/TS expressing the aforementioned mutated otoferlin. Unexpectedly, through Auditory Brainstem response (ABR) recordings, immunohistofluorescence analyzes, and IHC membrane capacitance measurements, we found that the Otof TS/TS mice suffer from profound deafness, regardless of their body temperature. This deafness results from both low expression levels and abnormal subcellular distribution of the otoferlin protein, which is found outside of the presynaptic active zone, leading to a failure in IHC Ca2+-dependent synaptic exocytosis. The objective of this study was to determine whether gene augmentation therapy in Otof TS/TS mutant mice can counteract the defects caused by abnormal otoferlin TS expression and restore normal auditory function.
Results: We showed that a single unilateral injection of dual AAV-vector in Otof TS/TS mouse at mature stages restores normal otoferlin production as well as its cellular basolateral distribution. As a result, IHC Ca2+-dependent exocytosis was rescued to wild level in Otof TS/TS mice leading to a sustained restoration of normal hearing in these mice. Furthermore, we were able to show using a Go/NoGo auditory behavior task that these injected mice were able to discriminate tones with different frequencies indicating normal central auditory processing and hearing performance.
Conclusions: In conclusion, we report here that peripheral gene therapy, even when administered well after hearing onset in mice, can successfully restore both hearing and central auditory processing in Otof TS/TS mice that were otherwise profoundly deaf.

M60. In Vivo Cellular Tropism of Adeno-Associated Viruses (AAVs) Within the Inner Ear: From Embryonic to Adult Stages

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Category: Gene Therapy

Background: Adeno-associated virus (AAV)-based gene therapy offers promising treatment opportunities for inner ear diseases, including deafness and balance disorders, due to their various serotypes allowing a large range of tropisms. However, the late onset of hearing in mice (at P12), which is the commonly used model in preclinical research, as compared to humans occurring during in utero period, suggests that any relevant gene therapy in mice should ideally be conducted at a mature stage. However, cellular tropism in the cochlea of AAV varies over time and often declines as the inner ear matures. Therefore, conducting a comparative analysis of AAV cellular tropism in the cochlea as it matures is critical for identifying the most efficient serotype(s) during the developmental stage of interest.

This study precisely aims to compare the transduction rate and target specificity of various AAV serotypes known for their inner ear sensory cells transduction efficiency when administered at different developmental stages.

Methods: Accordingly, two naturally occurring AAV serotypes, AAV2 and AAV8, and two engineered ones, AAV9-PHP.eB and AAV-Anc80, expressing GFP under the control of a ubiquitous promoter were delivered into the inner ear of wild-type mice at three developmental time points: embryonic, neonatal, and adult stage.

Results: At the embryonic stage, all serotypes except AAV9-PHP.eB, demonstrated a higher efficiency in transducing OHCs compared to IHCs, revealing a baso-apical gradient, with higher number of transduced cells in the basal region. The highest transduction rate of cochlear sensory cells was achieved using Anc80L65 serotype infecting both IHCs and OHCs, with an average of 41±8.2% and 62±7.7%, respectively. The transduction rate of vestibular hair cells (VHCs) was similar for all serotypes except AAV2, which showed a much lower rate. At neonatal stage, all four vectors were effective in transducing inner ear sensory cells, and the AAV9-PHP.eB serotype appeared to be the most efficient for cochlear hair cells, with an average of 85±6% and 85±4.9% for IHCs and OHCs, respectively. In contrast to the embryonic stage, we observed an apico-basal gradient, with higher number of transduced cells in the basal region for all serotypes except AAV2. Finally, at the mature stage, the transduction rate for all serotypes was significantly higher in IHCs compared to OHCs, regardless of the cochlear region. AAV9-PHP.eB and Anc80L65 achieved the maximum IHC transduction rate (almost 100%). For OHC transduction rate, AAV9-PHP.eB and AAV2 were the most effective serotypes, with 26±4.7% and 29±5.5% of OHC transduced. The percentage of transduced VHCs was reduced compared to neonatal stage, with great variability across all serotypes.

Conclusions: We concluded that the transduction efficiency and targeting of AAV serotypes is developmental stage-dependent, which may impact the favorable therapeutic outcomes of gene therapy.

M61. Developmental Impact on AAV-DJ-GFP Expression Profile

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Category: Gene Therapy

Background: Viral delivery to the cochlea is an important technique to develop and optimize because of its dual use as a scientific tool for investigating molecular components of function and for translational work related to gene therapy. Here we investigate how sensitivity to viral infection varies based on developmental state and based on frequency location (tonotopically). We measure efficacy and expression levels of GFP in hair cells and supporting cells in the mouse cochlea when injected via semicircular canal in neonatal and adult mouse

Methods: AAV-DJ CMV-hrGFP was injected through posterior semicircular canal of the left ear in neonate (P1) or adult (P35) c57BL6 animal (of either sex). The right ear was not operated on. Auditory Brainstem responses (ABR)s and distortion product otoacoustic emissions (DPOAEs) were obtained prior to injection for adult mice and again after 5 weeks post injection for both adult and neonatal mice. After the 5-week ABR and DPOAE mice were sacrificed and the cochleae were collected (separated tonotopically), stained with phalloidin and DAPI, mounted and imaged using a confocal microscope. The imaging settings were maintained the same for all the samples. Images were analyzed by drawing a region of interest (1.5 um diameter) on the interested cell types and measuring their intensities on a z-stack plotting z-step against intensity. The background rms signal was measured and cells that were mean+3SD above the background

were considered to be transfected. We then compared the GFP intensities and transfection percentages of Inner (IHCs) and Outer Hair Cells (OHCs), Pillar and Deiter cells in three tonotopic regions (apex, middle and base) between adult and neonates. Analysis: two sample t-test.
Results: No differences were observed in ABR or DPOAE between groups.
1: Adult injections resulted in higher transfection rates and expression levels in IHCs at each cochlear location.

2: Adult injections resulted in lower intensity but similar transfection percentages in OHCs.

3: Adult injections resulted in lower transfection rates in the middle turn but higher expression levels in the apex and base for Pillar cells

4: Adult injections resulted in lower transfection rates and lower expression levels across all regions for Deiter cells.

Conclusions: 1. Transduction in the IHC suggests that IHC have a high capacity for protein expression that does not get regulated or saturated over time.
2. IHC expression difference between neonate and adult may be developmental or may be a difference in infection efficacy due to access of the virus to the cells.

3. The reduced transduction rates in supporting cells in the adult injection scenario, suggest that neonatal animals have more active synthetic capacity, are less able to prevent translation of nonfunctional proteins or the virus has easier access and infection capabilities in neonatal systems.

M62. In Vivo Characterization of Supporting Cell Responses to Hair Cell Damage in the Zebrafish

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Category: Inner Ear: Damage and Protection
Background: Hair cells (HCs) carry a high metabolic load, making them both exquisitely sensitive to sound and highly susceptible to damage. In mammals, where hair cells do not meaningfully regenerate, this damage can result in permanent auditory and vestibular dysfunction. Understanding how hair cell sensory systems respond to damage is essential for developing new strategies to prevent hair cell loss. In explants from the mammalian cochlea, HC damage triggers waves of calcium activity in surrounding supporting cells (SCs). Importantly, this activity can regulate HC death. However, a major limitation of this previous work is that all experiments were performed ex vivo. Here, we leverage the power of the zebrafish lateral line to examine SC responses to HC damage in vivo.
Methods: HCs in the lateral line are organized into clusters, called neuromasts, with each HC surrounded by multiple SCs. Neuromasts are located superficially along the body of the fish, making them optically and physically accessible in an intact, living fish. For our studies, we utilize transgenic zebrafish expressing the calcium indicator GCaMP6s specifically in SCs. Using a laser scanning confocal microscope, we can induce laser damage to a single HC while simultaneously imaging changes in SC intracellular calcium across the entire neuromast. This enables us to characterize how SCs respond to HC damage both locally, near the damaged HC, and globally within the neuromast.
Results: Preliminary work suggests that SCs near the site of HC damage respond with robust calcium transients. Less frequently, extended calcium waves propagate through the SC network in response to HC damage. Ongoing experiments seek to the elucidate the molecular mechanisms that drive the initiation and propagation of SC damage responses, with a particular focus on purinergic signaling and SC gap junctions. Ultimately, we hope to explore the functional significance of SC damage responses by blocking these responses and asking how this alters both HC turnover and changes to the functional circuitry post-damage.
Conclusions: Our work characterizes a novel in vivo model for studying how SCs sense and respond to HC damage, and how these SC responses impact the function of hair cell sensory systems. This work could inspire new therapeutic approaches to preventing HC damage.

M63. Open Board

M64. Development of the Auditory Pathway Depends on the Strength of the Transient Efferent Synapse and is Altered by Early Noise Exposure

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Category: Inner Ear: Damage and Protection

Background: In the developing auditory system, spontaneous activity generated in the cochleae propagates into the central nervous system to promote circuit formation. Before the onset of hearing, inner hair cells (IHC) are innervated by auditory nerve fibers and transiently by neurons of the medial olivocochlear (MOC) system. This synapse is cholinergic and mediated by the nicotinic acetylcholine α9α10 receptor (nAChR). It has been proposed that the MOC system may be a modulator of this spontaneous activity. In addition, it has an important role in the protection from noise-induced hearing loss in adult rodents. Here, we analyze the effect of the strength of the cholinergic component of the MOC synapse on the auditory system development and the consequences of exposure to loud noise at this early stage of development.

Methods: We used an α9 nAChR subunit knock-out (α9KO), which lacks cholinergic transmission between efferent neurons and hair cells; and a gain-of-function knock-in (α9KI) mice carrying an α9 point mutation that leads to enhanced cholinergic activity. Animals at P15 were exposed to 1-16 kHz noise at 100 dB SPL for 1 hour. We measured ABRs and DPOEAs before and after exposure. Cochlear sections were immunolabeled to assess cochlear synapses.

Results: We tracked the onset of ABRs and DPOAEs in mice with different levels of α9α10 nAChR activity. ABR onset was earlier in α9KI mice compared to WT. In contrast, ABR onset in α9KO ears was delayed, suggesting that cochlear maturation is slowed in the absence of pre-hearing efferent modulation. DPOAEs responses showed the same trend only at 11.33 kHz. At P16, ABR wave 1 amplitudes were not different from amplitudes at P21. However, at P16 waves 2 and 3 are lower than their amplitudes at P21 in all three genotypes, suggesting that the maturation of the central connections begins in the periphery and continues after the onset of hearing. Finally, we exposed mice at P15 and tested the animals 1, 7 and 60 days post-exposure. 1 day after, no changes in thresholds were observed in the three genotypes. 7 days later, thresholds were elevated in WT and α9KO which partially recovered after 60 days. In contrast, α9KI mice showed no changes in thresholds after acoustic trauma at any stage. ABR wave 1 amplitudes were reduced in WT and α9KO accompanied by a decrease in the number of afferent synapses per IHC. α9KI mice showed no changes in ABR W1 amplitudes at any point after exposure.

Conclusions: Auditory sensitivity in mice with enhanced or null MOC activity is altered at hearing onset. Exposure to loud noise at this early stage of development led to permanent changes in auditory thresholds in WT and α9KO mice, while no changes were observed in those with enhanced MOC function.

M65. Lead Exposure Induces Synaptopathy and Alters the Abundance of Synaptosomal Proteins in the Cochlea

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Category: Inner Ear: Damage and Protection

Background: Environmental exposure to lead can cause serious health effects such as developmental neurotoxicity in infants, cognitive impairment in children, and cardiovascular and nephrotoxic effects in adults. Hearing loss is one among the toxic effects induced by exposure to lead. However, the mechanism by which lead exposure causes hearing deficits is yet to be fully understood. Our recent study demonstrated that exposure to lead causes oxidative stress in the cochlea, disrupts ribbon synapses, and results in hearing loss. This study further investigates the underlying mechanism by evaluating the changes in the abundance of cochlear synaptosomal proteins that accompany lead-induced cochlear synaptopathy and hearing loss in mice.

Methods: CBA/J mice were given lead acetate (2 mmol/L) in drinking water for 28 days. The blood lead level was analyzed using inductively coupled plasma mass spectrometry (ICP-MS). Hearing threshold shift was evaluated by recording Auditory Brainstem Responses (ABR). Outer hair cell 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 wave-I amplitude and latency and the functional synapses in the cochlea were estimated by immunostaining with anti-GluR2 and -CtBP2. The abundance of cochlear synaptosomal proteins was analyzed using mass spectrophotometry.
Results: Lead exposure significantly increased the hearing thresholds, particularly at the higher frequencies (16, 24, 32 kHz; p less than 0.05; n=12), in both male and female mice, but it did not affect the activity of outer hair cells or induce hair cell loss. However, lead exposure decreased wave-I amplitude (8, 24, 32 kHz) suggesting lead-induced cochlear synaptopathy. In agreement, colocalization of pre- and post-synaptic markers indicated that lead exposure decreased the number of functional synapses (p less than 0.01; n=3) in the basal turn of the cochlea. Proteomics analysis indicated that lead exposure increased the abundance of 352 synaptic proteins and decreased the abundance of 394 synaptic proteins (p less than 0.05; n=4) in the cochlea.
Conclusions: These results suggest that outer hair cells are not the primary target in lead-induced ototoxicity. Cochlear synaptopathy caused by lead exposure is more pronounced in the basal turn of the cochlea. Lead-induced cochlear synaptopathy is accompanied by changes in the abundance of many synaptosomal proteins. The proteomics profile of the cochlear synaptosomes provides novel insights about underlying mechanisms and identifies potential targets for intervention.

M66. Repurposing Therapies Against Cisplatin-Induced Hearing Loss

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Category: Inner Ear: Damage and Protection
Background: Cisplatin, widely used to treat pediatric and adult cancer, has a high prevalence of ototoxicity, resulting in permanent hearing loss in over half of treated patients. There is a clear, demonstrated need for therapies to mitigate cisplatin-induced hearing loss (CIHL). In order to expedite the identification of potential therapies to treat CIHL, this study sought to perform high throughput screening, using a zebrafish model for cisplatin toxicity, of FDA-approved drugs currently used to treat long-term conditions.
Methods: Tg(pou4f3:GAP-GFP) zebrafish, at 5-6 days post fertilization (dpf), were randomized into 6 animals/group. For each treatment, six 5-6 dpf fish were pre-incubated for 1 hr with the drug at various concentrations between 0.5 nM to 50 µM. The fish were fixed after a 6-hour incubation with cisplatin (300-400 µM) and recovery. Neuromast hair cells were immunolabeled using otoferlin (anti-HCS-1). Three rostral neuromasts were inspected per fish by manually counting and were normalized to the control and cisplatin values to produce the relative percentage of hair cell protection. Nifedipine's effect was further classified using MitoTracker Deep Red FM, MitoTracker Red CMxROS 500nM, and FM3-1 dyes.
Results: Atenolol at 10 uM and nifedipine from 0.08 uM to 2 uM provided significant protection against cisplatin (P less than 0.05). Nifedipine was chosen to be further classified. Using MitoTracker dyes, nifedipine was not shown to prevent the hyperpolarization of mitochondrial membrane potential caused by cisplatin, suggesting it provides a protective effect in another manner. Additionally, nifedipine did not prevent the uptake of FM1-43 dye, suggesting that nifedipine does not prevent cisplatin entrance into hair cells through mechanotransduction channels. Candesartan, gemfibrozil, and nicotinamide showed no significant protection against cisplatin.
Conclusions: Out of five FDA-approved drugs, atenolol, and nifedipine were the only ones to show significant protection against cisplatin-induced hair cell loss in zebrafish. They showed potential for future investigation in more relevant hair cell models (i.e., mice) to investigate their potential for clinical use to prevent cisplatin-induced hearing loss. Since nifedipine did not significantly reduce the hyperpolarization state of mitochondrial membrane potential due to cisplatin treatment nor prevent the uptake of cisplatin through mechanotransduction channels into hair cells, further investigation should be done to determine the mechanism of nifedipine protection against cisplatin.

M67. Genetic Polymorphisms in Human Fractalkine Receptor CX3CR1 Expressed by Macrophages is Associated With Weakened Immune Response and Impaired Recovery of Loss of Hearing and Ribbon Synapses After Noise-Induced Cochlear Synaptopathy: Study in a Humanized Mouse Model

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Category: Inner Ear: Damage and Protection

Background: Fractalkine receptors, CX3CR1 expressed on macrophages play a significant role in inner hair cell ribbon synaptic repair and spiral ganglion neuron survival after a synaptopathic noise trauma (Kaur et al., 2019). Notably, 20-30% of Caucasians carries single nucleotide polymorphisms in CX3CR1 gene known as CX3CR1I249/M280 whereas most individuals carry the common wildtype CX3CR1V249/T280 allele. These polymorphisms play a detrimental role in pathogenesis of several neurodegenerative disorders like multiple sclerosis, age-related macular degeneration, and diabetic retinopathy. However, the impact of these polymorphisms on noise-induced hearing loss and cochlear damage is largely unknown. Thus, the objective of the present study was to examine the association of human CX3CR1I249/M280 (hCX3CR1I249/M280) variant on normal hearing as well as after noise-induced hearing loss and cochlear synaptopathy.

Methods: Young (5 weeks of age) CX3CR1 wild type, CX3CR1 knockout and hCX3CR1I249/M280 mice of both sexes were used. Following pre-noise hearing function test, mice of all three genotypes were exposed for 2 hours to an octave band (8-16 kHz) noise of 93 dB SPL. Mice of same genotypes exposed to ambient room noise served as controls. Auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE) were measured at 1- and 15- days after noise trauma following which mice were euthanized and temporal bones were processed for hair cell, synapse, neuron, and macrophages immunolabeling and confocal imaging. Data was compared among the three genotypes at baseline and at different time points after noise exposure.

Results: By 2 months of age, unexposed hCX3CR1I249/M280 mice showed early elevation in hearing thresholds at higher frequencies when compared to age-matched CX3CR1-knockout and wildtype mice. At 2 weeks after synaptopathic noise trauma, hCX3CR1I249/M280 and CX3CR1-knockout mice failed to show recovery of elevated hearing thresholds at stimulus frequencies of 16 kHz and above when compared to CX3CR1 wildtype mice where thresholds nearly recovered to baseline levels. Also, ABR peak I amplitude at 16 and 32 kHz were reduced by 54 % and 94% in hCX3CR1I249/M280 mice respectively that was associated with a significant loss of inner hair cell synapses in middle (21 %) and basal (32 %) cochlear regions, whereas synaptic density and peak I amplitude were restored in exposed wild type mice. Last, CX3CR1 wildtype mice exhibited an increase in macrophage density in the spiral ganglion after noise trauma however, such increase was not observed in exposed CX3CR1-knockout and hCX3CR1I249/M280 mice.

Conclusions: These findings reveal a novel immune-related polymorphism in human CX3CR1 gene that may contribute to an increased risk for sensorineural hearing loss and cochlear synaptopathy due to noise trauma or normal aging.

M68. Variants in the vWFA2 Domain of the COCH Gene Cause Proteinopathy

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Category: Inner Ear: Damage and Protection

Background: COCH (coagulation factor C homology) mutations cause both autosomal dominant (DFNA9) and autosomal recessive (DFNB110) nonsyndromic hearing loss. The COCH gene encodes cochlin, which contains an N-terminal LCCL domain and two copies of Von Willebrand factor A (vWFA) domains. When bacteria invade the inner ear, cochlin is cleaved by aggrecanase-1, and the cleaved LCCL domain is secreted to the perilymph space, where it stimulates bacterial aggregation and phagocyte recruitment in the scala tympani. However, neither the function of the C-terminal vWFA domains nor the pathogenicity of mutations in these domains are well understood.

Methods: We performed whole exom sequencing (WES) from patients with hearing loss.

Results: We identified six variants in seven families with DFNA9 and four of them (p.A76T, p.F230L, p.G403C, and p.G447D) were not previously reported. Our functional study revealed that mutations in the vWFA2 domain significantly decreased cochlin cleavage. This result, however, does adequately explain the autosomal dominant inheritance of DFNA9 caused by these variants. To address this issue, we generated a Coch p.G449D knock-in (KI) mouse model.
Conclusions: The KI mice exhibited moderate-to-severe hearing loss at 6 months of age. p.G449D cochlin accumulated in the inner ear as multimers. Compared to wild-type control, aggregated p.G449D cochlin resulted in impaired autophagy flux and ER stress. These findings suggest that mutations in vWFA2 domain of cochlin affect the cellular stress response and result in proteinopathy.

M69. AMPA Receptor Plasticity and Proteomics-Based Identification of Biological Pathways of Macrophage-Dependent Restored Inner Hair Cell Ribbon Synapses Following Noise-Induced Cochlear Synaptopathy

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Category: Inner Ear: Damage and Protection
Background: Inner hair cell (IHC) ribbon synapses are pivotal in glutamatergic neurotransmission, which underlies sound encoding. These ribbon synapses consist of presynaptic ribbons in IHC and postsynaptic AMPA receptors on afferent nerve terminals, which are composed of different subunits: GluA2, GluA3, and GluA4. The GluA2/GluA4 subunit governs calcium entry dynamics. Overexposure to moderate noise is correlated with synaptic damage. Eventually, the noise-damaged synapses are partially repaired spontaneously. Recently we have demonstrated the essential role of macrophages in promoting synaptic repair following noise-induced cochlear synaptopathy (NICS). However, the precise AMPA receptor stoichiometry of those repaired synapses and molecular mechanisms by which macrophages facilitate synaptic repair remains unexplored. Thus, this study aims to identify the key biological pathways underlying macrophage-dependent synaptic repair and the stoichiometry of the reformed AMPARs after NICS.
Methods: Young adults CX3CR1GFP/+ heterozygous mice were divided into two groups: no noise exposure (NNE) and noise exposure (NE). Mice in each group were fed with either control chow (macrophages present) or PLX5622 chow (macrophages absent) or initially fed with PLX5622 chow followed by its withdrawal and replacement with control chow (macrophages repopulate). The NNE group was exposed to ambient room noise, while the NE groups were exposed to an octave band noise (8-16 kHz) at 93 dB SPL for 2 hours. Subsets of mice from each group were euthanized at 1- and 7-day post-noise exposure (DPNE) and cochlear perilymph and protein lysates were collected for proteomics. Remaining mice from each group were euthanized at 30DPNE, and dissected temporal bones were subjected for immunolabeling for AMPARs (GluA2 and GluA4) and ribbons (CtBP2). Fluorescence intensity and volume for each marker per synapse were analyzed using Imaris and absolute intensity was used for computing GluA4/GluA2 ratio.
Results: At each ribbon synapse an increased GluA4 puncta intensity was observed in exposed control and PLX5622 to control groups relative to GluA2 puncta intensity. Additionally, GluA4/GluA2 intensity ratio was more elevated in exposed control (1.3) and PLX5622 to control (1.3) than PLX5622 (1.1) or unexposed control group (1). CtBP2 intensity in control and PLX5622 to control group was comparable to unexposed control group, but it was significantly decreased in PLX5622 group. Preliminary proteomics analysis of perilymph showed major upregulation in arginine metabolic pathway with increase in ornithine aminotransferase enzyme levels. Similarly, protein lysates, biological processes, including monoamine receptor signaling, synaptogenesis and axon guidance, THOP-1-mediated neuroprotection, endocannabinoid signaling, phagosome maturation, and ROS in macrophages were significantly altered at 7DPNE in control and PLX5622 to control groups where synapses are repaired.
Conclusions: Data reveal that macrophage-mediated repaired synapses manifest an altered AMPA receptor stoichiometry, suggestive of immature synapses. Proteomics-based identification of key proteins and associated biological pathways will allow investigation of the mechanisms that govern macrophage-mediated synaptic repair in NICS.

M70. Outer Hair Cell Motor Protein Prestin is Not a Reliable Biomarker for Mouse Cochlear Damage

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Category: Inner Ear: Damage and Protection

Background: Outer hair cell (OHC) motor protein prestin is the molecular basis for OHC electromotility, which is essential to producing high sensitivity and sharp frequency selectivity for our hearing (Zheng et al., 2000). OHCs are also one of the most vulnerable components in the cochlea and are extremely sensitive to and often damaged by different insults. Since prestin is the most abundant membrane protein in OHCs, several reports showed that prestin was detected in the bloodstream of humans, rats, guinea pigs, and mice using a sandwich enzyme-linked immunosorbent assay (ELISA). These data suggested that prestin could be a serological biomarker for idiopathic sudden sensorineural hearing loss and cochlear damage caused by noise exposure and ototoxic drugs. However, the reported data are inconsistent. Prestin is also expressed in the heart, raising the question of whether the prestin detected in the bloodstream is derived from OHCs. In this study, we measured prestin quantities in the bloodstream using ELISA kits obtained from different companies. Wildtype (WT) mice were exposed to different ototoxic treatments, including noise exposure and HPβCD, which rapidly kills OHCs and releases prestin into extracellular space. Prestin-knockout (KO) mice (Liberman et al., 2002) were used as a negative control, while WT cochlear homogenates were used as a positive control.

Methods: WT and prestin-KO mice were injected with NaCl or HPβCD. WT mice were also exposed to noise to create PTS (Permanent Threshold Shift). The serums and cochleae from WT and prestin-KO mice were collected at different time points after the injection or noise exposure. Prestin concentrations in the bloodstream and cochleae were measured using mouse prestin ELISA kits. The expression of prestin in cochleae was verified by immunofluorescence using anti-prestin antibodies targeting both the N-terminal and C-terminal of prestin.

Results: Prestin was expressed in WT-OHCs but not in prestin-KO-OHCs. Single high-dose administration of HPβCD resulted in greater than 60% OHC death in WT mice, but OHC loss was not observed in WT control mice treated with saline. Some Elisa kits detected prestin signals in WT cochlear homogenate samples but not in the serum samples despite a large amount of OHCs lost in cochleae after HPβCD injection. Other Elisa kits showed that prestin concentrations in the bloodstream and cochlear homogenate were similar between WT and prestin-KO mice regardless of whether mice were treated with HPβCD or not. In addition, the optical densities of samples, which correlate to prestin quantities, were significantly influenced by the severities of hemolysis in the samples.

Conclusions: Prestin concentrations in the samples are significantly affected by the quality of the collected serum. Prestin from OHCs is a not sensitive and reliable serological biomarker to detect cochlear damage. (Work supported by the Knowles Leadership Fund and NIH R56DC020542 to JZ and R01DC019434-01 to XT).

M71. Protective Effects of Y-27632 on Cisplatin-Induced Ototoxicity in Zebrafish

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Category: Inner Ear: Damage and Protection

Background: Cisplatin is a widely used chemotherapeutic agent for treating solid malignancies, but its clinical utility is limited by the development of irreversible sensorineural hearing loss. Cisplatin-induced hearing loss results from the apoptosis and necrosis of auditory hair cells, particularly affecting outer hair cells (OHCs) in the organ of Corti. Currently, there are no approved drugs to prevent cisplatin ototoxicity, highlighting the urgent need for preventive interventions. Rho-associated coiled-coil containing protein kinase (ROCK) is a serine-threonine protein kinase involved in various cellular processes, including apoptosis regulation. Its role in cell survival has yielded conflicting results in different cell types.

Methods: We utilized a transgenic zebrafish model (Brn3C: EGFP) with naturally occurring green neuromasts for our study. Zebrafish larvae were exposed to cisplatin alone or cisplatin in combination with various concentrations of Y-27632, a potent ROCK inhibitor. Hair cell counts, apoptosis assessments using TUNEL

assay, and behavioral analyses (startle response and rheotaxis) were conducted to evaluate the protective effects of Y-27632 against cisplatin-induced ototoxicity.
Results: Cisplatin treatment significantly reduced the number of hair cells in neuromasts, induced apoptosis, and impaired zebrafish larval behaviors. Y-27632 demonstrated a dose-dependent protective effect against cisplatin-induced hair cell loss, apoptosis, and alterations in startle response latency and distance moved. Although the protective effects of Y-27632 on rheotaxis were less pronounced, a trend toward preservation of this behavior was observed.
Conclusions: Our findings suggest that Y-27632, as a ROCK inhibitor, may mitigate cisplatin-induced hair cell loss and associated ototoxicity in zebrafish. Further investigations are warranted to elucidate the mechanisms underlying these protective effects and to explore the translational potential of ROCK inhibitors in preventing cisplatin-induced hearing loss in clinical settings.

M72. The Impact of Estrogen on Lateral Line Hair Cells in Adult Zebrafish

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Category: Inner Ear: Damage and Protection
Background: Estrogen is a steroid hormone commonly used as a signaling molecule in vertebrates. Estrogen has also been observed to modulate auditory sensitivity in several species. For example, in plainfin midshipman fish, estrogen fluctuations drive seasonal auditory plasticity associated with reproductive success. While an excellent model to understand the mechanisms underlying estrogen-dependent auditory plasticity, midshipman fish are challenging to obtain and maintain in the lab. By contrast, zebrafish are an excellent model for studies of hormonal auditory plasticity, in part because they have an external sensory system, called the lateral line, which contains clusters of hair cells. Our prior data suggests that estrogen increases hair cell number in larval zebrafish lateral line. Here, we hypothesize that estrogen will increase cell proliferation and hair cell addition in adult zebrafish and that estrogen sensitivity will differ by sex.
Methods: We exposed adult male and female zebrafish to estrogen for seven days using a bath incubation paradigm. We used BrdU to quantify cell proliferation, a TUNEL assay to quantify cell death, and labeled lateral line hair cells with DAPI, which serves as a specific marker of lateral line hair cells when used as a vital dye.
Results: Preliminary analysis suggests that estrogen increased cell proliferation in the adult zebrafish lateral line. We are currently investigating sex effects and the degree to which estrogen-induced cell proliferation is specific for supporting cell populations, as opposed to a more general effect of estrogen on all mitotic cells.
Conclusions: Our preliminary results demonstrate that estrogen alters the rate of hair cell turnover in the adult zebrafish lateral line, consistent with experiments in both larval fish and in other fishes. Future experiments will examine the specific mechanisms of estrogen-mediated hair cell addition and further explore age and sex-specific effects.

M73. Real-Time Measurement of Basilar Membrane Motion During Blast Exposure – A Preliminary Study

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Category: Inner Ear: Damage and Protection
Background: Sensorineural hearing loss induced by repeated exposures to blast and high-intensity noise reduces the quality of life of a major fraction of service members as well as civilians. Exposures to blast induce cochlear synaptopathy, hair cell loss, and subsequent pathological changes which originate from the mechanical damage induced by the abnormally large motion of the basilar membrane (BM) during the blast exposure. However, the BM motion induced by blast waves has not been measured experimentally, and it could not be extrapolated from the results measured under acoustic stimuli due to the nonlinearity of the ear tissues. The experimental setup based on laser Doppler vibrometers (LDVs) has been developed to simultaneously measure the motion of middle ear tissues and cochlear pressure under blast (Bien et al., 2023;

Jiang et al., 2021). The goal of this study is to measure the motion of BM under blast conditions – a preliminary study to establish the experimental measurement of the BM response to blast waves.
Methods: Five fresh human temporal bones (TBs) were included in this study. The BM base turn was accessed through a surgically opened window on the scala tympani (ST) through the inner auditory canal. Reflective microbeads with a diameter of 10 μm were placed on the BM to serve as laser targets. The ST was then sealed using microscopic cover glass to ensure the structural integrity of the cochlea. The TB was then mounted under the blast apparatus inside an anechoic chamber. The pressure sensor P0 was mounted approximately 1 cm lateral to the ear canal to monitor the blast overpressure (BOP) and the pressure sensor P1 was inserted into the ear canal to monitor the BOP near the tympanic membrane. LDV1 measured the TB movement as a reference and LDV2 recorded the BM movement. All signals were triggered by P0 and recorded at a sampling rate of 1 MHz.
Results: The BM velocity was successfully measured simultaneously with P0 and P1 under blast conditions. The peak-to-peak BM velocity ranged approximately between 0.2 to 0.6 m/s under a P0 input of approximately 10-20 kPa. The time delay between the P1 peak and the first positive peak of the BM velocity ranged between 0.6-1.2 ms, which was consistent with the time delay of the first peak of scala vestibuli pressure reported in the previous study (Bien et al., 2023). Results measured from 5 TBs were consistent with each other.
Conclusions: Movement of the BM in response to blast exposure was first-time measured at the BM base turn. This preliminary study indicated that the BM motion induced by blast exposure can be measured in TBs and the experimental results will be critical for validating the finite elemental simulation of the cochlea during blasts.

M74. Challenges of Rat Explant Culture for a Reliable Ototoxic Ex Vivo Model for Drug Screening

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[1]*CILCARE*

Category: Inner Ear: Damage and Protection
Background: Cisplatin is one of the most used chemotherapy medications, but its severe ototoxic consequences resulting in irreversible damage to hair cells and hearing impairment, limits its clinical application. The exact mechanism leading to ototoxicity remains unclear. However, cisplatin is well known to induce inflammation, oxidative stress, and apoptosis in the cochlea. This apoptosis takes place in hair cells, principally in the outer hair cells (OHC). In line with 3R principles, reliable in vitro models are of interest and need to be carefully developed. Regarding the cisplatin models, cochlear explant models allow a better selection of therapies against cisplatin's side effects, allowing drug screening and dose response studies to determine the most efficient drug and its concentration to counteract cisplatin ototoxicity. This ex-vivo model allows to limit animal use, by confirming the therapeutic strategy only with an efficient drug at an efficient dose, that could be used in later stages in vivo.
Methods: The objective of this study was to develop the most accurate ototoxic ex vivo model, using rat cochlear explants. In addition, the technical challenges are also presented and discussed.
As a result, we worked with different parameters characterizing the cisplatin explant model: age of the pups, the composition of culture medium, the dissection method, cisplatin concentration, duration of cisplatin exposure and culture period. In addition, to clearly define the effects of cisplatin on cochlear cells, several markers (Myosin 7A, Phalloidin, Hoechst, β-tubulin, V-Glut 3 and Sox2) were used and validated. This allowed the visualization of the various structures of the cochlea, such as hair cells, supporting cells, fibers, and neurons. Depending on the target structures, it is possible to choose more or less severe in vitro models of toxicity induced by cisplatin.

Results: In parallel, a method of image acquisition using a laser scanning confocal microscope as well as histological analysis methods based on a qualitative and quantitative assessment of hair cells (scoring of hair cell organization and counting of hair cell numbers) were developed and validated to provide the most relevant and reliable method to analyze cisplatin effects on the rat cochlea explants.
Conclusions: The development of reliable and consistent rodent explant cultures offers significant advantages for investigating cisplatin's mechanisms of toxicity and for the development of novel therapies. Preclinical testing is indeed a critical phase in the development of new drugs, making it essential to continually refine and expand tools, including in vivo and in vitro models, to advance the progress of new treatments. In this study,

we assessed the toxic effects of cisplatin, but similar investigations can be conducted for various other ototoxic drugs, such as gentamicin, kainate, and more.

M75. A Corticotropin Releasing Factor Response in the Contralateral Cochlea Following Monaural Cochlear Ablation

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Category: Inner Ear: Damage and Protection

Background: Monaural hearing loss may be caused by genetic factors, bacterial or viral infections, head trauma or tumors, including intralabyrinthine (Green et al., 1999) or intracochlear schwannomas (Grayeli, et al., 1007). In the United States, 1 child out of 1000 is born with unilateral hearing loss and approximately 13 million adults have unilateral hearing loss, a.k.a. single-sided deafness.

Corticotropin releasing factor (CRF) and its receptors, well known for hypothalamic-pituitary-adrenal (HPA) axis signaling that mediates the systemic fight versus flight response, are also expressed in cochlea, where they are involved in cellular stress responses (Graham et al, 2010), and along the central auditory pathway. CRF-expressing neurons form a dense terminal field in the inferior colliculus (IC) that is coincident with the greatest density of cytochrome oxidase within the IC, indicating the highly metabolically active nature of this region. We have shown that unilateral cochlear ablation decreases the density of the CRF-positive terminal field contralaterally relative to no ablation. Since unilateral cochlear ablation alters central CRF-positive connectivity in the brain, we asked whether a concomitant change/response exists within the remaining cochlea.

Methods: 8-13 week-old 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 transtympanic 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 cyanoacrylic glue. Age-matched CRFcre:tdTomato male and female mice without cochlear ablation served as controls. Following a 7-day survival, animals were transcardially perfused with buffered 4% paraformaldehyde. Temporal bones were processed for cryostat sectioning and immunostained with primary antibodies against red fluorescent protein and appropriate secondary antibodies conjugated to Alexa 594 or Cy3. Sections were counterstained with DAPI and imaged (Zeiss LSM880 Confocal Microscope).

Results: Seven days following unilateral cochlear ablation, inner hair cells of the opposite, previously presumed normal, cochlea upregulated tdTomato in CRFcre:tdTomato mice.

Conclusions: These results show that the sensory inner hair cells, directly or indirectly, respond to denervation of the opposite cochlea by upregulating CRF. These results further indicate that a potential stress-response occurs in the opposite ear following unilateral deafening. Because CRF signaling in the brain impacts glutamate neurotransmission, these results suggest the potential for effects on basic physiology for hearing in the remaining, intact, ear. It is not known whether this 7-day survival time point reflects the peak of inner hair cell CRF protein levels post-ablation or whether this represents a sub-maximal level. Further studies will investigate this, whether other CRF signaling molecules are also modulated, and what functional effects may ensue following unilateral hearing loss.

M76. The Role of MyD88 Signaling in Cochlear Ototoxin Trafficking and Ototoxin-Induced Hearing Loss

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Category: Inner Ear: Damage and Protection

Background: Lipopolysaccharide (LPS)-induced inflammation mimics gram-negative bacterial infections and exacerbates aminoglycoside-induced hearing loss. LPS activates TLR4 signaling via MyD88 and TRIF adaptor proteins, with each pathway having distinct functions in combating bacterial and viral infections. To

investigate whether the MyD88 adaptor protein signaling pathway plays a more significant role in exacerbating drug-induced hearing loss, we examined whether gardiquimod, which agonizes TLR7 signaling utilizing only MyD88, modulates cochlear or serum levels of ototoxins (gentamicin and chloroquine) and ototoxin-induced hearing loss. This study also offers the advantage of exploring the impact of viral infections on drug-induced hearing loss.
Methods: C57BL/6 mice received either DPBS (control) or gardiquimod (5.0 mg/kg; s.c.; N≥4 per group). After three or 24 hours, we collected blood and cochlear tissues to determine cytokine expression levels using qRT-PCR or Luminex ELISA assays. The cytokines measured included IFNα, IFNβ, IFNγ, MCP1, MIP1α, NFκB, TNFα, IP10, IL1α, IL1β, IL2, IL6, IL10, IL12α, and IL12β. To assess the impact of gardiquimod on cochlear and serum concentrations of gentamicin and chloroquine, C57BL/6 mice were administered a single injection of DPBS or gardiquimod (5.0 mg/kg; N=6 per group). After 24 hours, the mice received gentamicin (20 mg/kg) or chloroquine (20 mg/kg) for 1 hour (i.p.). Subsequently, we collected blood and cochlear samples to measure the concentration of gentamicin (ELISA) and chloroquine (UPLC). To track ototoxin trafficking into the cochleae, we employed fluorescently-tagged ototoxins, namely, Texas Red-conjugated gentamicin (GTTR) and NBD-labelled chloroquine (NBD-CQ), with a specific focus on the stria vascularis. To evaluate the effect of gardiquimod and/or ototoxins on mouse hearing, the mice received DPBS or gardiquimod every other day (5.0 mg/kg; s.c.; N≥6 per group) and/or ototoxins (kanamycin, 700 mg/kg, twice daily; or chloroquine, 40-100 mg/kg, daily). Auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs) were measured before treatment and at 1, 7, and 14 days after treatment.
Results: Gardiquimod induced viral-like inflammatory responses, resulting in increased serum and cochlear levels of cytokines compared to DPBS-treated mice. Gardiquimod also increased cochlear uptake of gentamicin and chloroquine compared to controls, without affecting serum levels of gentamicin and chloroquine. The assessment of gardiquimod's effect on ototoxin-induced hearing loss is currently ongoing.
Conclusions: Our data demonstrate that gardiquimod-induced TLR7 signaling initiates robust inflammatory responses that mimic viral-like inflammation in humans. Furthermore, gardiquimod increased cochlear levels of gentamicin and chloroquine but did not affect serum levels, potentially exacerbating ototoxin-induced hearing loss. Thus, MyD88 signaling alone appears to be sufficient to exacerbate ototoxin-induced hearing loss.

M77. Viral Mediated Selective and Timed Ablation of Spiral Ganglion Neurons in Parvalbumin-Cre Mice

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Category: Inner Ear: Damage and Protection
Background: Direct reprogramming of non-neuronal cells to neurons is an emerging field of regenerative medicine. Hearing loss conditions such as auditory neuropathy (AN) may be restored by inducing cellular reprogramming with gene therapy. Spiral ganglion glial cells are potential sources of in-situ direct reprogramming given the proximity to the native auditory nerve and its proliferative capacity upon nerve injury. One of the challenges in studying direct reprogramming of spiral ganglion neuron (SGN) is the lack of a mouse model with selective and temporally controlled SGN ablation. We sought to generate a SGN degeneration model that reflects auditory neuropathy in mice.
Methods: We delivered AAV2/Retro-flex-DTA-mCherry driven by ubiquitous EF1a promoter (AAV-EF1a-DTA) or neuronal hSYN promoter (AAV-hSYN-DTA) in neonatal (P1-2) and adult (P30) Parvalbumin-Cre (PVCre), Ai9-Tdtomato;PV/Cre (Td/PVCre), and wild-type C57 strain. Vectors were microinjected via the posterior semicircular canal. Neonatal mice were euthanized at one-week and one month post-injection while adult mice were euthanized at one-week post-injection. We performed immunohistochemistry with whole-mount and cryosection tissue staining with anti-beta-tubulinIII (TUJ1), anti-myosin7A (myo7a), and anti-cleaved-caspase-3. SGN degeneration was quantitatively assessed by analyzing the cellular density of double positive TUJ1 and Tdtomato spiral ganglion cells in Td/PvCre cochlear cryosection tissue. ABR and DPOAE were measured at one-month post-injection for neonatal mice and one-week post-injection for adult mice.
Results: PVCre neonatal mice injected with AAV-EF1a-DTA showed a dramatic decrease of SGN at one week in whole-mount and cryosection tissue while preserving hair cells in all turns. Disorganized and thinning of afferent neurons with cleaved caspase-3 expression was detected in both the osseous spiral lamina and

Rosenthal's canal. Wild-type C57 injected with the same vector demonstrated intact neurites and SGN in Rosenthal's canal. We quantified the cell density of the SGN in mid-modiolar sections following the injection of AAV-EF1a-DTA and AAV-hSYN-DTA in neonatal mice. Compared to uninjected Td/PVCre, we observed an average of 60% and 61% degeneration in all turns at one week, respectively. At one month post-injection, we observed a near-total loss of TUJ1-positive neurons in all three turns. In adult mice injected with AAV-hSYN-DTA, we observed a similar degenerative pattern as neonates at one week. The ABR result showed significantly elevated thresholds at 8, 16, 24, and 32 kHz at one-month post-injection for neonatal mice and one-week post-injection for adult mice.
Conclusions: We demonstrate a novel mouse model of selective and temporally controlled primary SGN degeneration in a Cre-dependent fashion. The Cre-dependent degeneration following the viral injection is observed in all ages and is not promoter dependent. However, a longer incubation period permits near-total ablation of SGNs. In conclusion, we generated a mouse model that mimics AN and can be used to study the degenerative and regenerative cell biology of SGNs in vivo.

M78. Synaptopathic Insults Cause Degradation of Axonal-Coat-Like Extracellular Matrix Structures Around the Junction of Auditory Nerve Fibers and Inner Hair Cells

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Category: Inner Ear: Damage and Protection
Background: Perineuronal nets (PNNs) are specialized extracellular matrix (ECM) structures that surround neuronal cell bodies, nodes of Ranvier, and neurites. PNNs and PNN-based axonal coats are known to affect synaptic maintenance and plasticity, neurite fasciculation, and conductance. Despite the established roles of PNNs throughout the CNS, less is known about these ECM structures in the inner ear. PNN proteins have been shown to be present around the fibers that innervate cochlear hair cells, and developmental deletion of a key PNN protein, brevican, leads to increased incidence of mispairing between pre- and post- synaptic components. Recent studies suggest that insults to the inner ear can cause dramatic losses of properly paired inner hair cell (iHC) synapses and that this cochlear "synaptopathy" may cause auditory impairments. Our main goal here is to investigate the degradation of PNN components following noise or trauma as a first step toward determining whether ECM remodeling may play a mechanistic role in synapse loss or subsequent recovery.
Methods: To investigate the effects of synaptopathic insults on cochlear tissues, 4-month old CBA/CaJ mice were subjected to 100db noise (8-16 kHz) for 2 hours and cochleae were collected at 1, 7, and, 14-days post-noise. In a separate cohort, 4-month-old C57BL/6J mice underwent mild Traumatic Brain Injury (mTBI) and cochleae were collected at 2-3 hours and at 1, 7, and 14 days post mTBI. Tissues were immunolabeled with antibodies against the known PNN proteins: brevican, aggrecan, HAPLN4, and tenascin-R. IMARIS software was used to measure changes in volume and/or fluorescence intensity of the PNN components around the iHCs. Tissues were also immunolabeled for pre-synaptic ribeye (anti-CTBP2) and post synaptic GLUR2 to confirm synapse loss after insult.
Results: Our data suggest there is a significant decrease in the volume of brevican positive ECM structures around inner hair cells as a result of noise and as a result of mTBI. Immunolabeling of aggrecan, haplan4, and other PNN components suggest similar reductions following synaptopathic insults. Triple labeling for GLUR2, CTBP2, and brevican also suggests close apposition of axonal coats to properly paired synapses. In both insult and control conditions we noted increasing PNN volumes from apex to base along the cochlear tonotopic axis.
Conclusions: Overall, the data suggest that PNN volume is positively correlated with the numbers of paired synapses that are present on inner hair cells. Preliminary data suggest no change in expression of matrix metalloproteinases (MMPs) around the iHCs, but current and future directions are focused on other enzymes known to degrade PNNs such as ADAMTS family members. Understanding PNN remodeling after cochlear insult will provide a better understanding of whether manipulation of PNN complexes in the inner ear can help to prevent or reverse synaptopathy.

M79. Open Board

M80. Optimization of Pharmacological Interventions in the Guinea Pig Animal Model – A New Approach to Calculate the Perilymph Volume of the Scala Tympani

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Category: Inner Ear: Drug Delivery

Background: The guinea pig is a well-established animal model for inner ear research, providing valuable insights into the anatomy, physiology, and therapeutic interventions of the auditory system. However, the heterogeneity of results observed in both in vivo experiments and clinical trials poses a challenge in understanding and optimizing pharmacotherapeutic outcomes. This heterogeneity may be due to individual differences in the shape of the guinea pig cochlea and thus in the volume of the scala tympani (ST), which can lead to different drug-volume concentrations in the ST, a fact that has been largely overlooked. To address this issue, we aimed to develop an approach to calculate the individual volume of perilymph within the ST based on radiological images of the Individual cochlea.

Methods: In this study, high-resolution µCT images of guinea pig temporal bones were used to determine the volume of the ST and compare the inter-individual variability found in ST volume as well as variability compared to human studies. Different temporal bone conditions from freshly extracted to paraformaldehyde fixed to frozen as well as colored and Albino sources were compared.

Results: Our results show a variability in mean ST volume with a relative standard deviation (RSD) of 14.7%, comparable to human studies (range RSD: 5% to 20%). This suggests that the guinea pig cochlea show variability similar to that of the human cochlea. Therefore, it is critical to account for this variability when designing and conducting studies using the guinea pig as an animal model. We have successfully developed a tool capable of estimating ST volume without the need for manual segmentation, using two geometric parameters, basal width (A) and basal diameter (B) of the cochlea, corresponding to the cochlear footprint.

Conclusions: This novel approach provides researchers with a valuable tool to calculate individual ST volume in guinea pigs, allowing for more precise dosing strategies and optimization of drug concentrations in pharmacotherapy studies. Furthermore, our study highlights the importance of recognizing and accounting for inter-individual variability in animal models to increase the translational relevance and applicability of research findings in the field of inner ear research.

M81. Intracochlear Drug Delivery in Combination With Dexamethasone Eluting Electrode Provides Efficient Otoprotection for Cochlear Implant Trauma

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Category: Inner Ear: Drug Delivery

Background: Cochlear implantation is a standard of care for providing auditory rehabilitation to individuals having severe to profound hearing loss. However, insertion of cochlear implant (CI)induces electrode insertion trauma (EIT) in inner ear activating host inflammatory and apoptotic pathways resulting in loss of residual hearing. Otoprotective drugs modulating these host inflammatory and apoptotic pathways can facilitate in the preservation of residual hearing leading to better clinical outcome and adoption of electroacoustic system (EAS) leading to better listening experience as well as music appreciation. However, delivery of drugs especially in the apical turn where speech recognition regions are located is very challenging. The aim of this study was to determine the feasibility and safety of a custom designed cochlear catheter (cannula) for inner ear drug delivery using a rat model of cochlear implantation. We also evaluated the efficacy of a novel drug

(Drug A) delivered through cannula for preservation of residual hearing in providing and the underlying molecular mechanisms behind otoprotection.
Methods: The rats were divided into 9 groups: 1) control; 2) animals with CI (CI); 3) animals with dexamethasone eluting electrode (Dexel); 4) artificial perilymph elution through cannula (Can + AP); 5) ringer lactate cannula elution (Can + Ringer); 6) ringer lactate cannula elution + CI (Can+CI); 7) ringer lactate cannula elution and implanted dexamethasone eluting electrode (Can + Dexel); 8) Drug A alone through cannula elution and implanted with CI (CI/Can+Drug A (2mM)) ; 9) Drug A cannula elution at 2mM and implanted dexamethasone eluting electrode (Can A2 + Dexel). Pre-operative auditory brainstem responses (ABRs) were conducted to determine baseline hearing thresholds, as well as ABRs at days 7 and days 30 post implantation. Organ of Corti dissections were performed for each group on day 30 and were subsequently immunostained to visualize hair cell damage and oxidative stress markers.
Results: Animals implanted with dexamethasone eluting electrode and receiving drug A (2 mM) showed significantly lower hearing thresholds compared to all the groups. On par with these findings, the number of surviving hair cells were significantly higher in animals implanted with dexamethasone eluting electrode and receiving Drug A (2 mM) through cannula compared to all the groups. The underlying molecular mechanisms behind otoprotection involve down regulation of oxidative stress that are activated in response to cochlear implant trauma.
Conclusions: The result of our study suggests that intracochlear drug delivery through cannula is safe and has no adverse effects. The delivery of Drug A in combination with dexamethasone eluting electrode provides efficient otoprotection for CI trauma. The cannula and drug A should be explored further to develop novel treatment modalities for the preservation of residual hearing post-implantation in pursuit of improving quality of life of implanted individuals and their families.

M82. Investigations on Safety and Biodistribution of Vesicle-Enriched Secretome Fraction in Mice After Cochlear Implantation Trauma

Odett Kaiser*[1], Susanne Sasse[1], Jennifer Harre[1], Mario Gimona[2], Eva Rohde[2], Hinrich Staecker[3], Thomas Lenarz[1], Athanasia Warnecke[1]

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Category: Inner Ear: Drug Delivery
Background: After cochlear implantation, immune response can impair hearing performance or even lead to loss of residual hearing. So far, there are no approved pharmacological therapies available to treat hearing loss or to compensate for the immune response. As a complex biological substance, vesicle-enriched secretome fraction (VSF) derived from human umbilical cord mesenchymal stromal cells is a novel drug candidate exerting immunomodulatory or neuroprotective effects. Neuroprotection in dissociated rat spiral ganglion cell culture as well as attenuation of hearing threshold shifts and protection of hair cells in mice has been shown previously. To prepare a clinical study, safety and biodistribution of VSF in mice after cochlear implantation trauma have been investigated.
Methods: Preclinical studies on immunocompetent 4-8 weeks old NMRI mice for safety (n=90, both sexes; non-labelled VSF) and biodistribution (n=25, females; DiD-labelled VSF) were performed. Under general anesthesia, test substance (Ringer's solution, therapeutic dose or 10-fold therapeutic dose of clinical grade VSF, randomized) was applied locally into the cochlea into the oval window after implantation trauma. Animals were sacrificed on days 1, 7, or 14 (safety) as well as after 1 or 24 hours (biodistribution). All organs underwent general histological assessment. For all inner ears of the biodistribution study, a separate preparation of basilar membrane and stria vascularis followed by staining with Alexa488-linked phallodin was perfomed and specimen were evaluated microscopically.
Results: General behavior and locomotor activity of animals revealed no abnormalities. While only few clinical symptoms were observed (none was related to the substance) and a reversible granulocyte infiltration was found after day 1 in all treatment groups, no safety concerns of the single local application of VSF in mice after day 1, 7 or 14 days post application could be raised. Labelled DiD-VSF were traced in a time dependent manner. DID-labelled VSF could be detected at the applied cochlea in hair cells, supporting cells and spiral ganglion neurons while signal on contralateral ears was on an autofluorescent level. Ten-fold therapeutic dose of VSF underwent physiological degradation process.

Conclusions: Single application of human umbilical cord mesenchymal stromal cells derived VSF could not raise any specific safety concern in this cochlear implantation trauma animal model. VSF is able to enter inner ear specific cells enabling a possible direct modulation of homeostasis. Thus, VSF seems to be a promising new drug and needs to be evaluated in phase one clinical trial.

M83. Biosafety and Biodistribution Study of Vesicle-Enriched Secretome Fraction for Prevention of Cochlear Implantation Trauma

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Category: Inner Ear: Drug Delivery

Background: Vesicle-enriched secretome fraction (VSF) derived from human umbilical cord mesenchymal stromal cells is a new class of biological therapeutic for the modulation of pathways and targeting cochlear cells in order to prevent or modulate immunological processes. Previous in vitro tests have shown the efficacy in spiral ganglion cell culture and first in vivo tests in mice and guinea pigs with research grade VSF indicate attenuation of threshold shifts, prevention of fibrosis formation and protection of hair cells. In three different animal study designs with guinea pigs we evaluated safety, biodistribution and neuroprotective effects of VSF to maintain gross structural integrity of the cochlea after electrode implantation trauma and associated conditions.

Methods: Mimicking the clinical setting in humans, we implanted a group of hearing guinea pigs (n=6) with a cochlear implant (implantation trauma group - IPT) followed by administration of a therapeutic dose of clinical grade VSF for 4 weeks. Investigation included auditory brainstem response (ABR) and compound action potential (CAP) measurements pre and post implantation as well as weekly impedance measures until sacrifice on day 28. Another group of hearing guinea pigs (n=11) was administered a 10-fold therapeutic dose of VSF with a six month follow up of ABR measurements. A third group of hearing guinea pigs (n=3) receiving DiD-labelled VSF in both ears were sacrificed 1 hour after the last administration confirming uptake and distribution of VSF in the inner ear. All groups underwent detailed histological assessment.

Results: Treatment with VSF improved hearing after cochlear implantation in the IPT group when compared to control animals treated with artificial perilymph. Histological data of this group also confirmed fibrosis 4 weeks post implantation at a level similar to control animals, but less in extension when compared to data of control groups with artificial perilymph. Electrophysiological and histological findings of the long term group revealed no adverse effects of the 10-fold therapeutic dose of VSF after six month. ABR thresholds in this six month group remained on a similar level compared to a control group treated with ringer lactate. Positive fluorescent signalling of labelled VSF was confirmed in the supporting cells of the organ of Corti and stria vascularis.

Conclusions: Uptake of human umbilical cord mesenchymal stromal cells derived VSF into cells of the guinea pig cochlea has been demonstrated and treatment with VSF seems to mediate immunological processes to a healthier state and maintain gross structural integrity. Application of VSF associated with cochlear implantation seems to be a safe and solid combination to prevent post implantation trauma and preserve residual hearing.

M84. Implanted Injection Port for Chronic Drug Delivery to the Middle Ear in Mice

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Category: Inner Ear: Drug Delivery

Background: Small molecule agents designed to rescue disorder-specific mutant proteins causative of sensorineural hearing loss, such as clarin-1N48K in Usher syndrome type IIIA (USH3A), need to be delivered regularly. In these cases, local delivery has significant advantages over systemic delivery. However, genetic predisposition to sensorineural hearing loss may render surgery to access the cochlea counter effective.

Alternatively, delivering therapeutic agents close to the round and oval window via the middle ear space may be a viable option. A transtympanic approach is ill suited for chronic/repeat injections due to repeated trauma to the tympanic membrane. Pressure equalizer (PE) tubes, implanted in the tympanic membrane to relieve otitis media in humans, could be repurposed to provide routine access to the middle ear space in patients to treat genetic hearing loss. However, implanting PE tubes in mice has not been reported. An approach for long term testing of candidate drugs in genetic models is necessary. Here we describe surgically implanting a catheter with an injection port for drug delivery to the middle ear. The goal of this pilot study was to determine feasibility and assess the impact of implantation of an injection port on hearing in mice.

Methods: For this study, we used ~2- to 3-month old C57BL/6J and USH3A mice. We repurposed the Vascular Access Button or VABTM (Instech Labs, PA) – made for access to the vein for sampling or drug infusion in mice. For the surgery, the bulla was accessed following post auricular incision of the left ear. A ~2 mm hole was created in the bulla, and a specially designed catheter was inserted and secured with veterinary glue. The other end of the catheter was tunneled under the skin until it reached the 'exit site' at the junction of the shoulder blades (mid-dorsal line). Here, another incision was made, the catheter threaded, and the catheter was connected to an injection port button before suturing both incision sites. The right ear served as non-surgical control ear for all mice. At 2 to 4 weeks post-operation, behavior, physical attributes, balance and hearing functions were monitored.

Results: The implant was generally well-tolerated in mice. Injection of saline through the port was tested for two weeks post-operation without issue. Post-operation, no sign of earwax accumulation, otitis media or vestibular dysfunction was noted. Importantly, no statistically significant difference in hearing thresholds between the left (implanted) and right (control) ears was noted (n=6 mice).

Conclusions: Here, we present a fully implantable method in mice for chronic drug delivery to the middle ear, with minimal damage or implant-induced hearing loss. With further investigation and optimization, this approach could be used to investigate drug therapies in preclinical models of sensorineural hearing loss.

M85. The Impact of Proteostasis on Neuronal Diversity in the Developing Inner Ear

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Category: Development: Cellular/Systems

Background: Recently, it has been shown that spiral ganglions neurons not only differ at the physiological and morphological level but also at the molecular level (Shrestha et. al, 2018; Sun et al, 2018; Petitpré et al, 2018). Growing evidence suggest a preferential vulnerability of the Ic subtype during age and noise trauma, strongly affecting the ability of patients to understand speech in a noisy background and thus highlighting the importance of the normal functioning of each SGN subtype. As well, single-cell RNA sequencing analysis could show that neuronal differentiation and diversification already starts during embryonic development between E14.5 and E16.5 (Petitpré et el., 2022; Sanders et al., 2022). Unnatural protein aggregation, as it is the case during age, some genetic diseases and after drug administration, could already be linked to sensorineural hearing loss. In our proteotoxic model of Elp3-deficient mice, we could show that there is an ER-stress induced neuronal cell death during development resulting in complete hearing loss. Thus, we plan to unravel the importance of proteostasis during development on the emergence and maintenance of the spiral ganglion neurons, as well as the vestibular counterpart, the vestibular ganglion neurons (VGNs).

Methods: We used a Foxg1Cre mouse model to induce protein aggregation from E8.5 onwards in the developing sensory epithelia and the spiral ganglion neurons. By performing RNAscope experiments on E14.5, E16.5, E18.5, P0 and P15 Elp3cKO mice by using subtype specific markers, we wanted to know how disrupted proteostasis impacts the emergence of the 3 neuronal subtypes.

As well, by performing Immunostainings and 3D reconstructions of the sensory epithelium, we analyzed the position of the Ribbon synapses in WT and KO animals.

Results: The results show that at E14.5 and E16.5 there is no obvious difference regarding the expression pattern of subgroup specific markers. In contrast, at E18.5 and P0, there is a predominance of Lypd1-positive cells at the apical turn whereas at the base and mid turn we found predominantly Calb2-positive cells. Surprisingly, at P7 and P15 this tonotopic pattern has completely vanished showing a predominance of Calb2-positive cells in all three turns, suggesting that they belong to the Ia subgroup. Interestingly, Ribbon synapse

position analyses show that the remaining neurons preferentially innervate the hair cell at the modiolar side, characteristic of the Ic subgroup.
Conclusions: These results suggests that proteostasis during development is at least crucial for neuronal survival and for the normal development of spiral ganglion neurons and that proteostasis disruption impacts the molecular signature starting prior to birth as well as the innervation of their target cells.
To further investigate if the phenotype results from the neuronal or sensory compartment, we'll use the Sox2CreERT and Ngn1CreERT mouse model to specifically induce protein aggregation in one or the other compartment.

M86. Development Expression of Proteins in Neurons to Innervate Brainstem and Hair Cells

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Category: Development: Cellular/Systems
Background: The vestibular and spiral ganglion neurons innervate the vestibular and cochlear hair cells in the ear and project to the vestibular and cochlear nuclei in the brainstem. Spiral ganglion neurons develop into three distinct Type I neurons to innervate the inner hair cells, whereas type II neurons innervate the outer hair cells, overlapping the tonotopic cochlear nuclei. At least two types of vestibular neurons have been identified, but their innervation to the five vestibular end-organ hair cells and the selective innervation of vestibular nuclei remain to be seen.
Methods: We use transgenic mice to selectively express in calbindin (Calb1), calretinin (Calb2) and peripherin (Prph) while nearly all people worked with antibodies to Calb1, Calb2, and Prph.
Results: Peripherin (Prph) is positive in VGN and SGN, reducing to only Type II SGNs in older mice. VGN remains positive and differentiate into different size of neurons. Type II SGNs show a progression from the middle turn that reaches out at P4 in the apex and disappears with fibers after P15 or older mice. The brainstem initially has Prph-positive fibers from VGN and SGN but is reduced and absent in the cochlear nuclei in older mice.
Calretinin is expressed in a substantial number of SGNs and is positive in fewer VGNs. Calbindin is highly expressed in a large population of VGNs and is slightly reduced in number in SGNs. Cochlear nuclei mix Calb1 and Calb2, whereas vestibular nuclei are highly positive for Calb1 that shows a small Calb2 positive VGNs.

Calbindin expression is upregulated in older mice. In contrast, canal cristae are highly positive for calbindin initially, which will reduce calbindin expression and become more positive for calretinin. Cochlear HC is positive for early calretinin, progressively expressing calbindin in a base-to-apex progression. In the adult, mice show only expressed calretinin for IHCs that remain positive for calbindin. Vestibular fibers reach Calb1 and Calb2, whereas cochlear nuclei innervate mostly the anteroventral and posteroventral cochlear nuclei. Vestibular hair cells are positive for calretinin in the utricle and the saccule and eventually are upregulated in the striola.

Central projections are positive for Calb1 and Calb2 that overlaps in cochlear nuclei and vestibular nuclei. In addition, a selective expression of Calb1 and Calb2 labels AVCN, PVCN and DCN that is highly positive for Calb2 vestibular nuclei, in particular the lateral vestibular nuclei (LVN).

Conclusions: Using transgenic expression of Calb1/2 and Prph can demonstrate a longitudinal expression of proteins of embryos and adult mice that has been previously used for antibodies. Our data compares and expand the previous work,

M87. A Novel Cytoskeletal Regulator That Blocks Cell Proliferation in the Mammalian Cochlea

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Category: Development: Cellular/Systems

Background: In the developed mammalian cochlea, cells are unable to re-enter the cell cycle and regenerate to replace damaged or missing cells. The expression of p27kip1, marking the cells that are exiting the cell cycle, begins on the apical end on mouse embryonic day (E)12.5 and progresses towards the base of the cochlea. This is known as the zone of non-proliferation (ZNP). Molecular blocks on proliferation in the ZNP occur very early in development. However, cells in the IS domain continue to maintain their proliferative capacity longer. The Wnt pathway is known to regulate stem cells and cell proliferation. Our previous studies suggest that the Bmp pathway antagonizes the Wnt pathway. Others have shown that loss of Bmp signaling increased cell proliferation. We predicted that the Bmp pathway specifically regulates genes that block cell proliferation. To identify these potential 'blockers' of cell proliferation, we performed transcriptomic analysis of cochleas treated with a Bmp inhibitor. Previous studies by the Corwin lab suggested that the F-actin stiffening in the mature mammalian cochlea disrupted cell cycle re-entry, creating a physical barrier for regeneration.
Methods: We screened for Bmp-regulated cytoskeletal regulators that are associated with epithelial organization. From this, we identified a novel cytoskeletal regulator, Shroom1. Shroom1 was also identified amongst the genes that were downregulated by the Wnt pathway. We compared the spatial pattern of proliferating cells with the expression of Shroom1 on E14.5 when genes that associated with cell proliferation and differentiation are expressed during the same stage of development, but spatially are segregated across the radial axis of the cochlea. Consistent with this, we found that on E14.5, the progenitor niche lies in the medial domain and in situ hybridization of Shroom1 showed it is expressed in the lateral domains. Shroom1 expression in the progenitor niche is absent; thus, Shroom1 shows an asymmetric expression pattern. SHROOMs are actin cytoskeletal regulators, but the role of Shroom1 remains unknown in any system. However, based on its expression, we hypothesized that SHROOM1 acts as an inhibitor of cell proliferation. To test this, we created Shroom1 KO mice. Due to embryonic lethality, we cultured E13.5 cochleas in vitro and on the second day, we added BrdU, a thymidine analogue, for 24 hours. We fixed the cochleas on the third day (~E15.5) and analyzed cell proliferation.
Results: Surprisingly, we found proliferating cells with increased BrdU incorporation in the ZNP. Conversely, when we overexpressed Shroom1 by electroporation on E14.5, we see reduced Ki67-positive cells in the IS domain by E15.5.
Conclusions: These data suggest that progenitors intrinsically suppress Shroom1 levels in order to allow cell cycle progression and conversely, the deletion of Shroom1 allows cell cycle re-entry.

M88. Expression of Ebf1 and Ebf3 in the Vestibule and Semicircular Canals During Mouse Development

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Category: Development: Cellular/Systems
Background: Our in silico re-analysis of the mouse whole-body single-cell RNAseq data suggested that early B-cell factors 1 and 3 (Ebf1 and Ebf3) were expressed in the prosensory domain of the developmental inner ear (Yamamoto R et al., 2021). The expression level of Ebf3 within the vestibular prosensory domains was significantly higher than within the cochlear prosensory domains in this study. Ebf1 and Ebf3 are members of the Ebf family, a basic helix-loop-helix (bHLH) transcription factor highly conserved in all metazoans. To confirm our in silico study findings, we examined the morphological expression patterns of Ebf1 and Ebf3 within the mouse vestibule and semicircular canals during developmental stages.
Methods: In situ hybridization was performed using Ebf1 and Ebf3 probes on the mouse inner ears from embryonic day (E)9.5 to E18.5. Immunohistochemistry for Ebf1 and Ebf3 was performed on E16.5 mouse saccule.
Results: At E9.5, both Ebf1 and Ebf3 were not expressed in the epithelia of otocysts, although both were expressed in the cochleovestibular ganglion area. Ebf1 started to be expressed in both cochlear and vestibular primordial regions, that is, ventromedial and lateral sides of the otocysts, respectively, at E10.5. In contrast, Ebf3 was mainly expressed at this stage in the lateral side of the otocyst, where vestibular organs will develop. At E11.5, both Ebf1 and Ebf3 were expressed in the primordial region of cochlear sensory epithelia, but only Ebf3 was detected in the vestibular primordial area. After E12.5 in the vestibular region, Ebf1 was expressed

in the parts of vestibular sensory epithelia. In contrast, Ebf3 expression was found in the whole vestibular sensory epithelia of the saccule, utricle, and crista ampularis.
Immunohistochemistry at E16.5 showed that Ebf1 and Ebf3 were expressed in hair and supporting cells of the utricle, respectively.

Conclusions: The expression patterns of Ebf3 mRNA confirmed our previous in silico study that suggested its higher expression in vestibular sensory epithelia. As predicted in our previous study, Ebf1 expression was detected within both cochlear and vestibular sensory epithelia. These results suggest that Ebf3 may have some roles in the development of vestibular sensory epithelia. In contrast, the functions of Ebf1 in the development of vestibular organs may be limited because of its partial expression within the sensory epithelia of vestibular organs. However, the different and reciprocal expression patterns of Ebf1 and Ebf3 in the mature utricular sensory epithelia suggested that both Ebf molecules have essential roles in the vestibular organs.
In conclusion, Ebf3 was expressed explicitly in the vestibular organs within the mouse inner ear. The expression of Ebf1 and Ebf3 in hair and supporting cells suggested the contribution of these two molecules to the development of vestibular sensory epithelia.

M89. Ebf1 is Necessary for Sensory Domain Establishment Within the Organ of Corti and Essential for Hearing in Mice

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Category: Development: Cellular/Systems
Background: Hearing depends on the precise establishment and 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 these developmental processes. Recent work from our lab identified Ebf binding motif enrichment in the open chromatin of prosensory cells collected from embryonic cochleae. Ebfs are generally expressed in overlapping patterns, allowing for compensation following loss of a single member of the transcription factor family. Our single cell RNAseq analyses, however, revealed that Ebf1 is strongly expressed in the developing cochlear epithelium while there is little to no expression of the other family members (Ebf2-4). Here, we further investigate the role of Ebf1 in cochlear development.
Methods: To identify Ebf1's role in cochlear development and auditory function, our lab designed a conditional knockout (cKO) model in which the Slc26a9 promoter directs Cre-mediated excision of Ebf1 in the otocyst at embryonic day 9.5 (E9.5). Ebf1-cKO mice are fertile and survive into adulthood. We treated E15.5-17.5 littermate control and Ebf1-cKO mice with EdU to identify Ebf1-dependent changes in the timing of sensory cell cycle exit and domain establishment. To characterize changes in sensory cell patterning, we performed immunolabeling on postnatal day 1 (P1) cochlear wholemounts and sections. We also tested auditory brainstem response to assess hearing in the adult mice.
Results: Our single cell RNAseq, in situ hybridization, and immunolabeling experiments involving E12-18 cochleae revealed Ebf1 is expressed in the Kölliker's organ, prosensory cells, HCs, and SCs. Loss of Ebf1 leads to a dramatic expansion of the sensory domain and formation of ectopic sensory patches, randomly distributed throughout the Kölliker's organ. Embryonic Ebf1-cKO cochleae exhibit sensory cell proliferation beyond the stages that these cells typically drop out of the cell cycle in addition to a delay in differentiation in the apex relative to littermate controls. By P1, Ebf1-cKO cochleae possess approximately triple the number of inner HCs and double the number of outer HCs observed in littermate controls. These supernumerary HCs continue to direct SC patterning. At least one Fabp7+ inner phalangeal cell can be found adjacent to each supernumerary inner HC. Similarly, multiple discontinuous rows of CD44+ outer pillar cells are concentrated between the inner HC and outer HC regions and Prox1+ Deiters' cell nuclei reside underneath the rows of supernumerary outer HCs. Ebf1-cKO inner pillar cells are present in a single row interrupted by supernumerary inner HCs. 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, and ABR testing shows the Ebf1-cKO mice are deaf.
Conclusions: Ebf1 restricts cochlear sensory epithelium establishment and is necessary for hearing.

M90. Time of Delamination of Inner Ear Neurons Specifies Their Topography and Target Innervation

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Category: Development: Cellular/Systems

Background: Neurons are the first cell types to differentiate in the inner ear. These form from the pro-neurosensory domain (PNSD) of the otic vesicle (OV). They delaminate from the otic epithelium forming a ganglion, the cochleovestibular ganglion (CVG), medio-ventral to the otocyst, proliferate and then undergo differentiation to innervate specific targets in the developing inner ear. Recent studies have highlighted the role of the auditory component of the CVG in directing the base to apical differentiation of hair cells in the cochlea. This implies a pre-pattern in the auditory ganglion.

Methods: We investigated how positional identities of neurons in the ear ganglion are specified. By using the chicken embryo as our model system, we found that differentiation within the ganglion follows a proximal to distal pattern. This was confirmed by EdU pulse labelling at different developmental times and we found that neurons became post-mitotic following a proximal to distal direction. This implies a conserved direction of differentiation followed by inner ear neurons of birds and mammals. Intrigued by the relationship between birth-dates and topography of inner ear neurons, we asked when positional identity was specified. We targeted the OV prior to the delamination of neurons, introducing a fluorescent protein expressing plasmid using electroporation at different times.

Results: Interestingly labelled cells occupied positions in the ganglion depending on when they left the OV. The earliest cells to delaminate populated the vestibular neurons. Cells that delaminate later were found to be both saccular neurons and in the proximal part of auditory ganglion. We also observed cells that were likely neurons that innervated the lagena, which even though are spatially closer to auditory ganglion were temporally related to other vestibular neurons. Neurons which delaminated afterwards primarily occupied more and more distal regions of the auditory ganglion. Lastly, we asked if the time of delamination of a neuron specified target selection. By using a Cre-Lox system to sparsely label the delaminating neurons we found that target innervation is specified during delamination. Here we observed that fibres from early and late delaminating cells were spatially segregated in the eight nerve. Early delaminating neurons, innervated hair cells that were more proximal, and innervated region of the developing auditory nucleus that was more posterior, whereas late delaminating neurons innervated more distal hair cells, and sent projections to more anterior regions of the auditory nucleus.

Conclusions: Our work shows that positional information, innervation choice and nerve topography are specified at the time of delamination of neurons from the otocyst.

M91. TMEM30A is Essential for Hair Cell Polarity Maintenance in Postnatal Mouse Cochlea

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Category: Development: Cellular/Systems

Background: Phosphatidylserine is translocated to the inner leaflet of the phospholipid bilayer membrane by the flippase function of type IV P-tape ATPase (P4-ATPase), which is critical to maintain cellular stability and homeostasis. Transmembrane protein 30A (TMEM30A) is the β-subunit of P4-ATPase. Loss of P4-ATPase function causes sensorineural hearing loss and visual dysfunction in human. However, the function of TMEM30A in the auditory system is unclear.

Methods: P4-ATPase subtype expression in the cochlea was detected by immunofluorescence staining and quantitative real-time polymerase chain reaction (qRT-PCR) at different developmental stages. Hair cell specific TMEM30A knockout mice and wildtype littermates were used for the following functional and morphological analysis. Auditory function was evaluated by auditory brainstem response. We investigated hair cell and stereocilia morphological changes by immunofluorescence staining. Scanning electron microscopy was applied to observe the stereocilia ultrastructure. Differentially expressed transcriptomes were analyzed based on RNA-sequencing data from knockout and wild-type mouse cochleae. Differentially expressed genes were verified by qRT-PCR.

Results: TMEM30A and subtypes of P4-ATPase are expressed in the mouse cochlea in a temporal-dependent pattern. Deletion of TMEM30A in hair cells impaired hearing onset due to progressive hair cell loss. The disrupted kinocilia placement and irregular distribution of spectrin-α in cuticular plate indicated the hair cell planar polarity disruption in TMEM30A deletion hair cells. Hair cell degeneration begins at P7 and finishes around P14. Transcriptional analysis indicates that the focal adhesion pathway and stereocilium tip-related genes changed dramatically. Without the TMEM30A chaperone, excessive ATP8A2 accumulated in the cytoplasm, leading to overwhelming endoplasmic reticulum stress, which eventually contributed to hair cell death.
Conclusions: Deletion of TMEM30A led to disrupted planar polarity and stereocilia bundles, and finally led to hair cell loss and auditory dysfunction. TMEM30A is essential for hair cell polarity maintenance and membrane homeostasis. Our study highlights a pivotal role of TMEM30A in the postnatal development of hair cells and reveals the possible mechanisms underlying P4-ATPase-related genetic hearing loss.

M92. Epithelial Morphogenesis Begins With the Cochlear Progenitors

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Category: Development: Cellular/Systems
Background: The structure of the cochlea is asymmetrically organized, influenced by the crosstalk of several signaling pathways during development. The sensory epithelium, the organ of Corti (OC), is comprised of inner hair cells (IHCs) and outer hair cells (OHCs), which are intercalated by support cells (SCs) across the radial axis. Cells on embryonic day (E)12.5 across the cochlear radial axis continue to proliferate, but on E14.5, only the cells in the future inner sulcus (IS) domain continue to proliferate. The Wnt pathway is essential for stem cell/ progenitor proliferation. On E14.5, Wnt secretion by PORCN is enriched in the IS domain where the "niche of proliferating/progenitor cells" is located. We predict that since the progenitors themselves secrete important morphogens, they influence cochlear epithelial morphogenesis.
Methods: Through transcriptomic analysis, we identified two genes encoding transcription factors, Mybl2 and Foxm1 that are expressed in the progenitor niche of the cochlea between E12.5 and E14.5.
MYBL2 and FOXM1 are known to be important for cell cycle progression in progenitor cells in other tissues but their functions have not been investigated in the cochlea. Both Mybl2 and Foxm1 are expressed across the radial axis on E12.5, but expression becomes restricted to the future IS on E14.5. We investigate novel roles for MYBL2 and FOXM1 on influencing epithelial morphogenesis in the cochlea. To study the function of MYBL2, we generated Mybl2 cKO embryos.

Results: Our data show that Ki67 labeling is decreased in the IS domain of Mybl2 cKO cochleas, suggesting a decrease in proliferation. Our data also show that the loss of Mybl2 resulted in a two-fold expansion of the JAG1 domain on E14.5 accompanied by an increase in SOX2-positive cells. By E18.5, Mybl2 cKO cochleas showed an expanded sensory epithelium with additional, ectopic IHCs. Surprisingly, the sensory epithelium was disorganized suggesting that epithelial morphogenesis was disrupted. Hair cell morphology was also disrupted. To test whether the morphological changes of the epithelium are due to modifications on the cellular/epithelial cytoskeleton, we examined the F-actin cytoskeletal network. Phalloidin labeling showed that F-actin content was increased across the sensory epithelium, despite the restricted expression of Mybl2 to the progenitor cells. Accompanying the increase in F-actin, we also saw an increase in E-cadherin across the radial axis. Next, we examined whether FOXM1, the binding partner of MYBL2 during cell cycle regulation, also influences epithelial morphogenesis by generating Foxm1 cKO embryos. Foxm1 cKOs also show decreased proliferation in the IS domain. Our data show severe disruption of the F-actin structure and organization of the IS domain.
Conclusions: These data demonstrate that progenitors play an important role in establishing cochlear epithelial morphology inside and outside of the progenitor niche.

M93. TUBB4B, a Protein Linked to Sensorineural Hearing Loss, is Needed for the Development of Motile Cilia in the Middle Ear and for the Cytoskeleton Architecture of Supporting Cells in the Inner Ear

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Category: Development: Cellular/Systems

Background: Microtubules (MTs) are cytoskeletal elements comprised of αβ-tubulin heterodimers. MTs are essential for cell division, intracellular trafficking, motility, and cellular shape. The incorporation of specific α- and β-tubulin isoforms into the MTs has been proposed to regulate MT function. Indeed, an association between mutations in tubulin isoforms with tissue-specific disorders suggests a unique function for each tubulin isoform. Interestingly, mutations in TUBB4B have been linked with sensorineural hearing loss and Leber congenital amaurosis, a severe childhood blindness. However, the mechanism underlying the diseases caused by TUBB4B mutations and the unique role of TUBB4B in cochlear and photoreceptor cells remains to be elucidated and is the focus of this study.

Methods: Tubb4b knockout (KO, Tubb4b−/−) mice were used to understand the importance of TUBB4B. Auditory Brainstem response (ABR) and distortion product otoacoustic emissions (DPOAE) were used to assess auditory function. Electroretinography was conducted to assess retinal function. Fluorescence and electron microscopy were utilized to assess cellular structure in the middle and inner ear.

Results: We discovered that Tubb4b−/− mice are profoundly deaf, as neither ABR nor DPOAE responses were detected in 1-month-old Tubb4b−/− mice. TUBB4B is localized to motile cilia of ciliated cells in the middle ear, and is expressed in cochlear sensory hair, pillar, and Deiters cells. Mice lacking TUBB4B developed otitis media due to defects in motile cilia in the middle ear. We found that motile cilia were shorter, and the number of cilia was reduced in the middle ear of Tubb4b−/− mice, suggesting a unique function for TUBB4B in ciliary MTs. In the inner ear, we found that in the absence of TUBB4B, MTs of the pillar and Deiters cells were disorganized and reduced, suggesting a critical role for TUBB4B in providing mechanical support for auditory transmission. Interestingly, our analyses show that TUBB4B is not needed for vision in mice, as retinal function and morphology were unaffected in Tubb4b−/− mice.

Conclusions: Our study demonstrates that Tubb4b−/− murine model faithfully recapitulates deafness but not blindness resulting from human mutations in TUBB4B. Furthermore, we propose that TUBB4B has a unique role in motile cilia formation in the middle ear, and TUBB4B is needed for providing supporting cells with mechanical properties to transmit sound through the cochlea.

M94. Characterizing the Role of Stereocilia Links During Early Hair-Bundle Development in Mouse Inner and Outer Hair Cells

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Category: Development: Cellular/Systems

Background: CDH23 and PCDH15 play essential roles in coordinating lengths of adjacent stereocilia in developing inner hair cells1 (IHCs). In addition to constituting tip links, CDH23 and PCDH15 also form kinocilial links and transient lateral links seen in mouse IHCs and outer hair cells (OHCs) during embryonic and early postnatal development. To examine the roles of CDH23 and PCDH15 links in early hair-bundle growth, we examined cadherin localization in embryonic IHCs and OHCs. In addition, we compared hair-bundle structure and link distribution between control and mice mutant for Cdh23 and Pcdh15.

Methods: Cochleas were collected from embryonic day 15.5 (E15.5) to postnatal day 0.5 (P0.5) mice and imaged using scanning electron microscope (SEM) and lattice structured illumination microscopy (lattice SIM). Images were collected from both C57BL/6 mice and Cdh23^v2J and Pcdh15^av3J heterozygous and homozygous mice. Antibodies against CDH23, PCDH15, and acetylated tubulin were used to localize cadherin links and the kinocilium during embryonic development.

Results: While stereocilia lengthening proceeds at progressively faster rates from row 4 to row 1 (Krey et al., ARO 2024 abstract), stereocilia tips remain close together, tightly coupled with links between the stereocilia. The tight coupling of stereocilia tips occurs in the context of the apical surface's domed shape, so that the hair bundle forms an acute angle (~45°) with respect to the protruding surface. This three-dimensional organization allows stereocilia lengthening to manifest at the stereocilia base, even though actin monomer incorporation presumably still occurs at the tips. By P0.5 at mid and base, the dome flattens and the bundle is restored to a

90°angle with respect to the apical surface; at the same time, acetylated-tubulin immunolocalization showed that the kinocilium base moved laterally away from the base of the bundle. Because both CDH23 and PCDH15 were concentrated between adjacent stereocilia in patches at the tip and likely form the links holding the tips together, we examined the morphology of Cdh23^v2J and Pcdh15^av3J bundles. Cdh23^v2J/v2J and Pcdh15^av3J/av3J bundles still had 3-5 rows of elongating stereocilia, but stereocilia lengths were highly variable and had variable orientation with respect to the apical surface.
Conclusions: Because shorter stereocilia still lengthen in Cdh23^v2J/v2J and Pcdh15^av3J/av3J hair bundles, tip links are unnecessary for early stereocilia lengthening. Instead, CDH23 and PCDH15 links coordinate stereocilia height in embryonic bundles, presumably through formation of transient lateral links. These links appear to draw embryonic stereocilia tips together in a purse-string fashion, which in conjunction with the hair cell's apical dome and kinocilium, allows for coordinated growth of stereocilia. Lateral movement of the kinocilium base at the end of embryonic development may help restore the bundle to a 90° angle with respect to the apical surface. These results begin to describe how the first stages of stereocilia lengthening are controlled.

M95. P2RX4 Signaling in Auditory System Maturation and Alzheimer's Disease-Related Sensorineural Hearing Loss

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Category: Development: Cellular/Systems
Background: Ionotropic purinergic receptors (P2RX family) play an essential role in hair cell innervation through ATP signaling before hearing onset. During this period, synaptic maturation and refinement occur and tonotopic organization is established. Previous studies have shown that P2RX3 loss in spiral ganglion neurons (SGNs) leads to increased SGN branching and activity. However, when P2RX3 is down-regulated around postnatal day (P) 6, its branching phenotype disappears. Interestingly, during this phase, P2RX4 expression dramatically increases, possibly as a compensatory factor for P2RX3. P2RX4 signaling has also been reported as a degradation mechanism for one of the critical molecules for SGN survival, ApoE, which is a hallmark of Alzheimer's disease (AD). It has also been reported that AD patients are more likely to develop hearing loss. Thus, we predict that P2RX4 in the cochlea may be a key factor in hair cell innervation, and may also be a causative player in AD-related hearing loss.
Methods: We used immunostaining and IMARIS to examine SGN branching phenotypes in P2rx4 conditional knockout (CKO) cochleae with SGN sparse labeling at P4 and P8. For these experiments, Bhlhb5-Cre was used to delete P2rx4 in SGNs; Rosa26-MORF3 was used for SGN sparse labeling. Additionally, we performed APOE antibody staining on wild-type and P2rx4 CKO cochleae at different stages to determine APOE expression patterns. In a parallel line of research, we are also examining how ApoE4, the dementia-related variant of ApoE, generates different phenotypic defects in the cochlea.
Results: Our data suggest that P2RX4 is expressed by inner and outer hair cells, SGNs, and macrophages until P8. We are currently doing further expression analyses in later stages. P2RX4 loss in SGNs leads to increased branching in type I and type II SGNs at P4. In ongoing research we are determining the developmental and adult expression patterns of APOE in the cochlea.
Conclusions: According to our data, P2RX4 may compensate for the loss of P2RX3, as both are required for SGN branch refinement during early development. We are currently investigating type I SGN subtype differentiation, as well as type I and type II SGN branching phenotypes at P8 in mice that have had P2RX4 conditionally knocked-out of their SGNs. Future results may yield new strategies to protect and/or repair cochlear synapses in the context of sensorineural hearing loss, which can occur due to AD, trauma, and aging.

M96. Molecular Characterization of Vestibular-Like Hair Cell Formation in Inner Ear Organoids

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Category: Development: Cellular/Systems

Background: In 2014, a method was introduced to direct the differentiation of mouse embryonic stem cells (mESCs) toward sensory epithelial supporting cells (SCs) and hair cells (HCs) within inner ear organoids in vitro. These inner ear organoids represent an inexhaustible source of otic tissue for investigating inner ear development, function, and responses to ototoxic and regenerative treatments. However, there has been limited exploration into how closely the molecular characteristics of organoid-derived HCs align with those that naturally develop in vivo. To address this gap, we employed 10X Genomics single-cell RNA sequencing (scRNA-seq) to profile all cells from dissociated inner ear organoids at six developmental stages, spanning the formation of definitive ectoderm to the emergence of HCs. Here, we utilize this dataset to investigate the developmental progression of organoid otic vesicle (OV) cells to SCs and HCs.
Methods: Inner ear organoids were generated using Fbxo2-Venus-Hygromycin-Cre mESCs following established protocols with minor modifications. Organoids were dissociated in biological duplicate at six developmental time points: 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, we used Cell Ranger for read mapping and quantification, followed by further analysis using Seurat and Slingshot.
Results: At DIV11, OV cells were distinguished from surrounding organoid tissues by the co-expression of OV markers Sox2, Jag1, and Fbxo2. At DIV16 and DIV21, the correct formation of inner ear sensory epithelia was confirmed by the presence of HCs that co-express Atoh1, Gfi1, and Myo7a. We next combined otic lineage cells from DIV11, DIV16, and DIV21 to define the dynamic changes in gene expression that occur as OV cells mature into SCs and HCs. This analysis revealed the presence of a population of nascent HCs in DIV11 organoids, which are distinguished from DIV16 and DIV21 HCs by the expression of neuronal markers such as Neurod1 and Tubb3. Finally, we compared our organoid HC profiles with published single-cell profiles of immature and mature HCs from the mouse utricle to assess the molecular differences between organoid-derived and utricle-derived HCs. This analysis revealed that DIV16 and DIV21 organoid HCs co-cluster with immature utricular HCs and lack many molecular markers that distinguish mature utricular HC subtypes.
Conclusions: We have produced a comprehensive scRNA-seq dataset of sensory tissue formation in inner ear organoids. Our analyses suggest that organoids between DIV11 and DIV21 are suitable models for studying and manipulating early vestibular-like HC development. It is likely that additional culture time, modified culture conditions, and/or the induced expression of vestibular HC subtype-specific transcription factors are necessary for the maturation of organoid HCs in vitro.

M97. The Common Marmoset as Suitable Non-Human Alternative for the Analysis of Primate Cochlear Development

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Category: Development: Cellular/Systems
Background: The rodent model has derived much of the understanding of cochlear development. This knowledge, especially from the mouse model, is useful for understanding basic human cochlear development and would be beneficial for treating hearing loss, such as regenerative therapy. However, there are several gaps in applying the rodent model to humans. First, it is known that some mouse model fails to reproduce the human congenital hearing loss model. Secondly, the difference in gene expression patterns between rodents and human fetuses during cochlear development has been reported. These previous observations mean that the cochlear development factors have different rules between rodents and humans, at least in some cases.
However, there is more limited information about human cochlear development, especially from the molecular biology viewpoint, because of the rarity of chance to assess human fetal samples or ethical hardness in some countries. Moreover, if human fetal samples were available, they would be suitable for only anatomical or histopathological analysis and not be used for molecular biological analysis for tissue preparation problems. This study analyzed the cochlear development of the common marmoset, a non-human primate model animal.

Methods: Cadaverous temporal bone samples of common marmosets from embryonic day=70 to neonate were used in this study. The gene expression patterns were examined with immunohistochemistry. The animal experiments were approved by the Animal Experiment Committee of Keio University (number: 11006, 08020)

and were performed following the guidelines of the National Institutes of Health and the Ministry of Education, Culture, Sports, Science, and Technology of Japan. This animal's important steps of cochlear development, including the organ of Corti, spiral ganglion neurons, the stria vascularis, and lateral wall fibrocytes, were examined.
Results: Much of the point of the important steps of cochlear development has been preserved between humans and this primate model animal. Moreover, several inter-species between the rodents and this primate could be unveiled.
Conclusions: Our study confirms that cochlear development in the common marmoset is similar to that in humans and is suitable for furthering our understanding of human cochlear development. The time study established in this report will aid in studying the primate-specific developmental biology of the inner ear, which could eventually lead to new treatment strategies for hearing loss in humans.

M98. Regulation of YAP/ERM Signaling Cascade by Itga8 during Inner Ear Development

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Category: Genetics B: General
Background: Usher syndrome (USH) is a genetic heterogeneous disorder affecting neurosensory cells in the inner ear and retina. It is considered the leading cause of combined deaf-blindness for which there are currently no treatments. Just recently, our laboratory has identified a functional association between the Usher protein, protocadherin-15 (Pcdh15), and integrin alpha 8 (Itga8), during hair cell (HC) maturation in zebrafish. Based on this and previous work showing defects in the hair cell bundles in Itga8 knockout (KO) mice, we decided to further characterize Pcdh15-Itga8 complex function in the mammalian cochlea.

Methods: We generated an Itga8 knockout epithelial cell line to study the downstream signaling cascade in the absence of the Pcdh15-Itga8 complex. We created a hair-cell specific Itga8 conditional knockout (Itga8 cKO) to assess inner ear development and function. Hearing function (auditory brainstem responses [ABRs] and distortion product otoacoustic emissions [DPOAEs]) was assessed in these animals after noise exposure.

Results: We identified two main players associated with Pcdh15-Itga8 complex function: YAP and Ezrin-Radixin-Moesin (ERM), two signaling molecules involved in cytoskeletal rearrangements and cell proliferation. Cells lacking the Pcdh15-Itga8 complex showed inactivation of YAP and ERM. Likewise, immature organs of Corti from Itga8 cKOs not only showed defects in the cytoskeletal structures but also defects in YAP and ERM activities. Moreover, although under basal conditions, Itga8 cKO animals have normal hearing when challenged with a low noise stimulus (94 dB SPL), they developed permanent hearing loss. These results suggest that although Itga8 is developmentally expressed, lack of function results in an increase in susceptibility to inner ear damage. We are currently developing Itga8 conditional KOs for supporting cells and spiral ganglion neurons to identify the contribution of each cell type to the Itga8 phenotype.

Conclusions: Itga8 is involved in inner ear development via regulation of, at least in part, YAP and ERM signaling cascades.

M99. Phospho-Regulation of PCDH15 Plays a Role in Inner Ear Hair Cell Planar Polarity

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Category: Development: Cellular/Systems
Background: Our sense of hearing relies on the planar polarized structure of the hair bundle atop auditory hair cells. The normal V-shape of the hair bundle is controlled by a microtubule-based kinocilium, which is tethered to the tallest stereocilia at the vertex of the hair bundle via kinociliary links. Similar to genetic ablation of the kinocilium {1,2}, deletion of the CD2 isoform of PCDH15 (PCDH15-CD2), a component of kinocilial

links, caused defects in hair bundle shape and orientation {3}. Recently, we showed that a non-canonical Wnt signaling pathway regulates kinocilium positioning and hair bundle shape {4,5}. Some of the signaling components of this pathway are localized to the kinocilium, including active AKT, raising the possibility that local signaling within the kinocilium compartment may play a role in regulating kinocilium positioning and hair cell planar polarity. We hypothesize that PCDH15-CD2, which is trafficked into the kinocilium to form the kinociliary links, is a potential cytoskeletal target regulated by phosphorylation downstream of non-canonical Wnt signaling.
Methods: To identify phosphorylation sites on the cytoplasmic domain of PCDH15-CD2, we expressed GFP-tagged C-terminal region spanning the transmembrane and cytoplasmic domains of PCDH15-CD2 in HEK293T cells and performed immunoprecipitation followed by tandem mass spectrometry. Candidate phosphorylation sites that map close to the C-terminal PDZ-binding motif (PBM) of PCDH15-CD2 were investigated further. First, both phospho-deficient and phospho-mimetic mutations were generated using site-directed mutagenesis (hereafter referred to as CD2-SA and CD2-SD, respectively) and tested by in vitro binding assays with a CD2-PBM ligand. Next, mice carrying equivalent mutations that affect CD2-ligand interaction were generated using CRISPR-Cas9. Following backcrossing, we analyzed the hair bundle morphology, kinocilium positioning, and hair cell mechanotransduction in these mutant mice. Auditory brainstem responses (ABR) were measured to assess the changes in hearing ability.
Results: Several phosphorylated serine residues map to the region surrounding the PBM of PCDH15-CD2. This led us to hypothesize that phosphorylation could play a role in modulating the interaction of PCDH15-CD2 with PDZ-domain-containing ligands. Using in vitro binding assays using Whirlin as a surrogate CD2-PBM ligand, we found that CD2-SA decreased whereas CD2-SD increased the binding interaction. Preliminary analysis of mutant mice showed mild but significant kinocilium positioning and hair bundle defects. Notably, a small number of inner hair cells in both CD2-SA and CD2-SD mice had split hair bundles. Furthermore, CD2-SD mice presented slightly elevated ABR thresholds at higher frequencies when compared to their littermate controls. We are currently conducting experiments to analyze CD2 localization in CD2-SA and CD2-SD mice using super-resolution microscopy, assess hair cell mechanotransduction, and identify CD2 interaction partners that regulate kinociliary link dynamics.
Conclusions: Overall, this research will elucidate how the phosphorylation of PCDH15-CD2 influences hair cell planar polarity and hearing function.

M100. The Role of AQP4 in Developmental Spontaneous Activity

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Category: Development: Cellular/Systems
Background: In the developing auditory system, spontaneous bursts of electrical activity initiated by inner supporting cells in the cochlea, propagate from the periphery to sound-processing circuits in the brain. In rodents, this spontaneous activity emerges before birth at approximately embryonic day 16 and lasts until the onset of the hearing (~ postnatal day 12), providing a prolonged time window for activity-dependent circuit refinement and maturation. Inner supporting cells in the developing cochlea also exhibit osmotic decreases in cell size (crenation), a result of enormous ion efflux from supporting cell triggered by purinergic receptor activation. Although these events are correlated with spontaneous bursts of action potentials, and these changes in extracellular space influence inner hair cell excitability, the functional significance and molecular mechanisms responsible for this water movement have not been determined. We hypothesize that this rapid water movement requires flux through dedicated water channels. Using publicly available single-cell RNA sequencing data from developing cochleae, we identified aquaporin 4 (AQP4) as a possible candidate responsible for this water transport, due to its high expression level in the sensory epithelium during development. Notably, a mutation impairing the channel's water transport capability has been identified in patients with hearing loss, suggesting that there may be a link between this aspect of supporting cell function and cochlear output.
Methods: In this study, we generated AQP4 conditional knockout mice (Tecta-Cre; Aqp4fl/fl), in which this water channel was specifically removed from the organ of Corti, and performed both imaging of crenation events and whole-cell recording of spontaneous currents in supporting cells to investigate the role of AQP4 in developmental spontaneous activity.

Results: Unexpectedly, assessment of hearing function using ABR just after hearing onset indicated that the AQP4 cKO mice have normal ABR thresholds, while AQP4 null mice are deaf.
Conclusions: Together, these studies will help define the role of AQP4 in auditory function and circuit development, with relevance to restoration of hearing in individuals with congenital hearing loss.

M101. Exploring Sex-Based Differences in the Constitutive Expression of Cochlear Genes Linked to Auditory Dysfunction: Findings From a Mouse Study

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Category: Genetics A: Genomics and Gene Regulation
Background: Gene mutations are a significant contributing factor to auditory dysfunction. To date, researchers have identified over two hundred genes associated with genetic hearing impairment. It is well-established that sex plays a crucial role in influencing gene expression and function. This current study aims to explore sex-based differences in the constitutive expression of established genes associated with hearing loss in mice.
Methods: Mice with C57BL/6J genetic background (n=4 samples per sex, aged 2 months) were used in this study. Female cochleae were specifically collected during the estrus stage of the estrous cycle, determined through vaginal cytology analysis. Cochlear tissues were harvested, including sensory epithelia, spiral ganglions, and lateral wall tissues. Total RNAs extracted from the tissues were used for RNA sequencing to assess the expression levels of deafness genes. The list of genes associated with auditory dysfunction was obtained from two online sources: the Hereditary Hearing Loss Homepage (https://hereditaryhearingloss.org/) and OtoSCOPE (https://morl.lab.uiowa.edu/genes-included-otoscope-v9). Differentially expressed genes were examined for their functional implications using multiple bioinformatics tools, including DAVID, IPA Analysis Match Explorer, and MitoCarta.
Results: We compiled a list of 255 deafness genes from the two online sources. Among these genes, 55 exhibited sex-specific differences in their expression levels, as determined by an adjusted p-value of less than 0.05. Within this subset, 28 genes demonstrated male-biased expression, while 27 genes displayed female-biased expression. Notably, a substantial proportion of these genes (25 out of 55) belonged to non-coding RNAs (ncRNAs), specifically inactive X-specific transcripts. The remaining genes encompassed various functional categories, including 5 protein-coding genes, 6 miRNA genes, 8 lincRNA) genes, 10 lncRNA genes, and 1 lncRNA gene. Within this gene set, 8 genes had direct associations with mitochondrial functions. Among them, 5 genes were classified as mitochondrial genes, namely mt-Rnr1, mt-Nd1, mt-Tl1, mt-Ti, and mt-Co1, which primarily function in mitochondrial energy production and cellular respiration. Regarding sex-based differences in expression levels, the most significant differences were observed in male-biased genes. The top 5 male-biased genes included mt-Rnr1, Lars2, mt-Nd, mt-Tl1, and mt-Ti, all of which play pivotal roles in cellular energy production and overall cellular function. The top 5 female-biased genes comprised Sema3e, Dmxl2, Atp6v1b2, Atp2b2, Ppip5k2. These genes have roles in diverse cellular processes, such as neural development, modulation of cellular signaling pathways, regulation of cellular pH balance, and maintenance of cellular calcium homeostasis.
Conclusions: Our expression analysis unveiled a group of deafness-related genes displaying sex-associated expression differences. This finding offers valuable insights for future research efforts aimed at exploring potential sex-specific differences in hearing loss linked to these genes.

M102. A Gene Regulatory Landscape and Network That Drives Hair Cell Regeneration in Zebrafish

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Category: Genetics A: Genomics and Gene Regulation
Background: Sensory hair cells in the vertebrate ear are responsible for hearing through the transduction of vibrations into nerve impulses, but do not regenerate when damaged or killed, leading to permanent hearing loss. Zebrafish and mammalian sensory hair cells are functionally and genetically homologous, however

zebrafish hair cells rapidly regenerate after damage or death. Our previous studies characterized transcriptional changes in the lateral line in a fine time scale using scRNA-seq, and we identified three core modules that drive regeneration of hair cells. A key missing perspective, however, is the epigenetic and regulatory landscape during regeneration that direct these transcriptional changes.
Methods: To understand the genetic program driving hair cell regeneration, we completed a finely spaced ATAC-seq and ChIP-seq time course of the epigenetic regulatory environment and combined this information with existing scRNA-seq data. Histone modifications assayed include H3K4me3, which marks active promoters, and H3K27ac, which marks both active promoters and distal enhancers. We used bioinformatic tools, including ANANSE, to find the most active and dynamic regulatory regions and transcription factors in the genome. Additionally, we performed DNA motif searches to identify highly enriched transcription factor binding sites to reveal a regulatory code at different stages of regeneration. Finally, we explored the role of individual enhancers by both cloning them driving a GFP reporter and deleting them to assay their necessity in gene expression and hair cell development and regeneration.
Results: Chromatin accessibility and regulatory histone marks rapidly change, matching the expression dynamics of genes during regeneration. Using hierarchical clustering, we found co-regulated enhancers form ten "regulatory groups" across time. Motif enrichment analysis and use of bioinformatics tools reveals that enhancer groups are defined by a unique core set of transcription factors necessary at steps of regeneration/regulatory modules in regeneration. We identified specific enhancers from the ten regulatory groups and show that they drive reporter gene expression. Functional analyses show that their deletion causes hair cell regeneration defects, demonstrating that these regulatory links identified are essential for hair cells. Importantly, injury/stress response genes, such as AP-1 genes fos and jun have highly enriched binding sites in enhancers of hair cell regeneration genes. When we inhibit the AP-1 pathway, we see a drastic reduction of target gene expression and hair cell regeneration defects, indicating a direct regulatory link between the injury response from dying hair cells and the genes necessary to regenerate new hair cells.
Conclusions: Our epigenetic data has allowed us to build a GRN to describe hair cell regeneration. By understanding the regulatory landscape and how links and binding motifs have broken or evolved between zebrafish and mammals, our data provide key insight and gene targets for hair cell regeneration in mammals, and the centrality of linking injury response with regeneration.

M103. Multiomic Changes in Sensory Hair Cells After Ablation of Chromatin Remodeling Protein CHD4

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Category: Genetics A: Genomics and Gene Regulation
Background: The chromatin helicase binding protein 4 (CHD4) is an ATP-dependent nucleosome remodeler that alters chromatin accessibility. CHD4 is a core component of the nucleosome remodeling and deacetylase (NuRD) complex component that represses transcription. Pathogenic variants of human CHD4 correlate to Sifrim-Hitz-Weiss (SIHIWES) syndrome. Patients with SIHIWES show delayed development, intellectual disability, facial dysmorphism, ear abnormalities, and hearing loss. Although expression of CHD4 has been reported in hair cells and supporting cells, the molecular and cellular consequence of how CHD4 mutations lead to hearing loss is poorly understood. Since cochlear hair cells are essential for hearing, we wanted to clarify the function of CHD4 in hair cells.
Methods: Two different Chd4 conditional knockout (cKO) animals were generated using the Atoh1-CreERTM or Otoferlin-Cre drivers. The Chd4 cKO animals were crossed to the Ai9 tdTomato reporter line to monitor Cre activity in hair cells. Cochlea sensory epithelia tissues from Chd4 cKO and control animals were collected and subjected to immunostaining to confirm hair cell-specific ablation of CHD4 protein. Cochleae were subjected to single-cell RNAseq (scRNAseq) and single-cell ATACseq (scATACseq) to determine molecular changes in the transcriptome and chromatin accessibility.
Results: CHD4 was not required for early hair-cell development but was needed for maturation and synaptogenesis. Cochleae from Chd4 ckO animals displayed hair cell death starting between postnatal day (P)5 to P10. Accompanying hair cell loss in Chd4 cKO cochleae, the hair cells also showed decreased nuclear foci numbers, suggesting fewer heterochromatic regions in the nucleus. The results indicate that loss of CHD4 altered chromatin structure and caused hair cell death. Single-cell multiome analysis showed genome-wide

changes in chromatin accessibility at regulatory regions in Chd4 cKO hair cells. Decreased chromatin accessibility near hair cell genes was observed, while increased accessibility in supporting cell and neuronal genes was seen in Chd4 cKO hair cells. These molecular changes are likely the underlying cause of hair cell death. The results implicate CHD4 in establishing a chromatin signature essential for hair cell identity. Interestingly, changes in hair cells indirectly altered the chromatin accessibility of other cochlear cells in Chd4 cKO animals.
Conclusions: These results suggest that CHD4 epigenetically regulates genetic programs essential for proper hair cell function and maintenance. Further, defining the epigenetic changes in damaged cochleae cells will accelerate efforts to convert supporting cells into hair cells for hearing restoration.

M104. Rare Coding Variants in Patients With Non-Syndromic Vestibular Dysfunction

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Category: Genetics B: General
Background: Vertigo due to vestibular dysfunction is rare in children. The elucidation of its etiology will improve clinical management and the quality of life of patients. Genes for vestibular dysfunction were previously identified in patients with both hearing loss and vertigo. This study aimed to identify rare, coding variants in children with peripheral vertigo but no hearing loss, and in patients with potentially overlapping phenotypes, namely, Meniere's disease or idiopathic scoliosis.
Methods: Rare variants were selected from the exome sequence data of 5 American children with vertigo, 226 Spanish patients with Meniere's disease, and 38 European–American probands with scoliosis.
Results: In children with vertigo, 17 variants were found in 15 genes involved in migraine, musculoskele-tal phenotypes, and vestibular development. Three genes, OTOP1, HMX3, and LAMA2, have knockout mouse models for vestibular dysfunction. Moreover, HMX3 and LAMA2 were expressed in human vestibular tissues. Rare variants within ECM1, OTOP1, and OTOP2 were each identified in three adult patients with Meniere's disease. Additionally, an OTOP1 variant was identified in 11 adolescents with lateral semicircular canal asymmetry, 10 of whom have scoliosis.
Conclusions: We hypothesize that peripheral vestibular dysfunction in children may be due to multiple rare variants within genes that are involved in the inner ear structure, migraine, and musculoskeletal disease.

M105. Audiologic Measures in an Indigenous Community With A2ML1- And FUT2-Related Otitis Media

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Category: Genetics B: General
Background: Indigenous populations are at higher risk for otitis media, however there is limited information on hearing loss due to otitis media in indigenous communities. An indigenous Filipino community has a high prevalence of otitis media due to rare A2ML1 variants and a common FUT2 variant. In this study, we describe the audiologic profiles in A2ML1- and FUT2-related otitis media and the validity of otoscopy and genotyping for A2ML1 and FUT2 variants in screening for otitis media and hearing loss.
Methods: We analyzed A2ML1 and FUT2 genotypes together with demographic, otologic and audiologic data from tympanometry and hearing level assessments of 109 indigenous individuals.
Results: We confirmed previous findings of a spectrum of nonsyndromic otitis media as associated with A2ML1 variants. A2ML1 and FUT2 variants were associated with high-frequency hearing loss at 4000Hz. As expected, young age was associated with flat tympanograms, and eardrum perforations due to chronic otitis media were associated with severe-to-profound hearing loss across frequencies. Adding A2ML1 or FUT2 genotypes improved the validity of otoscopy as a screening test to rule out moderate-to-profound hearing loss.

Conclusions: Continued multi-disciplinary management and audiologic follow-up using tympanometry and screening audiometry are needed to document and treat otitis media and prevent permanent hearing loss in the indigenous community.

M106. HDAC Inhibitor, Trichostatin a Inhibits Hearing Loss in a Model of Alport Syndrome

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Category: Genetics B: General

Background: Alport syndrome (AS) is caused by a mutation in type IV collagen, one of the major proteins in the basement membrane. Kidney and cochlea are frequently involved causing glomerulonephritis and hearing loss. Hearing in AS is usually normal at birth, progressively deteriorating thereafter. To evaluate the impact of acquired genetic changes on disease progression, we tested the effects of treatment with Trichostatin A (TSA), an HDAC inhibitor, on hearing in an AS model.

Methods: Knockout (KO) mice of type IV collagen alpha 3 chains (Col4α3) were used in the AS model (Jackson lab, 129x1/SVJ). The treatment group was intraperitoneally injected with TSA from 3 weeks of age. Hearing levels were measured by auditory brainstem response audiometry (ABR) from 4 weeks of age to 9 weeks when homozygous KO began to die of end-stage renal failure. Cochlear were harvested at 9 weeks, and underwent 4-Hydroxynonenal (4-HNE) immunostaining. Basement membrane thickness was checked using TEM (Transmission Electron Microscope). TSA(1uM) was treated to HEI-OC1 cell and Western blot was done to see the molecular changes.

Results: From week 4, the hearing threshold gradually increased in AS mice, reaching a plateau of approximately 50±5 dB at 5 weeks of age. The KO treated with TSA had a better ABR threshold of 40±3 dB at 7 weeks of age in AS mice, while the untreated group was 50±5 dB. Col4α3 KO showed dispersed and enlarged lateral wall, especially in the stria vascularis. Spiral ganglion neurons and hair cells in the organ of Corti appeared to be normal. Basement membrane thickness of the TSA-treated group was comparable to the control in Col4α3 KO mice. Instead, 4-HNE was significantly elevated in KO mice and TSA reduced it. In HEI-OC1 cells, inflammatory molecules TGFβ1, TNFα, IL-6 and IL-1β were down-regulated by TSA treatment. Hearing thresholds were gradually increased in Col4α3 KO mice and HDAC inhibitor TSA inhibited this hearing impairment in AS mice. Oxidative stress marker 4-HNE was increased in AS mice in which TSA reduced it. TSA reduced inflammatory cytokines in HEI-OC1 cells.

Conclusions: TSA is a candidate molecule for inhibiting hearing deterioration in AS model.

M107. Reinterpretation of Pathogenic Variants Associated With Hearing Loss in Korean Population

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Category: Genetics B: General

Background: In clinical practice, the accurate interpretation of rare variants presents significant difficulties. As the number of case reports continues to rise, the potential misinterpretation of previously reported pathogenic or likely pathogenic variants becomes a significant obstacle. This misinterpretation can lead to inaccurate genetic diagnoses. Due to inadequate sample sizes in commonly used population databases, rare variants, especially those identified in under-represented populations, can contribute to misinterpretation. In this study, we attempted to reevaluate previously reported pathogenic or likely pathogenic variants by providing accurate allele frequencies of Koreans (n = 1,916 individuals in gnomAD), a population underrepresented in major databases.

Methods: We evaluated rare genetic variants associated with non-syndromic hearing loss (NSHL) that have been reported as pathogenic or likely pathogenic in the Deafness Variation Database (https://deafnessvariationdatabase.org/) utilizing 9,839 whole exome or genome sequence data of Korean individuals and patient cohort of 473 individuals with sensorineural hearing loss.

Results: Of the 8,173 pathogenic or likely pathogenic variants in the Deafness Variation Database, 302 (3.69%) were identified in Korean population data and 31 exceeded the minor allele frequency cutoff for

hearing loss (BA1 and BS1 criteria of the ACMG guideline). In accordance with previous reports, four variants were identified as pathogenic founder alleles in our patient cohort. In addition to the identification of novel founder alleles, seven disease-causing variants remained. Our pipeline identified 17 pathogenic or likely pathogenic variants that should be reclassified as benign or likely benign variant and three variants as variant of uncertain significance. Following a reevaluation of pathogenic variants, we were able to identify 1.35% of Koreans who are potentially diagnosed with an inherited form of hearing loss.
Conclusions: This study illustrates the significance of using minor allele frequencies from under-represented populations to interpret rare variants more precisely.

M108. MYH1 Variants Cause Autosomal Recessive Hearing Loss by Disrupting Outer Hair Cell Electromotility

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[1]*Yonsei University College of Medicine*

Category: Genetics B: General
Background: Hearing loss is a prevalent sensory impairment affecting individuals worldwide, with diverse genetic factors contributing to its etiology. In this study, we explored the genetic underpinnings of hearing loss by conducting whole exome sequencing on a cohort of 437 families. Within this cohort, we identified biallelic variants in the myosin heavy chain 1 (MYH1) gene in five unrelated families. Based on multiple functional studies, we suggest MYH1 as a novel gene responsible for syndromic hearing loss.
Methods: Whole exome sequencing was employed to screen 437 families with hearing loss for genetic variants. Phenotypic data, including the onset and progression of hearing loss, as well as the presence of osteopenia, were collected from affected individuals. Structural predictions of MYH1 variants were conducted using AlphaFold2, followed by molecular dynamics (MD) simulations with GROMACS. Auditory assessments such as auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE) were performed on Myh1 knockout (KO) mice. Voltage clamp electrophysiology was performed in outer hair cells of wild-type and Myh1 KO mice to measure Prestin activity. Prestin activity and traction force were measured with mutant MYH1 proteins in heterogeneous overexpression systems.
Results: Among the 437 families with hearing loss, we identified biallelic MYH1 variants in five unrelated families. The hearing loss observed in individuals with these variants ranged from congenital to childhood onset and showed a non-progressive course. Osteopenia was present in two out of the five affected patients. Structural analysis revealed that the MYH1 variants exhibited structural abnormalities compared to the wild-type MYH1. Myh1 knockout mice exhibited elevated auditory threshold, absence of distortion product optoacoustic emission threshold, and decreased bone mineral density. Myh1 was expressed in multiple inner ear cell types, including hair cells, supporting cells, and spiral ganglion neurons. Direct recording from outer hair cells (OHCs) indicated compromised Prestin activity in Myh1 KO mice, reflecting abnormal OHC electromotility. In heterogenous overexpression systems, MYH1 variants reduced Prestin activity and membrane traction force.
Conclusions: Our findings suggest that MYH1 variants are associated with syndromic autosomal recessive hearing loss, accompanied by decreased bone mineral density. These results provide insights into the structural and functional consequences of MYH1 variants, shedding light on their potential role in hearing loss pathology. The expression of Myh1 in various inner ear cell types and its impact on Prestin activity highlight the intricate mechanisms involved in auditory function and underscore the importance of MYH1 in hearing-related processes.

M109. PKHD1L1, a Gene Involved in the Stereociliary Coat, Causes Autosomal Recessive Nonsyndromic Hearing Loss

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Category: Genetics B: General

Background: Identification of genes associated with nonsyndromic hearing loss is a crucial endeavor, given the substantial number of individuals who remain without a diagnosis after even the most advanced genetic testing. PKHD1L1 was established as necessary for the formation of the cochlear hair-cell stereociliary coat and causes hearing loss in mice and zebrafish when mutated. We sought to determine if biallelic variants in PKHD1L1 also cause hearing loss in humans.

Methods: Exome sequencing was performed on DNA of three families segregating autosomal recessive moderate to severe nonsyndromic sensorineural hearing loss. In vitro functional analysis of two missense variants was performed using purified recombinant PKHD1L1 protein fragments. We then evaluated protein thermodynamic stability with and without the missense variants found in one of the families.

Results: Compound heterozygous missense p.[(Gly129Ser)];p.[(Gly1314Val)], homozygous missense p.(His2479Gln) and nonsense p.(Arg3381Ter) variants were identified in PKHD1L1 that were predicted to be damaging using in silico pathogenicity prediction methods. In vitro functional assessment indicated that both engineered PKHD1L1 mutant p.(Gly129Ser) and p.(Gly1314Val) constructs significantly reduced the folding and structural stabilities of the expressed protein fragments, providing further evidence to support pathogenicity of these variants. In silico molecular modelling using AlphaFold2 and protein sequence alignment analysis were carried out to further explore potential variant effects on protein folding and stability and exposed key structural features that might suggest PKHD1L1 protein destabilization.

Conclusions: Multiple lines of evidence collectively associate PKHD1L1 with nonsyndromic moderate to severe sensorineural hearing loss. PKHD1L1 testing in individuals with moderate hearing loss may identify further affected families.

M110. Defects in Exosome Biogenesis Are Associated With Deficits in Auditory Sensorimotor Transformation in Zebrafish VPS4A Mutants

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Category: Genetics B: General

Background: Haploinsufficiency of human vacuolar protein sorting homologue 4A (VPS4A) is associated with neurodevelopmental defects, including hypotonia, ataxia, and sensorineural deafness. VPS4A is an AAA-ATPase that is required for membrane scission within cells, which is key to several biological processes. How mutations in VPS4A lead to sensory deficits is currently unknown.

Methods: The vps4a mutant was originally isolated from a forward mutagenesis screen and identified using Whole Genome Sequencing and SNPTrack software. The behavioral assays (AEBR, VSR, and VIEM) have been previously described (Maeda et al., 2017; Gao and Nicolson, 2020). Standard LysoTracker labeling and RNA-FISH methods were used to characterize gene misexpression in the mutant. Transient expression of CD63pHluorin was used to study the release of exosomes and calcium imaging was used to quantify neuronal activity in response to tone stimuli in the vps4a mutant.

Results: We identified a recessive mutation in zebrafish vps4a, T248I, that results in defects in acoustic startle and vestibular spinal reflex. Consistent with the established role for Vps4a in the formation of intraluminal vesicles in endosomes that are subsequently released as extracellular vesicles, we observed enlarged acidic membrane compartments in the central nervous system and decreased numbers of circulating exosomes in mutant brain ventricles in vivo. As exosomes are implicated in the regulation of transcription, we analyzed bulk RNAseq data and found pronounced changes in vps4a mutants. Genes associated with transcription (atf3 and jun) and neuronal growth cones (gap43) are highly upregulated in the photoreceptors and retinal ganglion cells in the eye, and throughout the mid- and hindbrain regions of vps4a larvae. To investigate how neuronal activity may be affected by the loss of vps4a function, we used a pan-neuronal GCaMP7a calcium indicator to visualize sound-evoked activity. Consistent with the lack of changes in acidic compartments or gene regulation in saccular hair cells and afferent neurons in vps4a mutants, these two cell types show normal

activity in response to pure tones. Notably, neurons of the torus semicircularis, a key region for relaying auditory cues and akin to the mammalian inferior colliculus, also show sound-evoked activity in vps4a mutants that is comparable to wild-type siblings. In contrast, mutant motor neurons in the spinal cord have strongly reduced calcium transients in response to tone stimuli compared to wild-type motor neurons. Considering that vps4a mutants have a robust touch startle reflex, these results imply that sensorimotor transformation of auditory cues is specifically affected.
Conclusions: Together, our data suggest that the T248I mutation in vps4a reduces membrane scission of endosomes and biogenesis of exosomes, resulting in the dysfunction of distinct cell types in central neural circuits that transform auditory cues into motor behaviors.

M111. The Pathogenesis of Common Gjb2 Mutation Associated With Human Hereditary Deafness in Mice

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Category: Genetics B: General
Background: Mutations in Gjb2 are the most common genetic cause of hereditary deafness in humans, especially the 35delG and 235delC mutations. Owing to the homozygous-lethal of Gjb2 mutation in mice, there are currently no perfect mouse models carrying Gjb2 mutation derived from patients to mimic human hereditary deafness and unveil the pathogenesis. Here, we constructed Gjb2 +/35delG and Gjb2 +/235delC mice for studying hearing function in heterozygous carriers and a homozygous mutant mouse model, Gjb2 35delG/35delG, completely mimicking the phenotypes, and revealing the pathogenesis of the DFNB1A-related human hereditary deafness.
Methods: The heterozygous mice were generated using androgenic haploid embryonic stem cells mediated semi-cloning technology. The homozygous mice were constructed via tetraploid embryo complementation. The auditory functions were assessed by ABR and DPOAE. Immunostaining was used to observe the expression of Cx26 and Cx30, the survival of hair cells, and supporting cells. Transmission electron microscopy was employed to observe the tunnel of the organ of Corti. RNA-Seq was performed to analyze the transcriptome data for the cause of hearing loss. The endocochlea potential (EP) was performed to study the electrophysiology of the cochlea.
Results: Gjb2 +/35delG and Gjb2 +/235delC mice show normal hearing at a young. The Gjb2 35delG/35delG mice exhibited normal development of hair cells and supporting cells at E17.5, P0, and P14, but complete hearing loss occurred with the reduction of the expression of Cx26 and Cx30 at P14 when the mouse cochlea approaches maturity. The supporting cells did not show a significant change in the cochlea of the homozygous 35delG mice at P35, while OHCs but not IHCs were partially lost. A significant reduction of EP in Gjb2 35delG/35delG mice happened at both P14 and P35. The tunnels of the organ of Corti were closed in Gjb2 35delG/35delG mice in contrast to the open tunnels in the wild type. Mechanism analysis showed that Gjb2 35delG disrupts the formation of intercellular gap junction channels, resulting in the dysregulation of genes enriched in synapse organization and axonogenesis, rather than the intactness of hair cells and supporting cells.
Conclusions: Collectively, our study provides ideal mouse models for understanding the pathogenic mechanism and opens up a new avenue for investigating the treatment for DFNB1A-related hereditary deafness.

M112. Identifying CHD4 Enrichment at Enhancers During Inner Ear Stem Cell Differentiation

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Category: Regeneration
Background: Spiral ganglion neurons (SGNs) are essential for transmitting sound information. SGNs cannot regenerate, and the loss of these neurons causes hearing loss in mammals. Stem cell replacement by directed differentiation of otic progenitors to SGNs has been proposed as a promising approach to repopulate SGNs. Understanding the molecular mechanisms that govern SGN differentiation will accelerate regenerative therapies. Using the immortalized multipotent otic progenitor (iMOP) cell line as a cellular model system, we identified the chromodomain helicase DNA-binding protein 4 (CHD4) as a candidate that promotes the differentiation of otic progenitors into neurons. CHD4 regulates cell fate decisions through its function at regulatory elements such as enhancers to control gene expression. Epigenetic changes at enhancer elements contribute to the different gene expression patterns and help confer cell identity. CHD4 function at enhancers during otic progenitor differentiation into SGNs is unknown.
Methods: Stable iMOP cell lines expressing scrambled shRNA and Chd4 shRNA were used to identify the role of CHD4 in iMOP neuronal differentiation. Cleavage Under Targets and Tagmentation (CUT and Tag) was used to identify CHD4 binding sites and deposition of histone marks in proliferating iMOPs and iMOP-derived neurons. Putative enhancers with histone marks (H3K4me1 and H3K27ac) in iMOPs were used to define enhancer states. Bioinformatics tools were employed to investigate the CHD4-enhancer network during the differentiation of proliferating iMOPs into iMOP-derived neurons.
Results: Knockdown of Chd4 perturbed the expression of the neuronal marker (TUBB3) and shortened neurites relative to controls. These results suggest that CHD4 contributes to the neuronal differentiation of iMOPs. CUT and Tag analysis enabled us to define active and inactive/poised enhancers based on the H3K27ac or H3K4me1 enrichment. The correlation of CHD4 binding at neuron-specific enhancers suggests that CHD4 alters the chromatin state at enhancers to modulate gene expression during neuronal differentiation.
Conclusions: Knockdown of Chd4 affects neuronal differentiation of iMOPs. CUT and Tag analysis shows CHD4 enrichment at distinct and common enhancers in proliferating iMOPs and iMOP-derived neurons. Identifying changes in the CHD4-enhancer network allows a better understanding of the epigenetic changes that occur during neuronal differentiation. The molecular changes will accelerate strategies to repopulate SGNs using stem cell replacement therapies.

M113. Overexpression of SIRTUIN1 Protein by N1-Methylnicotinamide (MNAM) Promotes Age-Related Cochlear Damage

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Category: Aging
Background: Age-related hearing loss (ARHL) is the common form of hearing loss associated with aging. Sirtuin 1 (SIRT1) is associated with the most complex physiological processes, including metabolism, cancer onset, and aging. SIRT1 proteins are stabilized by the conversion of nicotinamide to N1-methylnicotinamide (MNAM), independent of its mRNA levels. Moreover, MNAM has implications in increased longevity achieved through its mitohormetic effects. Our previous study showed that administration of MNAM to high-fat diet-induced age-related hearing loss model mice inhibited SIRT1 protein expression in the cochlea, thereby preventing the onset of ARHL. However, it has also been reported that the relationship between SIRT1 expression level and aging is controversial (Alcendor, 2007), and opinions regarding the effect of SIRT1 on ARHL have been controversial. In this study, we aimed to determine the relationship between hearing function and SIRT1 expression levels in the inner ear, and cochlear morphology in normal diet manner.
Methods: Female wild-type C57BL/6 mice were used as a model of age-related hearing loss (Miwa ,2021). Mice were divided into two groups. One group received MNAM mixed with normal diet orally for 12 months (LFD+MNAM group). The other served as a control, consuming only normal food (LFD group). The effects of MNAM and SIRT1 on the development of age-related hearing loss were evaluated audiology and morphologically by comparing these mice.
Results: In the LFD+MNAM group, hearing loss progressed more gradually than in the LFD group from the age of 6 months, and endocochlear potentials decreased. In addition, increased SIRT1 protein expression was observed in fibroblasts of the spiral ligament in the LFD+MNAM group, while SIRT1 protein expression was slightly decreased in the LFD group.

Conclusions: MNAM treatment of mice models of age-related hearing loss increased SIRT1 protein expression in the cochlea and reversed the onset of age-related hearing loss; although SIRT1 has been shown to have a role in ameliorating age-related hearing loss caused by high-fat dietary stress (Miwa, 2021), excess SIRT1 protein SIRT1 protein excess may inversely cause cochlear injury.

M114. Impact of Aging and Temporal Salience on Unit Responses to SAM Stimuli in the Inferior Colliculus of the Awake Rat

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Category: Aging

Background: Age-related hearing loss (ARHL) is a complex disorder affecting between 50-70% of the United States population aged 65 or older. In public settings, seniors frequently have great difficulty understanding speech, which can lead to withdrawal from social activities, depression and cognitive decline. All individuals and especially the elderly can maintain speech understanding in difficult listening conditions by engaging attentional, cognitive and mnemonic (top-down) resources to help disambiguate speech from a degraded ascending acoustic code. Unit recordings from awake rat auditory thalamus (MGB) have shown age-related repetition-enhancement, as opposed to sensory adaptation, in response to repeating/predictable temporally rich/sinusoidally amplitude modulated (SAM) stimuli. Repetition enhancement was shown to reflect increased use of corticofugal/higher-order resources. Enhanced coding of repeating stimuli was engaged in young MGB by degrading the temporal clarity of the SAM stimuli.

Methods: Here we examine the impact of aging on the coding of repeating/predictable SAM in the awake rat inferior colliculus (IC). SAM stimuli were modulated between 2 to 1024 Hz with 16 trials per fm delivered randomly across trials (RAN) or in a repeating/predictable fashion (REP). Modulation depth could be decreased from 100% to 25% effectively reducing the temporal clarity of the SAM stimulus. "Noisy SAM", the sum of the envelope and low-pass filtered (1000 Hz) broadband noise, was also used to reduce temporal clarity.

Results: In response to SAM stimuli, preliminary single unit recordings from awake rat IC showed notable age- and salience-dependent differences in sequence preference. Virtually all units from young IC show a preference for randomly presented more salient SAM (100% modulation depth) or showed no preference for RAN or REP SAM stimuli (within ±10%). While a majority of IC units from aged animals were also random preferring, decreasing SAM modulation depth increased the percentage of aged units preferring repeating/predictable SAM. Decreasing salience by using noisy SAM stimuli showed smaller preference changes in aged unit responses relative to decreasing modulation depth. In contrast to aged animal, noisy SAM stimuli increased unit responses to predictable SAM stimuli in young rats. Decreasing modulation depth had minor effects on the preference ratio (REP/RAD) in our limited population of units from young rat IC. The preference ratio responses from aged IC units increased with decreasing SAM modulation depth. Significant differences were seen in responses to SAM at 25% modulation depth between young and aged group.

Conclusions: The present study shows that, similar to auditory cortex and auditory thalamus, repetition enhancement is present in the auditory midbrain of aged rats. These findings suggest that loss of temporal stimulus salience likely engages top-down resources to help disambiguate less-salient modulated signals as early as auditory midbrain.

M115. Electrophysiological Insights Into Age-Related Neural Changes in the Aging Auditory System and Speech-In-Noise Decline

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Category: Aging

Background: Age-related auditory changes at the cochlear level can influence neurophysiological responses across the central auditory pathway. Such alterations may be related to declines in speech-in-noise perceptual capabilities in older adults. The study intends to explore electrophysiological markers associated with age-

related hearing loss and neural correlates of declines in speech-in-noise perceptual capabilities with advancing age. Therefore, the study proposes two specific aims: (1) to investigate the differential and compound effects of aging and hearing loss on auditory neural processing; and (2) to identify electrophysiological correlates of declines in speech-in-noise perception in older adults.

Methods: The study included 30 young normal-hearing adults (YNH, M=21 years, range=18-28 years), 26 older adults with normal or near normal hearing (ONH, M=63.9 years, range=56-75 years) and 26 older adults with age-related hearing loss (OHL, M=72.8 years, range=59-89 years). Auditory middle latency responses (AMLR) and auditory late latency responses (ALLR) were monaurally and ipsilaterally recorded at rates of 11.1/s and 0.71/s, respectively, in separate runs. The synthesized speech /ba/ was presented at 100 dB peSPL in quiet and 12-talker babble noise conditions set at 65 dBA. Using a two-channel setup, one channel recorded responses with a non-inverting electrode at Cz, while the other monitored eyeblink artifacts. Analysis highlighted Pa of AMLR and N1-P2-N2 of ALLR. To determine correlation with these electrophysiological outcomes, the revised Speech Perception in Noise (R-SPIN) test was assessed and low-predictability scores were only computed.

Results: The effects of aging revealed significantly enhanced Pa and N1 amplitudes and significantly prolonged Pa, P2 and N2 latencies in ONH relative to YNH group. The effects of hearing loss revealed significantly prolonged N2 latencies in OHL relative to ONH group. The compound effects of age-related hearing loss revealed significantly enhanced amplitudes and significantly delayed latencies across all AEP components in OHL relative to YNH group, indicating a stronger impact on age-related auditory neural processing. In babble noise, all groups demonstrated reduced AEP responses with marked delay in latency, highlighting noise-induced neural disruption. This effect was more pronounced in older groups, especially in the OHL group. Enhanced Pa amplitudes and prolonged N2 latencies were significantly correlated with lower R-SPIN scores in both older groups. Age-related enhancements of suprathreshold Pa amplitudes and age-related prolongations of N2 latencies may serve as electrophysiological correlates of age-related declines in speech-in-noise perceptual ability.

Conclusions: The study suggests that when aging interacts with hearing loss, it amplifies the age-related deterioration in auditory neural processing at subcortical and cortical levels. Pa of AMLR and N2 of ALLR indicate electrophysiological markers of age-related neural changes in the aging auditory system. Age-related enhancement of suprathreshold Pa amplitudes may reflect suprathreshold auditory processing deficits. Age-related prolongation of N2 latencies may reflect the slowing of temporal processing speed.

M116. Speech Discrimination in Aging: MMN Study

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Category: Aging

Background: Previous research suggests age-related deteriorations of central auditory processing, reduced cognitive capacity, and decline in speech discrimination abilities and memory (e.g., Choi et al., 2022). Aging is a risk factor for language comprehension decline, impacting social functionality and quality of life. Several studies have shown that mismatch negativity (MMN), an automatic neural response recorded with in electroencephalography (EEG) might reflect discrimination at a pre-attentive, automatic level of processing (e.g., Näätänen et al., 2007; Näätänen et al., 2012; Bartha-Doering et al., 2015; Partanen et al., 2011).

This study aimed to understand how cognitive exercises impacts speech processing differences associated with aging and automatic electrophysiological response. The goal was to identify differences in vowel sound discrimination between monolingual and bilingual in different aging groups. The study examined the following: 1) the impact that bilingualism has on neurophysiological effects of the aging process on speech perception with the contribution of cognitive demand, 2) the effectiveness of innate ability versus bilingual experience as predictors of speech perception in older adults.

Methods: We evaluated neural encoding of the acoustic features and neural discrimination using the same modified oddball paradigm and stimuli previously reported in Hisagi et al. (2010). Age-related speech processing deficits were studied from three different aging groups with varied language backgrounds: younger (18-39y), middle-age (40-59y), and older (60y+). The MMN responses (amplitude/latency) were recorded

utilizing a 32-channel BrainVision actiCAP system (Brain Products GmbH). A phonological memory repetition task (cognitive exercise) was inserted between two EEG sessions (Part 1 and Part 2).
Results: There was no change in MMN before and after the phonological task in the younger group as expected, while there was a larger MMN in Part 1 compared to the younger group and significantly diminished MMN in Part 2 for the older group.
Conclusions: Compared to baseline expectations of MMN difference between Part 1 populations, some unanticipated results emerged. Part 1 findings of the EEG task align with studies suggesting that lifetime accrual of sensory experience optimizes functional brain architectures (Moran et al., 2014). Accordingly, Part 1 results indicated a larger MMN in older populations. Furthermore, optimized function of the brains in the older population was significantly affected by the repetition task. This age group displayed a diminished MMN in Part 2, possibly due to this task's additional burden on overall cognitive capacity, or to the general fatigue from the experiment.
Our study contributes to the understanding of auditory processing in aging persons with cognitive decline and in those who speak English as a second language. Results have implications for future speech understanding, language assessments, and intervention processes. Ultimately, the goal of this experiment is to improve the likelihood of success of auditory rehabilitation and aural therapy techniques.

M117. A Machine-Learning-Based Approach to Predict Early Hallmarks of Progressive Hearing Loss

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Category: Aging
Background: Age-related hearing loss (ARHL), the most common health condition in older adults, is usually diagnosed only after patients start losing key hearing abilities, such as being able to distinguish speech in noisy environments. This is usually an indication that some irreversible damage has already happened to the sensory cells and neurons. Therefore, as we develop therapies to target ARHL, we also need to improve the diagnostic tools to detect the disease at an early stage.
Advanced computational techniques such as machine learning (ML) are increasingly being employed towards improving the diagnosis and treatment of diseases. These techniques use advanced algorithms applied to datasets to uncover patterns that would otherwise be elusive even to well-trained experts in the field.

Here, we provide a proof-of-principle study that ML techniques applied to electrophysiological recordings of hearing function (auditory brainstem responses, ABRs) are well suited to detect early signs of ARHL in mice, and to forecast the future progression of the disease.

Methods: We used the common C57BL/6N mouse strain (6N), which suffers from early-onset ARHL due to an hypomorphic allele of Cadherin23 (Cdh23ahl). The ARHL phenotype becomes evident from around 3 months of age, when these mice develop a threshold shift in ABRs compared to a co-isogenic mouse strain in which the normal Cdh23 has been reinstated (6N-Repaired).
We recorded ABRs from a cohort of 1-month-old 6N and 6N-Repaired mice, with some of them going through repeated measurements over a year to follow the progression of hearing loss. We then trained ML algorithms through supervised learning using the ABR data as input features. The hyperparameters of the models were optimised using random grid search and the performances were evaluated using repeated k-fold cross validation.

Results: Despite no shift in auditory thresholds at 1 month of age, several ML algorithms were successful in identifying the mice that had the Cdh23ahl allele (6N), and therefore will develop early-onset ARHL. Interestingly, feature importance analysis indicated that subtle differences in ABR wave I were employed by one of the models to discriminate between the two genotypes.
We also successfully trained regression models that forecast the progression of hearing loss at old ages using ABRs from the same mice as healthy young adults (1-3 months of age) as input. This is achieved by predicting the average threshold shift across all frequencies.

Conclusions: Our strategy highlights the power of ML for the early diagnosis of ARHL. This approach could dramatically improve the success rate of future treatments if translated to humans.

M118. Voice Familiarisation Delivered Online Improves Speech Intelligibility for Older and Younger Adults

Wansu Zhu[1], Emma Holmes*[1]

[1]*UCL*

Category: Aging

Background: People often face the challenge of understanding speech when other speakers are present, and this becomes increasingly difficult with older age. Previous studies have demonstrated a large speech intelligibility benefit in noise when people listen to familiar voices—even when voices are familiarised artificially in the lab. This finding has practical applications for delivering voice training to improve speech intelligibility in everyday life. Yet, we do not currently understand whether older adults benefit from this type of training, or whether it could be delivered effectively outside the laboratory, in the comfort of people's own homes.

Methods: We recruited 20 older (55–73 years) and 20 younger (18–34 years) adults for an online study. Participants were trained with three novel voices for approximately 20 minutes (166 sentences) each. During training, they heard a sentence and were asked to identify the speaker's voice, which varied randomly from trial to trial. Next, we tested their ability to report a target sentence in the presence of a competing sentence. The target voice could be one of the three trained voices or one of two novel, unfamiliar voices. The competing voice was always unfamiliar and different to the target voice. The target-to-masker ratio was either -3 or +6 dB and varied randomly from trial to trial. Voices were counterbalanced across participants, so that each voice served as familiar for equal numbers of participants and were unfamiliar for the remaining participants. Open-set sentences were used for training, whereas closed matrix sentences were used for testing, meaning that a benefit from familiar voices can only arise if it generalises to new speech materials.

Results: We found that both groups benefited significantly from voice training: In other words, participants reported more sentences correctly for voices with which they had been trained than for unfamiliar voices (even though the voices themselves were counterbalanced across participants). Interestingly, the magnitude of the benefit—which amounted to approximately 30% improvement in percent correct sentence report—did not differ between older and younger adults. A Bayesian equivalence test provided evidence in favour of a similar-magnitude benefit from voice training in both groups, suggesting that older adults gain as much benefit from voice training as do younger adults. For both groups, familiar voices presented at -3 dB TMR were as intelligible as unfamiliar voices presented at +6 dB TMR, which demonstrates a benefit of approximately 9 dB from voice training.

Conclusions: Our results demonstrate that even short durations of voice training (approximately 20 minutes for each voice) have great potential to improve speech intelligibility for a variety of people, including older adults who often find it difficult to understand speech when competing speech is present. Furthermore, this type of training is effective when delivered online, outside of a laboratory setting.

M119. Purinergic Signalling in the Supporting Cells of the Ageing Cochlea

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[1]*University of Sheffield,* [2]*University College London*

Category: Aging

Background: The unique physiological environment within the mammalian inner ear is crucial for the optimal function of the auditory receptors known as 'hair cells'. A syncytium of specialised non-sensory 'supporting cells' surround the hair cells and drive the maintenance of these specific conditions. Among the different proteins expressed in the supporting cells, metabotropic purinergic receptors (P2Y) have been shown to be critical for cochlear development and function. Considering that purinergic receptors are known to undergo changes in expression during ageing in several other tissues, we hypothesized that similar changes could occur within the cochlea and underpin age-related hearing loss (ARHL), the causes of which are still largely unknown. Therefore, we investigated potential progressive changes in the expression and function of P2Y receptors in the supporting cells of the mouse ageing cochlea.

Methods: We investigated the localisation of P2Y1, P2Y2 and P2Y4 receptors in the aged cochlea by performing immunolabelling experiments. We also used ratiometric calcium imaging as a readout of purinergic receptor function in cochlear supporting cells. We compared both early-onset and late-onset ARHL mouse strains, and animals of three age groups: postnatal day 7 (P7), adult (P30) and aged (17-24 months).
Results: Immunolabelling and calcium imaging experiments revealed a downregulation of P2Y receptor expression and a decrease of purinergic-mediated calcium responses after the onset of hearing, which in mice occurs at around P12. Conversely, we observed an upregulation of P2Y2 and P2Y4 receptor expression in the supporting cells of aged mice when compared to P30 adults, but no difference was observed in P2Y1 receptor expression. Moreover, aged supporting cells had significantly larger calcium responses and displayed calcium oscillations to prolonged purine applications.
Conclusions: We conclude that the aged cochlea undergoes alterations in purinergic receptor expression and function, which may be involved in senescent mechanisms contributing to age-related hearing loss.

M120. Silence, Solitude, and Serotonin: Combined Effects of Noise-Induced Hearing Loss and Social Isolation in Older Mice

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Category: Aging
Background: Both hearing loss and social isolation decrease the densities of serotonergic fibers in the inferior colliculus (IC) of mice. Past studies were limited to males or to ages from postweaning to early adulthood, and did not examine the interaction of hearing loss and isolation. We conducted a pilot study to assess the effects of noise-induced hearing loss and a one-month period of individual housing in older female CBA/CaJ mice.
Methods: At ~12 months of age, mice were exposed to band-limited noise presented at 115 dB for 1 hour, or to a sham procedure in which noise was not played. Mice were then either returned to paired housing or housed individually for a month. These procedures created three experimental groups: sham-exposed, socially housed females; noise-exposed, socially housed females; and noise-exposed, individually housed females. After the housing treatment, mice were placed in a behavioral interaction with a male social partner, and vocal and nonvocal behaviors were monitored.
Results: Among groups, the density of serotonergic fibers in the IC was highest in the noise-exposed, individually housed group. Vocal and non-vocal behaviors were different among groups, with interactions including the noise-exposed, socially housed females producing the highest numbers of USVs and female escape behaviors.
Conclusions: These findings suggest that, in contrast to previous findings, social isolation but not noise exposure in older female mice increases the density of serotonergic fibers in the IC. Neither social isolation nor noise exposure were determinative factors for social behavior, suggesting an interactive effect between these on behavior.

M121. Mitochondrial Dysfunction in Strial Marginal Cells of Middle-Aged Mice

Tyreek Jenkins*[1], Hainan Lang[1]

[1]*Medical University of South Carolina*

Category: Aging
Background: Metabolic age-related hearing loss (ARHL) is associated with decreased endocochlear potential (EP) and elevated auditory thresholds due to degeneration and inflammation of the stria vascularis (SV). The SV, located in the lateral wall of the cochlea, is a highly active bioenergetic, vascularized epithelium that conducts essential functions for generating the battery-like electrochemical gradient (i.e., EP), which is necessary for driving hair cell transduction. In a recent study, we propose that aberrant macrophage activity, starting at middle age in a ARHL mouse model, is an initial contributor to strial dysfunction, cochlear pathology, and hearing loss. Although the underlying mechanisms of aberrant macrophage activity have yet to be elucidated, emerging evidence implies that dysfunctional mitochondria have a role as signaling

promoters of immune cell activation and inflammation. When mitochondria become bioenergetically unfavorable, due to high energy demand and/or cellular stress, they depolarize and undergo fission to prime for mitophagy (a selective form of mitochondrial degradation). If mitochondrial fission becomes excessive, mitophagy can become impaired due to the inability to efficiently form autophagosomes. This study addresses the hypothesis that, beginning at middle age, mitochondria within strial marginal cells are largely depolarized with exacerbated mitochondrial fission and impaired mitophagy. Here, we characterize age-dependent mitochondrial dysfunction in marginal cells of the SV using live-cell confocal imaging of ex vivo SV preparations.

Methods: The hearing sensitivity of young adult (2-4 months) and middle-aged (10-15 months) CBA/CaJ mice were assessed by measuring the auditory brainstem response. The SV was then dissected from the cochlea, cultured for 1-2 hours, loaded with either tetramethyl rhodamine methyl ester (TMRM) to assess mitochondrial membrane potential or mitoSOX superoxide indicator to assess mitochondrial superoxide production. Mitochondrial fission and mitophagy were assessed using a colocalization analysis of phosphorylated dynamin-related protein 1 (p-Drp1) and microtubule-associated protein 1A/1B light chain 3B (LC3B), respectively, with positively labeled mitochondria.

Results: Middle-aged mice exhibited reduced hearing sensitivity compared to young adult mice with mild threshold shifts at lower and middle frequencies and moderate threshold shifts at higher frequencies. In addition, mitochondria within strial marginal cells of middle-aged mice were largely depolarized compared to young adult mice, as indicated by reduced TMRM fluorescence intensity. Furthermore, mitoSOX indicated higher levels of mitochondrial superoxide within marginal cells of middle-aged mice, suggesting increased levels of oxidative stress compared to young adult mice. Lastly, mitochondrial fission, as indicated by p-Drp1, was exacerbated beginning in middle age with reduced LC3B expression.

Conclusions: The understanding of age-related mitochondrial alterations in marginal cells of the SV will aid in the development of therapeutic agents designed to combat inflammation within the aging inner ear via targeting mechanisms aimed at preserving mitochondrial function (i.e., mitophagy).

M122. Contrasting Intra-Cochlea Neural Health Variability Assessed Using Psychophysical Thresholds and Panoramic Evoked Compound Action Potentials

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Category: Auditory Prostheses

Background: There is growing evidence that variations in intra-cochlea spiral ganglion cell survival negatively impact cochlear implant (CI) user speech perception outcomes. When activating CI electrodes at regions with poor neural survival near regions with good neural survival, this variability in local neuron survival results in unintended activation of the neighbouring good regions, effectively masking and smearing the local frequency information, leading to poorer speech perception.

This study compares patient-specific neural health estimates obtained using broad (monopolar) and focussed (bipolar) psychophysical thresholds and electrophysiological evoked compound action potentials in a group of post-lingually deaf adult implant users. We hypothesize that: 1) neural health estimates from both methods correlate within and between participants; and 2) a larger neural variation along the electrode array is associated with poorer speech understanding outcomes.

Methods: Twenty-one (n=21) post-lingually deaf adult CI users with at least one year of implant experience were recruited. Seven participants were excluded from analysis due to ongoing data collection (n=3) or poor PECAP recording signal-to-noise ratios (n=4).

Psychophysical detection thresholds were measured in monopolar and bipolar stimulation modes. The monopolar thresholds were subtracted from the bipolar thresholds to obtain a psychophysical estimate for neural health. An alternative electrophysiological estimate of neural responsiveness was obtained using the Panoramic ECAP (PECAP) measure.

Speech understanding scores were measured using consonant-nucleus-consonant words in quiet.

Results: PECAP and psychophysical threshold neural health estimates were significantly correlated at the group level (p less than 0.001), but only for 5 recipients at the individual level. The within-subject variation in psychophysical threshold neural health estimates was negatively correlated with speech understanding outcomes (p less than 0.01). In contrast, the within-subject variation in PECAP neural responsiveness estimate was not negatively correlated with speech understanding outcomes.
Conclusions: While neural health estimates from both methods correlate at the group level, there is a significant difference in the ability for the two neural health measures to explain variance in speech understanding outcomes. Neural health derived from psychophysical thresholds is a better correlate for speech understanding outcomes (Pearson and Filon's z: -2.0943, p=0.0037). This difference can be attributed to differences between perceptual and electrophysiological responses and differences in stimulation parameters.

M123. Speech Encoding in the Midbrain of Normal Hearing Cats in Noisy Environments

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Category: Auditory Prostheses
Background: Originally reserved for the profoundly deaf, cochlear implantation is now common for people with partial hearing loss. Combining cochlear implants (electrical stimulation) and hearing aids (amplified acoustic stimulation) enhances speech comprehension and sound quality when compared to electrical stimulation alone, particularly in noisy environments. Our long-term aim is to elucidate the underlying physiological mechanisms of this improvement. As a first step in this process, we have investigated the degree to which the patterns of neural activity evoked in the inferior colliculus (IC) by speech sounds in various levels of noise allows discrimination between those sounds in normal hearing cats.
Methods: Neuronal responses were recorded simultaneously from 32 sites across the tonotopic axis of the IC in anaesthetised cats with normal hearing (n = 8). Speech sounds were presented at 20, 40 and 60dB SPL in quiet and with increasing levels of additive noise (signal-to-noise ratios (SNRs) -20, -15, -10, -5, 0, +5, +10, +15, +20dB). The speech sounds included CVC tokens that have been employed previously to investigate spectral and temporal resolution in implant users: 11 bVd words (bad, bed, bid, bod, bud, bead, board, bird, bard, bared, booed), 11 hVd words (had, hed, hid, hod, hud, head, hoard, hird, hard, hared, hooed) and 11 hVp words (hap, hep, hip, hop, hup, heap, hoarp, hirp, harp, harep, hooep). Neural discrimination was assessed using the Euclidean distance between the neural representations in multi-dimensional (recording electrode x analysis bin width) space, resulting in a function reflecting speech sound differentiation across various SNRs.
Results: Electrophysiological recordings demonstrated that the responses of IC neurons reliably encoded the speech stimuli when presented in quiet. The discrimination threshold depended not only on the stimulus (loudness of the stimulus or the background noise) but also on how the neural signals were processed. For soft (20dB SPL) stimuli, SNR thresholds exhibited a U-shaped response, with a minimum threshold at a 10ms bin width. In contrast, for moderate (40 and 60-dB SPL) stimuli, SNR thresholds were relatively flat below a cut-off of 100ms bin width, with a rapid increase for larger bin widths.
Conclusions: This study sheds light on how the auditory midbrain represents speech sounds and provides baseline data with which responses to electro-acoustic speech sounds in partially deafened animals can be compared.

M124. Cortex Developmental Plasticity and Relations to Behavior Outcomes of Congenital Deafness Children With Auditory Brainstem Implantations

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Category: Auditory Prostheses
Background: The auditory brainstem implant (ABI) is an auditory neuroprosthesis that provides a sense of hearing by stimulating the cochlear nucleus (CN). So far the performance of most ABI patients, as measured by speech comprehension, is poor. The unclear mechanism of neural development after ABI may be one

reason for these poor outcomes. The aim of this study was to investigate the cortical developmental and functional connection features after ABI in children, and their relations to behavior outcomes.
Methods: We recruited 95 consecutive pediatric auditory brainstem implant (ABI) recipients operated on between January 2019 and December 2022, with regular follow-up. Med-EL Concerto, Synchrony and Nurotron WH-01A ABI were implanted in this group. On each follow-up, participants will assess PTA, CAP, IT-MAIS/MAIS, MUSS, SIR, charge level of threshold of electrically evoked auditory brainstem responses (eABRs). The electroencephalogram and functional near-infrared spectroscopy (fNIRS) were used to record cortical neural responses to frequency mismatch negativity paradigm. The MMN amplitude, cortical oxygen concentration and cortex functional connections were analyzed.
Results: ABI was successfully performed in all cases without intra-operative and post-operative major complications. The average active electrode ratio was 83% ±19% at first activation (n=49) and 76% ±15% at 12 months after 1st fitting (n=21). At 24 months after 1st fitting, 61.5% reached CAP-II≥5; 69.2% reached IT-MAIS/MAIS≥35 and 38.5% reached IT-MAIS/MAIS 40; 46.2% reached MUSS≥25; 92.3% children presented improvement on SIR and 30.8% reached SIR≥3. The fNIRS oxygen concentration analyses revealed more efficient functional-connections between the left auditory cortex and other cortices with longer implantation duration. The functional coherence of left auditory and frontal cortex was positively correlated to CAP (Spearman r=0.67 P=0.0019), IT-MAIS (Spearman r=0.69 P=0.0020) and SIR (Spearman r=0. 47 P=0.017) scores.
Conclusions: Cortical plasticity in congenital deafness children after ABI may contribute to the auditory and speech behavioral development, especially the connection between left temporal and frontal lobe. The understanding of these mechanisms could be helpful in predicting the behavior outcomes and improving the speech comprehension, which should be further explored in future studies.

M125. Exploring Temporal Mechanisms of Pitch Perception Using the Apical Electrodes of a Cochlear Implant

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Category: Auditory Prostheses
Background: Recent experimental and computational studies have produced predictions that have important implications for auditory theory and for coding of pitch by cochlear implants (CIs). Computational models predict that encoding of place-of-excitation in response to stimulation of apical electrodes depends on survival of auditory-nerve (AN) peripheral processes (PPs). Recordings from cat inferior colliculus suggest that selective activation of apical AN fibres activates a pathway that accurately encodes the temporal structure of pulse trains over a wide range of rates. Influential models of pitch perception assert that listeners combine the temporal responses of AN fibres that innervate different cochlear regions, so as to perceive the fundamental frequency of harmonic sounds - suggesting that the same may occur with contemporary CI speech-processing strategies that present harmonically-related temporal cues to different apical electrodes.
Methods: Two experiments studied pitch perception by users of the MedEL CI, using the optimally- efficient "MidPoint Comparison" pitch-ranking method. Experiment 1 obtained (i) place-pitch ranking of 80-pps pulse trains applied to apical electrodes 1-4, (ii) an estimate of PP survival near apical and mid-array electrodes, using the "polarity effect (PE)" – defined as the difference in detection thresholds for 99-pps trains of triphasic pulses with cathodic vs anodic central phase, (iii) temporal-pitch ranking for 8 pulse rates between 80-981 pps for stimulation of the most-apical electrode, for electrodes 1-4 presented simultaneously, and for a mid-array electrode. In experiment 2 listeners ranked the pitch of pulse trains presented to electrodes 1-4. In four conditions the same pulse rate of 100, 200, 300, or 400 pps was presented to all 4 electrodes. The remaining "mixed-rate" conditions presented each electrode with a different pulse rate, differed only in the assignation of electrode to rate, and included configurations [100 200 300 400] and [400 300 200 100]. We also measured the effect of the relative timing of pulses between electrodes.
Results: Experiment 1 showed good place-pitch ranking in half the participants. The PE correlated positively with average detection thresholds, did not differ between apical and mid-array electrodes, and was not related to accuracy of place-pitch ranking. There was no significant difference in temporal pitch ranking between conditions. Preliminary results from experiment 2 showed good pitch ranking in the same-rate conditions, but

no evidence that listeners heard a clear pitch corresponding to the fundamental frequency in the mixed-rate conditions.
Conclusions: We found no evidence that selective stimulation of the most apical electrode of the MedEL CI can improve temporal pitch perception relative to other forms of stimulation, and no evidence that presenting multiple rates to different electrodes elicits pitch at the fundamental frequency. Place-pitch perception with apical stimulation was generally accurate but did not correlate with the PE, which has been proposed as an estimate of PP survival.

M126. Acute Speech Perception in Optimized Dynamically Focused Cochlear Implant Stimulation

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Category: Auditory Prostheses
Background: Dynamic tripolar (DT) is a cochlear implant (CI) strategy that focuses the current using three electrodes, whereby the degree of current focusing (σ) varies depending on the input level and the channel interaction coefficient (K) that determines the rate of change of σ. Previous studies demonstrated improved speech in noise identification using DT compared to a monopolar (MP) control program in participants with Advanced Bionics (AB) CIs. Those studies used all available channels for stimulation, however, other studies have demonstrated that deactivating or focusing channels with large amounts of channel interactions also improves performance. In the present study, we combine these approaches (channel deactivation and dynamic focusing) to create optimized programming strategies. Channels with relatively high focused thresholds and broad tuning are selected for deactivation. Three programs with a subset of channels deactivated are compared: 1) Remaining channels programmed with MP (all-MP) 2) remaining channels programmed with DT (all-focused), and 3) channels with low focused thresholds programmed in MP and relatively high threshold channels in DT (mixed-focused). We hypothesize that reducing channel interaction by deactivating some channels and using DT will result in improved speech in noise perception.
Methods: Six ears of five adults (aged 56 - 76) were available for an intermediate analysis. The programming strategies were implemented via AB's BEPS+ research platform and generated similarly to clinical practice, measuring thresholds and comfort levels. DT parameters σ and K were defined similarly to previous studies (σ = 0.8 at threshold and 0.5 at most comfortable level, fixed and individual K) and individually optimized. Open-set sentence recognition and closed-set vowel identification performance were assessed at 60 dB SPL in quiet and four-talker babble background noise. Speech materials were presented at two SNRs representing the speech reception performance of SNR80 and SNR60. The outcomes of the experimental strategies were compared to the study participants' everyday clinical strategy.
Results: Preliminary speech perception outcomes in quiet and background noise showed considerable individual differences over strategies and SNRs. Performance was highest for the clinical program, and subjects performed better in quiet than background noise with all strategies. The all-focused programs tended to be better than the mixed-focused and similar to the all-MP programs when tested in noise. Correlations of word and vowel scores showed weak relationships for mixed- and all-focused strategies.
Conclusions: The intermediate outcomes for optimizing programming strategies with all-MP, mixed- and all-focused strategies support prior findings that some individuals obtain perceptual benefits on acute word recognition and vowel identification. Performance was enhanced for the all-focused DT strategy in noise but did not exceed that of the subjects' everyday listening program. To further investigate individual differences, subject demographics and strategy parameters will be incorporated.

M127. A Study on the Development of Middle-Ear Microphone Package for Fully Implantable Hearing Devices Using Piezoresistive Sensor

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Category: Other, Hearing device

Background: Most conventional cochlear implants and hearing devices use microphones in the external device to capture the sound field. However, the external devices cause some challenges, social stigma, and restrictions on physical activity such as swimming. To overcome these limitations, studies have been conducted to develop totally implantable hearing aids and the development of implantable microphones is one of the main challenges. An implantable middle-ear microphone (MEM) that obtains acoustic energy from vibrations of the eardrum or ossicles has the advantage of being able to use the physiological amplifier effects and acquire the directionality of sound. In this study, we investigated to develop MEM package with high sensitivity, low equivalent input noise (EIN), and easier surgical method.

Methods: The MEM inserted between the umbo to promontory was designed. Umbo movements was measured and analyzed using a LDV and using fresh-frozen temporal bones (TB). The MEM package was inserted between the umbo and promontory through the facial recess. An average sound stimulus of 102 dB sound pressure level (SPL) was stimulated with 0.5Hz ~ 8 kHz pure tone and 100Hz ~ 10 kHz sweep sound through sealed artificial external auditory canal. The performance of the piezoresistive sensor such as signal-to-noise ratio (SNR), EIN, and sensitivity was measured and analyzed. FEM simulation was performed to compare the effect of insertion of the MEM package.

Results: We developed a novel implantable MEM package. This package is comprised of a mounted piezoelectric sensor and biocompatible materials. The vibration of the umbo to the microphone conduct to the center of sensor. To maximize the performance of the sensor and adjust the individual distance between umbo and promontory, biocompatible silicone was located at the bottom of this package. The effect of sensor package insertion resulted an average 7dB decrease in displacement transfer function. Which is a similar result compared to the FEM simulation result. The SNR for pure tone sound achieved a maximum of 55dB (obtained for the 0.8~1.5kHz). The best EIN is 48 dB SPL. As a result, a low bio noise and surgically simple bio middle-ear microphone was designed, but sensitivity improvement was required.

Conclusions: This study was investigated that the MEMS piezoresistive sensor has favorable sensitivity of performance. However, the high EIN and the low SNR are still insufficient to be used as a bio middle-ear microphone. To develop an ideal implantable ME microphone, the sensor needs to require 5 times improvement. We will develop the performances through the improvement of the sensor in the further study. We focus on a different shape of the sensor, and the manufacturing method of the changing structure of sensor. In addition, there is a need for a steady effort to develop a new middle-ear microphone with an easy-to-implant design.

M128. Tonal Language Experience is Associated With Utilization of Spatial Cues for Segregating Competing Speech in Bimodal Cochlear Implant Listeners

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Category: Auditory Prostheses

Background: Differences in spatial location and/or talker sex can facilitate segregation of competing speech. However, the spectro-temporal degradation associated with cochlear implants (CIs) greatly limits access to spatial and/or talker sex segregation cues. For bilateral CI users, electric hearing in both ears may allow for some spatial cue perception. For bimodal CI users, residual acoustic hearing in the non-implanted ear may benefit perception of talker sex cues. In bilateral and bimodal English-speaking CI users, utilization of spatial talker sex and spatial cues is generally poor. Long-term tonal language experience (e.g., Mandarin) has been shown to improve sensitivity to voice pitch cues. It is unclear whether Mandarin-speaking CI users may successfully utilize segregation cues, especially talker sex cues.

Methods: In the present study, speech recognition thresholds (SRTs) for target speech in two-talker masker speech was measured in Mandarin-speaking bilateral and bimodal CI users while listening with both ears. Maskers were either co-located or spatially separated from the target, and the talker sex was either the same

as or different from the target. Masking release (MR) was calculated relative to SRTs when no talker sex or spatial cues were available. Consistent with previous findings in English-speaking bimodal CI users, we predicted that Mandarin-speaking bimodal listeners would be able to segregate competing speech using talker sex cues but not spatial cues. We also predicted that Mandarin-speaking CI users would experience greater masking release (MR) with talker sex and/or spatial cues observed with English-speaking CI users.
Results: There was no significant difference in SRTs between bilateral and bimodal CI users. However, bimodal CI users significantly benefitted from spatial cues and combined talker sex and spatial cues, but bilateral CI users did not. Mean MR with spatial cues was 5.0 dB higher for the present Mandarin-speaking than for English-speaking bimodal CI users in a previous related study.
Conclusions: Different from our predictions, Mandarin-speaking bimodal CI users significantly benefitted from spatial cues, but not talker sex cues. While there appears to be a tonal language advantage for utilization of spatial cues, the source of the advantage is unclear. It is possible that better perception of voice pitch information in the non-implanted ear may make the maskers in each ear more distinct, allowing for better utilization of spatial cues for segregation.

M129. Effects of Older Age and Steroid Treatment on Inflammation and Survival of Neural Structures in the Aging Guinea Pig Cochlea After Cochlear Implantation

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Category: Auditory Prostheses
Background: Approximately 30% of cochlear implant recipients lose more than 30 dB of their residual hearing in the months after surgery. Postoperative inflammation is considered a strong candidate in pathophysiology of this hearing loss, so that steroid treatments are often used to reduce inflammation and thus loss of hearing. However, older age has been shown to be a risk factor for greater residual hearing loss after implantation, and it is unclear whether underlying mechanisms are different in older animals. Most previous research has used very young animals, and have not accounted for potential differences in inflammation and effects of steroid treatment with older age. The objective of this study was to examine how age and steroid treatment modulate the effects of cochlear implantation on inflammation and survival of neural structures.
Methods: 3 groups of guinea pigs- young (n=10), old (n=9) and old + steroids (n=9)- were implanted unilaterally in the left ear, with the contralateral ears used as controls. Auditory brainstem response (ABR) measurements were performed pre-operatively and at two and six weeks after surgery. After the final ABR, cochleae were collected, fixed, and decalcified. Immunohistochemistry was conducted using 100 µm slices of the mid modular cochlear sections. Neural structures were labeled using MYO7A for hair cells, NF-H for spiral ganglion neurons and axons, and Cellmask for myelination at the 1, 8, and 16 kHz regions. Chronic inflammatory markers including TNF alpha, IL-1 B, and IL-6, and macrophages (F 4/80 antibody) were labeled in alternate sections. Implanted ears will be compared to the contralateral, non-implanted controls, and all three groups will be compared with each other.
Results: ABR measurements indicated significantly greater hearing loss in old compared to young animals [F=5.273 (1,17) p=0.035], and that this hearing loss was reduced in old animals given steroid treatment [F=15.926 (1,16) p=0.001]. Comparisons of hair cell counts, SGN density, myelination, and chronic inflammatory markers between ears, between the young and old animals, and between old animals in the presence or absence of perioperative steroid taper will be described.
Conclusions: The findings indicate that older age leads to greater hearing loss and that perioperative glucocorticoid treatments can help reduce this hearing loss. However, it is unclear whether the benefit of glucocorticoid treatment is due to prevention of fibrosis and ossification, or prevention of chronic inflammation and subsequent loss of hair cells and spiral ganglion neurons (SGN), or a combination of both. The histology findings will provide insight into pathophysiology of residual hearing loss after cochlear implantation as it relates to the role of chronic inflammation and neural structures in old animals.

M130. Improved Tactile Speech Perception Using Audio-To-Tactile Sensory Substitution With Formant Frequency Focusing

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Category: Auditory Prostheses

Background: A new generation of haptic aids for hearing, which provide speech information to hearing-impaired listeners through tactile stimulation, could substantially improve outcomes for both cochlear implant (CI) users and for those unable to access CIs. Recent advances in wide-band haptic actuator technology have made new audio-to-tactile conversion strategies viable for wearable devices. One such strategy filters the audio into eight frequency bands, which are evenly distributed across the speech frequency range, and uses the amplitude envelopes from each band to modulate the amplitude of each of eight vibro-tactile tones. This tactile vocoder approach can effectively transfer some phonemic information, but vowels are poorly portrayed.

Methods: In 20 participants with normal touch perception, we tested whether focusing the audio filters of the tactile vocoder more densely around the first and second formant frequencies ("formant focusing") improved tactile vowel discrimination. Vibro-tactile stimulation was delivered at a single site on the dorsal wrist at an intensity that could readily be reproduced by a compact, wearable device.

Results: Formant-focusing improved performance across all phonemes by 3.9% on average ($F(1,19) = 27.5$, p less than .001). The size of this improvement differed for vowels and consonants ($F(1,19) = 13.2$, $p = .002$). Post-hoc analyses (corrected for multiple comparisons) revealed that the improvement was statistically significant for vowels ($t(19) = 4.9$, p = less than .001), but not for consonants (although this improvement was also close to significance; $t(19) = 1.9$, $p = .069$). For vowels, mean performance was 7.7% better with formant focusing and, for consonants, mean performance was 1.4% better.

Conclusions: The formant-focused tactile vocoder approach significantly improved phoneme discrimination for vowels, without impairing performance for consonants. The approach is computationally lightweight and can readily be implemented in real time on a compact wearable device to deliver real-world benefit. We will present our prototype real-world-viable device for those who visit the poster to experience. A device such as this could improve outcomes both for haptic hearing aid users who are unable to access CI technology and for CI users who do not achieve high levels of speech recognition even in optimal listening conditions.

M131. Relationship Between Cochlear Implant Electrode Position and Postoperative Residual Hearing

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Category: Auditory Prostheses

Background: The indication criteria for cochlear implantation have been extended in recent years to patients with residual hearing in the low frequency range. Despite the development of an electrode design that is as atraumatic as possible and a gentle insertion technique, loss or long-term deterioration of residual hearing after cochlear implantation is currently still seen in approximately 60% of patients. Therefore, other parameters such as the electrode position should be also taken into account for the preservation of residual hearing.

Methods: In this study, the relationship between the exact location of the CI electrode in the scala tympani and the preservation of residual hearing was investigated. For this purpose, guinea pigs were implanted with a pure silicon electrode array without stimulation electrodes or other metallic components. This prevented reflection artifacts during x-ray exposure and enabled the creation of high-resolution µCT images and thereby allowed an accurate determination of the electrode position. In the µCT images, the distance of the electrode array to the lateral wall as well as to the modiolar wall was determined. From this, the intracochlear positioning index (ICPI) was calculated, which characterizes the electrode position, relatively to the modiolus (higher values indicate a more lateral electrode position). For determining the residual hearing thresholds, brainstem audiometry (ABR) was performed preoperatively and four weeks postoperatively. Subsequently, a correlation analysis of the electrode position with the ABR-thresholds was performed.

Results: A significant negative correlation was found between electrode position (ICPI) and postoperative hearing thresholds especially in the low frequency range of residual hearing. A mid-modiolar electrode position seems to exert a significant effect on the preservation of residual hearing if compared to the peri-modiolar position.

Conclusions: The present data thus provide new impetus for further developments of electrode design to improve the preservation of residual hearing.

M132. The Effect of Pulse Phase Duration on the Electrically Evoked Compound Action Potential and Its Relation to Auditory Nerve Health

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Category: Auditory Prostheses

Background: Hearing with a cochlear implant (CI) relies on the condition of the auditory nerve. For optimization of CI parameter settings and prognosis of hearing performance, assessment of the auditory nerve is therefore important. Neural health may be assessed by recording electrically evoked compound action potentials (eCAPs). Relative eCAP measures by varying electrical pulse parameters can be useful in reducing confounding factors such as electrode position and intrascalar tissue growth. The inter-phase gap (IPG) of the biphasic pulse appeared to be a suitable parameter revealing strong correlations of relative eCAP measures with neural survival in animals (Ramekers et al., JARO, 2014) and with hearing performance in CI users (Schvartz-Leyzac and Pfingst, Ear Hear., 2018). The phase duration (PD) has a similar effect of separating phases as the IPG, and may therefore be another interesting parameter to derive relative measures. Here we examine in guinea pigs the effect of PD on the eCAP, its relation to neural survival, and the interaction of PD and IPG in their effect on the eCAP.

Methods: Nine normal-hearing and 31 ototoxically deafened guinea pigs were equipped with a short four-contact electrode array (MED-EL, Innsbruck, Austria). eCAPs were obtained by stimulating the most apical electrode and recording from the most basal electrode. Fixed-charge biphasic current pulses of 30, 50 or 100 µs/phase and IPG of 2.1 or 30 µs were presented, with alternating polarity to reduce stimulation artefact. Charge was varied from 0 to 24 nC. eCAP outcomes included amplitude, slope and level at 50% of amplitude growth function, dynamic range, threshold, and latency. Following the eCAP recordings the animals were sacrificed, and their cochleas were processed for histological quantification of spiral ganglion cells (SGCs).

Results: All eCAP measures significantly depended on PD, most notably the latency, which increased by about 1.5 times the increase in PD. Overall, these PD effects were less prominent than the IPG effects. A significant interaction of PD and IPG was found in that the IPG effect decreased with increasing PD, and the PD effect either decreased (amplitude, slope, dynamic range) or increased (threshold, level at 50%) with increasing IPG. The IPG effect and to a lesser extent the PD effect were both correlated to SGC survival; these correlations were typically largest when the other parameter was shortest.

Conclusions: The best relative eCAP measures for assessment of auditory nerve health can be obtained with varying IPG using short PDs.

M133. Determination of Implant Dynamic Range to Predict Speech Performance of Vibrant Soundbridge Users

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Category: Auditory Prostheses

Background: The dynamic range (DR) of hearing is the loudness input range accessible to the patient, defined as the difference between the hearing threshold and the uncomfortable loudness level. A minimum dynamic range to understand human speech is proposed to be in the range of 30 to 35 dB (French and Steinberg, 1947). In patients with acoustic hearing implants the maximum output (MO) of the implant is usually below the UCL of the patient. Thus, the covered DR by the device depends on the bone conduction threshold and the MO of the implant. Active middle ear implants such as the Vibrant Soundbridge (VSB) can be used to restore the hearing of patients with mild to moderate-severe hearing losses. Additionally, the floating mass transducer can be attached to the round window, bypassing the middle ear. The objectives of our study were to determine the covered DR of patients implanted with the VSB in round window application from clinical data, and to derive the relationship between patients' covered dynamic range and corresponding word recognition score.

Methods: The data of 66 patients implanted with the Vibrant Soundbridge in round window application were analyzed retrospectively. Thereto, individual frequency-dependent DR of the Vibrant Soundbridge was calculated for each patient based on the patients' direct threshold and specific technical specifications of implant and processor types provided by the manufacturer. Then, a weighted dynamic range (WDR) was estimated based on the Speech Intelligibility Index which weights audible speech cues by their importance at each frequency (ANSI S3.5-1997). Finally, the patients' monosyllabic word recognition score (WRS) in quiet was correlated to the absolute DR and weighted DR.
Results: The word recognition scores in quiet (n=66) improved with increasing dynamic range of the patients. The relationship between mean DR and WRS was described by a sigmoidal Chapman function with R^2=0.6371 and a maximum WRS determined at 93.5% which did not differ from the results of the mean WDR (R^2=0.6403, max 93.8%). A significant shift in speech performance was found from DR bin 2 (10-20 dB, median WRS 55%) to bin 3 (20-30 dB, median WRS 80%) and from DR bin 4 (30-40 dB, median WRS 82.5%) to bin 5 (40-50 dB, median WRS 90%). Only minor differences (-1.1 to 3.2 dB) were visible between the weighted DR and the absolute DR PTA4.
Conclusions: The individual DR could be easily determined from patients' clinical data only. The absolute DR as a 4-frequency average is a good predictor of the WRS in patients implanted with the VSB. A covered DR of 20 dB can provide a patient with sufficient word recognition but increasing DR further improves the mean speech outcome and reduces the variance within the outcome data.

M134. Modelling Consonant Discrimination by Users of Cochlear Implants

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Category: Auditory Prostheses
Background: Users of cochlear implants can vary widely in their ability to recognize isolated consonants or monosyllabic words in quiet. We hypothesize that this variability is partly due to the amount of speech information transmitted through the implant to the auditory nerve (AN) of the CI user, which can vary depending on multiple factors, including neural survival and/or the spread of electrical current excitation. Here, we use a recently published computational modeling framework (Leclére et al., 2023, Hear Res 432:108744. doi: 10.1016/j.heares.2023.108744) to investigate these hypotheses and predict consonant recognition for CI users.
Methods: For six CI users, we measured consonant discrimination in quiet, psychophysical masking patterns, thresholds (T levels) and maximum comfortable levels (C levels). Based on these measures, we constructed a model of the AN response to speech presented via the CI. In the model, the acoustic stimulus is first converted into sequences of electrical pulses (electrodogram) according to the audio processing strategy implemented in the participant's CI. A current spread model then simulates the spread of electrical current from each electrode to a simulated population of AN fibers distributed along the cochlea. Then, a point-process model (Goldwyn et al., 2012, J Neurophysiol 108:1430-1452. doi: 10.1152/jn.00095.2012) is used to simulate the response of every AN fiber to the electrical stimulation. The model parameters for spread of excitation, electrode-modiolar distance and neural survival were tuned based on group-mean T and C levels, and masking patterns. The model was used to simulate population neural responses for each consonant. Lastly, an information-theory algorithm was used to optimally reconstruct (decode) every consonant from the simulated neural responses.
Results: The model predicted the measured masking patterns reasonably well. Confusion matrices were simulated by plotting the reconstruction error for every possible pair of stimulus-response consonants. The confusion matrix simulated with the average model was correlated with the mean experimental confusion matrix.
Conclusions: A model of AN response for CI users can be constructed based on psychophysical masking patterns and the clinical T and C levels. This model can be useful to simulate the impact of parameters like neural survival and spread of excitation on speech discrimination. Future work will investigate if the model can be individualized to predict consonant confusion matrices for individual participants. (Work supported by Oticon Medical and the William Demant Foundation).

M135. Development of Stimulation Strategies for a New Auditory Nerve Implant for Hearing Restoration in a Guinea Pig Model

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Category: Auditory Prostheses

Background: A penetrating electrode array that directly stimulates auditory nerve fibers can provide more precise activation of auditory nerve fiber groups compared with scala tympani stimulation via a cochlear implant (CI). Since current spread is reduced due to a more optimal electrode-nerve interface, nerve stimulation can potentially provide more accurate transmission of sound signals relative to what is possible with CIs, including a greater number of frequency channels of information. As auditory nerve implants (ANIs) are translated for clinical usage, further characterization studies of auditory nerve stimulation in animal models are needed to inform future stimulation strategies that could lead to significant improvements in hearing performance, such as in noisy environments and for music appreciation.

Methods: Responses to electrical stimulation (charge-balanced, biphasic pulses) were recorded in the central nucleus of the inferior colliculus (ICC) of ketamine-anesthetized guinea pigs. A 32-site electrode array (NeuroNexus) was placed along the tonotopic axis of ICC. A transcochlear surgical approach was employed to expose the modiolar portion of the auditory nerve.

A single-shank 16-site electrode array (NeuroNexus) was inserted into the auditory nerve. Electrical current values ranging from 0 μA to 120 μA were presented for each stimulation site (60 μs/phase). Both monopolar and bipolar electrode configurations were tested. A proportional stimulation paradigm was also tested for simultaneous stimulation of multiple sites to investigate the effects on the patterns of spatial tuning curves recorded in ICC. In this paradigm, a set amount of current was distributed proportionally or differentially across multiple electrodes for various current levels tested.

Results: Bipolar stimulation showed more restricted spread of activation and significantly higher thresholds as compared with monopolar stimulation. Thresholds could be very low down to 10μA whereas those for bipolar stimulation were typically higher by 20μA or more. As the return electrode was shifted further from the active electrode, the stimulation pattern approached that of the monopolar case for the active electrode. For proportional stimulation, the tuning curve for a given combination of stimulation sites could be shaped and expanded in different directions by adjusting the relative proportion of current at each site.

Conclusions: Intraneural stimulation is capable of activating the auditory system in a systematic, controlled manner. By utilizing additional electrode configurations (such as bipolar or even tripolar stimulation) current spread may be reduced even further, allowing for more focal activation. Due to the spiraling nature of the tonotopic gradient in the auditory nerve, stimulation of two disjoint frequency regions (i.e., a very low and a very high frequency fiber group) can occur during stimulation. By shaping the current fields with multiple-site stimulation, it appears possible to prevent unwanted current spread to disjoint frequency fiber groups, which opens up new ways for enhancing accurate frequency information transmission to the auditory brain.

M136. Design Optimization of Piezoelectric Accelerometers for Totally Implantable Auditory Prostheses

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Category: Auditory Prostheses

Background: Moderate to severe hearing loss is a debilitating condition that affects over 5% of the world's population. Depending on the severity of the hearing loss and the location of the damage within the ear, hearing aids (HAs) and cochlear implants (CIs) are beneficial devices that aid their users to ameliorate their hearing loss. Both devices can have a positive impact in patients' lives, but their adoption rates are low. Several key limitations associated with both HAs and CIs have been identified as the culprits that impact device safety, appearance, acoustic performance, and ease-of-use (Calero, et al. (BioMedical Engineering OnLine 17(1), 23 (2018)). Other limitations include the external elements of these devices (e.g., the microphones and signal processors) 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 these issues by eliminating the external components. 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 studies have indicated that piezoelectric microelectromechanical systems (MEMS) accelerometers have the potential to function as implantable sensors within the middle ear meeting a 20-phon noise floor over a 100Hz–8kHz range. In the current study, we optimize our sensor designs to continue to meet specifications while minimizing their overall size.

Methods: Our ultraminiature accelerometer designs consist of proof-mass-loaded piezoelectric cantilever bimorph beams. Configurations include single-, dual-, and multi-bandwidth designs. To validate our analytical model, we test our sensors in benchtop experiments using a laser doppler vibrometer and determine their sensitivity. We also conduct a design optimization to identify the best dimensions and combinations of sensing elements that minimize the overall area and meet the frequency range (100Hz – 8kHz) and noise floor (20-phon).

Results: Previous experimental results have validated the analytical model for transverse and longitudinal excitations. We used our model to determine dual- and multi-bandwidth sensor designs that utilize rectangular plate areas and proof-mass configurations. With the numerical optimizations, a better and more compact design is identified for the multi-bandwidth configuration. Results show the sensor surface area associated with a multi-bandwidth design is 26% that of the dual-bandwidth design.

Conclusions: Our validated modeling and testing show that a small packaged piezoelectric MEMS accelerometer can meet the 20-phon threshold using rectangular sensing geometries. Multi-bandwidth designs significantly decrease the total surface area required for the sensor to operate at the specified frequency range and detect the necessary minimum acceleration to meet the 20-phon noise floor. We would like to acknowledge the NIH training grant (T32 DC00011) that funded this research.

M137. Modeling and Assessment of a Vowel Identification Task in Cochlear Implants Using Machine Learning: Preliminary Results

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Category: Auditory Prostheses

Background: Cochlear implants (CI) are implantable devices capable of partially restoring hearing loss by electrically stimulating the auditory nerve to mimic normal-hearing conditions. Resulting speech perception varies among CI users, depending mostly on their degree of deafness and ambient noise conditions. Novel electrical-stimulation strategies are often developed following phenomenologically-based approaches instead of being derived from known physiological functions of the auditory system. This pilot study assesses performance of a framework that provides an optimized electrical-stimulation strategy that maximizes similarity between simulated auditory nerve excitation elicited by acoustic and electrical means (Llico et al., ARO 2020). Pilot performance was assessed using a classifier trained to perform a closed-set vowel identification task, thereby modeling a post-lingually deaf CI user. Preliminary results demonstrated successful classification of neural responses derived from normal hearing conditions, with reduced performance for electrical stimulation.

Methods: The classifier was implemented using a 50-layer residual neural network (ResNet-50; He et al., 2016) to perform a closed-set vowel identification task. Transfer learning was used on a pre-trained network to identify new classes, i.e., 10 /hVd/ words. Neural responses were segmented and conditioned to be used as inputs to the classifier. The classifier was trained and cross-validated using neural responses derived from a model of normal hearing (Zilany et al., 2014) to simulate higher brain processes for vowel identification prior to the onset of deafness. Once trained, the classifier was presented with neural responses derived from electrical stimulation, simulating the scenario of a (new) CI user performing the vowel identification task.

Results: Confusion matrices were generated from classifications of normal hearing and electrical stimulation words. Average recognition ranged from 74-77% for normal hearing, despite some systematic misclassifications believed to be associated with proximity of first and second formants between vowels. For electrical stimulation, recognition was around 10%, with most words classified into only 2 or 3 categories.

Reduced fine detail in the neural responses from electrical stimulation is hypothesized to reduce features available for word discrimination.
Conclusions: Preliminary results suggest a pre-trained classifier can successfully identify simulated neural responses for normal hearing. Poor performance observed with neural responses from electrical stimulation may resemble that of a post-lingually deafened patient whose implant is activated for the first time, while the brain has yet to adapt to the new stimulation. Additionally, lack of fine detail in current electrical stimulation neural patterns may offer a path for improvement in the models used to simulate these responses. Future work will seek to gauge real-world performance of the framework using cochlear implant patients rather than a classifier.

M138. Optical Properties of the Human Cochlea Bone

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Category: Auditory Prostheses
Background: Optical stimulation has been proposed to offer superior benefits compared to electrical stimulation, due to its ability to directly excite neurons without contact and with high spatial precision. This enhanced precision allows for the activation of discrete and independent populations of neurons, resulting in a more precise control of neural firing patterns [1].
Light propagation in tissues is determined by optical properties of absorption and scattering, both being wavelength-dependent. These two events are quantified by the absorption (μa) and the scattering (μs) coefficients. These variables are defined such that, when a photon propagates over an infinitesimal distance ds, the probability for absorption and scattering is μa×ds and μs×ds, respectively. Because biological tissues are complex turbid media (multiple scattering events [2]), the spot size of the optical radiation at the target structure and the energy delivered to the target, can be affected by these properties. As a result, the decrease in spatial precision may nullify the advantage of optical stimulation over electrical stimulation.

To better predict the neural population in the cochlea that will be stimulated using certain optical parameters (wavelength, optical power, and beam profile), it is important to characterize the optical properties in the human cochlear bone, in the visible (for optogenetics) and near-infrared (for infrared neurostimulation) wavelengths, which are poorly explored in literature.

Methods: To determine the optical properties of the human cochlea bone, we will use the inverse Adding-Doubling method (IAD) [3] and several cochlea samples with thicknesses ranging from 300 to 1000μm. The IAD is an iterative procedure that generates the optical properties of a slab-layered material when three parameters are provided: the measured transmittance MT (light transmitted by the sample normalized by the incident light), the measured reflectance MR (light reflected by the sample normalized by the incident light) and the measured unscattered transmittance MU (light passing through the sample without being scattered or absorbed normalized by the incident light).
To determine these parameters, the cochlea sample is fixed in a double-integrating sphere system (IS200, Thorlabs, USA). Light of several wavelengths (450-1550 nm) illuminates the sample and the output of each sphere is connected to a power meter.

Results: The study is in progress.
Conclusions: With the scattering and absorption coefficients, we will work in a model that will provide insights into light distribution in the cochlea bone, of importance in optical neurostimulation and neuromodulation.

M139. Visualization System for Real-Time Monitoring of Electrode Array Insertion into the Human Cochlea

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Category: Auditory Prostheses
Background: Deafness and hearing loss are widespread in the world. Currently, more than 1.5 billion people live with hearing loss. By 2050, nearly 2.5 billion people are projected to have some degree of hearing loss and at least 700 million will require hearing rehabilitation.
Assistive technologies, such as cochlear implants (CIs) can help people with this disability. Despite being one of the most successful neural prosthetics, CI implantation surgery is not devoid of risks. A crucial component of successful CI surgeries, the cochlear electrode array, is surgically inserted into the cochlea to electrically stimulate the auditory nerve. Given the intricate inner ear anatomy, the insertion process demands the utmost precision and care. However, damage or potential misalignment of the cochlear implant electrode can trigger inflammation. Tragically, this leads to severe loss of residual hearing in at least 32% of implantations. Therefore, preserving this residual hearing is paramount as it can bolster the benefits of the CI.

In recent years, atraumatic electrode insertion has received increased interest among CI surgeons and researchers in order to optimize hearing preservation. Although there are several monitoring techniques that can facilitate the insertion procedure; the surgeon relies primarily on tactile feedback to determine the trajectory of the insertion.

In the present study, we describe a visualization system that can be integrated into a CI electrode.

Methods: The visualization system includes the world's smallest camera and a module for post-processing the images acquired. This allows for real-time monitoring and analysis of the images, and provides a high level of accuracy and detail in the visualization process.
The nanocamera used in our design is the OVM6948 (Omnivision, USA). It integrates image array, signal processing, timing, and control circuitry, all on a single chip. It measures 0.65 mm x 0.65 mm with a z-heigh of 1.16 mm and has a resolution of 200 x 200 pixels. It is capable of capturing high-quality images and video at up to 30 frames per second.

The nanocamera is affixed at the tip of conventional CI electrode arrays (Advanced Bionics, USA) and it is inserted into human cadaveric temporal bones.

Results: The device test is ongoing.
Conclusions: We successfully achieved full insertion of the electrode, equipped with the nanocamera, in five different cadaveric human temporal bones.

M140. Ensuring the Future of Otitis Media Research: Interest in and Feasibility of a Mentoring Network Program Within the Otitis Media Research Community

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Category: Other, Education
Background: The goal of this study was to assess the feasibility of the implementation of a mentoring network program based on expressed interest and the diverse composition of the otitis media research community in order to address attrition of the scientific workforce.
Methods: An online survey was sent to basic, translational, and clinical researchers with a known interest in otitis media.
Results: Of 509 eligible participants, 119 (24.4%) responded to the survey. Survey respondents had a diverse background by completed education, current job description, and membership in an underrepresented group in science. Most faculty respondents (76.4%) were willing to participate in the proposed program as mentors and faculty lecturers, or had early-career researchers or trainees in their research group who were willing to participate as mentees in the mentoring network. Scientific and non-scientific topics for inclusion in the training program were ranked, with immunology and inflammation, microbiology, science leadership and collaboration, mentoring, and grantsmanship as main foci of interest among respondents.
Conclusions: Our survey results showed enthusiastic participation among responding otitis media researchers, indicating the feasibility of implementing a mentoring network program that will address workforce attrition,

particularly among underrepresented groups in science. Key aspects of the proposed program will be presented, including linkages with ARO.

M141. Sexual Dimorphism in cVEMP Latency

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Category: Vestibular: Basic Research and Clinical

Background: Women have been identified more frequently than men in vestibular diagnoses. A review of healthcare data representing approximately 86% of the German population found women more frequently affected than men by dizziness and vertigo, with higher prevalence of vestibular-related diagnoses in women than men between the ages of 15 and 74 years. We examined whether a standard clinical test, the cervical vestibular-evoked myogenic potential (VEMP), could identify difference in vestibular function between women and men.

Methods: We compared 24 women aged between 16.6 and 21.1 years (mean 19.22, SD 0.70) to 24 men aged between 18.6 and 20.7 years (mean 19.72, SD 0.57). VEMP was recorded between the sternum and belly of the sternocleidomastoid, with muscular tension controlled via biofeedback in which participants pushed their foreheads against a padded bar to maintain background tension as close as possible to 50 μV root mean square. Body-conducted stimuli were delivered through a sequence of descending stimulus levels, enabling sex comparison using linear mixed-effects modelling.

Results: Women had significantly shorter VEMP interpeak latencies than men. We established a sex difference of 2.4 ms (95% CI [–0.9, –3.9], $p = 0.0020$), which is 21% of the mean 11.4 ms VEMP interpeak latency measured across women and men. We found no significant sex difference in VEMP interpeak amplitude ($p = 0.52$). Our findings were a reversal of several prior studies, which we reviewed and assessed for quality. Using simulation based on our data, we demonstrated how some of the prior studies were underpowered to detect the sex difference.

Conclusions: The finding of sex difference in VEMP interpeak latency suggests influence of sex hormones. These may have affected myelination or synaptic response, and thereby conduction through the vestibulo-collic reflex arc of which VEMP represents a short latency fragment. Those investigating vestibular-related diagnoses in women should consider the possibility that sex hormones contribute to vestibular dysfunction. We also discuss the possibility that a neck/head size which is on average smaller in women than men may have contributed to our findings. Such an effect may co-occur with an explanation involving sex hormones, complicating interpretation.

Combining our findings with data from our review of previous studies, the overall sex-specific indication is that between 17–21 years VEMP onset is prolonged and offset shortened in women compared to men. Across the lifespan, onset becomes prolonged in both sexes, but moreso in men than in women. By the age of 70 years or more, VEMP onset is prolonged in men compared to women – the opposite of the situation at ages 17–21 years. When used to assess sex difference, VEMP is a highly dynamic measure whose latency changes across the lifespan in a way that differs between women and men.

M142. Examination of Aspiration Prevention Surgery in Our Department

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Category: Clinical Otolaryngology and Pathology

Background: There are two types of surgical interventions for aspiration. One is surgery to improve swallowing function that preserves the larynx. The other method is to completely separate the respiratory tract and digestive tract, sacrificing voice function.

Methods: We conducted a retrospective study based on medical records of 25 patients who underwent aspiration prevention surgery at Yamaguchi University Hospital from May 2013 to October 2018.

Results: Separation of the larynx from the trachea was performed in 6 cases, laryngeal closure in 18 cases, total laryngectomy in 2 cases, and total laryngectomy + pectoralis major myocutaneous flap reconstruction in a case.
Regarding pre- and post-operative respiratory management, 10 of the 15 patients who did not use mechanical ventilation achieved post-operative no need of a tracheal cannula.

We evaluated status of pre- and post-operative nutrition intake. Regarding adult cases, 10 out of 16 cases improved from alternative nutrition to oral intake. Of the 16 cases, 11 no longer needed alternative nutrition.

Conclusions: In cases no ventilator was required, there was a high probability of achieving no need of a tracheal cannula postoperatively.
It was possible for many adults to discontinue alternative nutrition.

We considered aspiration prevention surgery as one of the major options in severe aspiration patients at risk of aspiration pneumonia.

M143. Potential Overestimation of Cognitive Decline in Older Adults With Hearing Loss

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Category: Clinical Otolaryngology and Pathology
Background: Hearing loss has been identified as a potential biomarker for cognitive decline, and most studies are focused on their causal relationship and preventive efforts. However, heterogeneity of evaluation methods for both auditory and cognitive function present challenges in their interpretation. The Korean Montreal Cognitive Assessment (K-MoCA) is a widely used tool to screen cognitive impairment in older adults, and includes items that utilize verbal information. In this study, we aimed to analyze the possible effect of hearing loss on the cognitive function test in older adults.
Methods: Twenty consecutive patients (M:F=1:2, age 72.151+/-13.73 years) who performed neurological examination and conventional audiometry were included in the study. K-MoCA results were scored, and an additional "auditory" scoring method was used that eliminated items that required the participants to listen to specific words or sounds. Participants were grouped as normal hearing and hearing impaired and the cutoff results were compared between the original and auditory scoring methods.
Results: Hearing thresholds in the better ear was 38+-16.83 dB (range 8-60 dB) in all participants. 25% passed the K-MoCA. In the normal hearing group, 50% had MoCA scores in the normal range, compared to 19% of those in the HL group. When the auditory scoring method was used, the mean score of the proportional scoring procedure in HL group increased by 7%, whereas it increased by 4% in normal hearing.
Conclusions: The results suggest that neglected hearing loss may be a confounding factor that leads to overestimation of cognitive impairment. Thus, implementation hearing screening would complement current protocol of cognitive function tests in older adults.

M144. Characteristics of Pure Tone Audiogram in Patients With Untreated Sporadic Unilateral Vestibular Schwannoma

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Category: Clinical Otolaryngology and Pathology
Background: Asymmetric sensorineural hearing loss (ASHL) is the most common symptom of sporadic vestibular schwannoma (VS). However, there is still no universally accepted MRI protocol for diagnosing VS. This study identified the characteristics of pure tone audiogram (PTA) in patients with VS.
Methods: We conducted a retrospective chart review of patients diagnosed with sporadic unilateral VS. In the analysis, we focused on the shape and interaural differences of PTA, stratified by the mode of onset and patient age. The audiogram shapes were defined as: (1) low-frequency, ascending form, (2) U-shaped form,

(3) high-frequency descending form, (4) flat form, (5) profound form, and (6) Dip form, (7) middle- and high-frequency descending form, and (8) other forms. Additionally, the patients were further categorized by age on the date of PTA into young or early adulthood (39 years old and under), middle adulthood (40-59 years old), and old age (60 years old and over).
Results: In total, 390 patients (195 males [50.0%] and 195 females [50.0%]) met the inclusion criteria. We categorized the patients into sudden sensorineural hearing loss (SSNHL) group (n = 87 [22.3 %]) and non-SSNHL group (n =303 [77.7%]).The U-shaped audiogram showed the highest proportion in patients with the onset of SSNHL. In patients with SSNHL, U-shaped audiograms were younger than other audiograms, and 86.7% of patients under 40 had U-shaped audiograms. Patients with VS were more likely to have interaural differences at higher frequencies than at lower frequencies. Patients with SSNHL had a significantly higher percentage of interaural differences at 500–4000 Hz than those with onset other than SSNHL (non-SSNHL patients). In addition, non-SSNHL patients had a significant trend toward a higher percentage of interaural differences at all frequencies with increasing age.
Conclusions: MRI screening can be considered in patients with SSNHL with U-shaped audiograms under 40 years of age. In ASHL, not SSNHL, MRI screening can be considered for older patients with interaural differences at wider continuous frequencies. Patients with interaural differences at high frequencies had a higher priority than those with interaural differences at low frequencies as indications for MRI screening for VS.

M145. Oto-Pathology of Sickle Cell Disease

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Category: Clinical Otolaryngology and Pathology
Background: Sickle cell disease (SCD) is associated with sensorineural hearing loss in both pediatric and adult populations. It is hypothesized that microvascular occulsion and hypoxic conditions during sickling events may cause cumulative neural damage to the inner ear. This study sought to compare spiral ganglion cell (SGC) populations amongst individuals with SCD and controls using temporal bone histology and to correlate these with clinical observations.
Methods: Thirty-six temporal bones including both ears from 6 cases of SCD (ages 6-28, 3 male, 3 female) and 12 controls (ages 8-29, 6 male, 6 female) age matched ± 5 years were scanned at 20x using an Olympus VS200 slide scanner. Controls did not have a history of otologic disease; 10/12 were also sex and race matched. To build a deep learning model, 12 images each containing multiple cochlear regions were used to train the algorithm by manually identifying SGCs. After running the algorithm, the checkpoint with the highest similarity value was selected (0.75), with a value closer to 1 signifying better neural network prediction.

The accuracy of our algorithm was further confirmed by favorable Intersection over Union (IoU) and Dice scores: IoU=0.77 (ratio of the intersection between the network's prediction and the training label to their combined union area), Dice coefficient=0.85. During the detection phase, objects identified with an area less than 40 μm2 were discarded after manual review; these were often artifacts mistakenly detected at the edge of larger SGCs. The auto-split function was also used to separate sets of SGCs counted as one due to proximity. Total SGC counts were multiplied by 10 to adjust for every 10th section being stained. SGC counts were estimated for missing or distorted slides by taking the average of the preceding and following slides.

Results: We found similar mean numbers of SGCs in the SCD and control groups, suggesting there is no substantial effect of SCD on SGN populations. The mean SGC counts for the SCD and control groups were 28,580 and 27,550, respectively. Upon review of slides, two SCD cases showed endolymphatic hydrops.
Conclusions: SGN populations remain intact in individuals with SCD. Final analyses correlating PTA values and morphological analysis of other cochlear structures in these cases is ongoing to better understand if cochlear pathology exists. Our current results suggest that sensorineural hearing loss in SCD cases is not primarily driven by neural loss. Notably, 2 SCD cases demonstrated endolymphatic hydrops, another possible explanation for the observed auditory symptoms.

M146. Investigating the Association Between SSHL Etiology and Hearing Outcomes Following Intratympanic Steroid Injection

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Category: Clinical Otolaryngology and Pathology

Background: Sudden sensorineural hearing loss (SSHL) cases often manifest abruptly, unilaterally, and idiopathically. Though the underlying cause of most SSHL cases are unknown, those with known etiologies may fall under a few categories including trauma, noise exposure, viral illness/vaccination, medication, genetically inherited, multifactorial, or other causes. In the clinic, SSHL is diagnosed with an audiometric evaluation and may be treated with either systemic oral corticosteroids, intratympanic corticosteroid injections (ITI), or combination therapy. For individuals following a SSHL diagnosis who do not meet criteria for systemic high-dose glucocorticoids, ITI is the first-line treatment. Prior work suggests that 20% recover from ITI alone, compared to 28% with combined therapy. In this work, we sought to identify whether specific underlying etiologies for SSHL were associated with improved hearing outcomes following ITI.

Methods: Through a retrospective chart review (#20230698), we reviewed the medical records (n=230) of individuals diagnosed with SSHL who received ITI. Our cohort consisted of n=150 responders, or those that experienced an SRT change of at least -10 dB and/or WRT recovery greater than 15% in the affected ear receiving ITI treatment, and n=80 non-responders. Patients with a past medical history of vestibular disorders, traumatic brain injury, or recent chemotherapy treatment were excluded. Pertinent information was collected from each patient's chart including basic demographics, as well as the origin of SSHL (if known), the timing of oral steroid administration in reference to ITI treatment, and speech recognition thresholds (SRT) and word recognition scores (WRS) in the affected ear pre- and post-ITI, if available. Fisher's exact tests were performed to assess relationships between responder status and categorical variables of interest.

Results: Of the 230 total patient charts reviewed, 112 were males (48.7%) and 118 females (51.3%) with a mean age of 52 (±12.7) years old. Most SSHL cases were either idiopathic (50.6%), of other identifiable causes (24.3%), or viral illness/vaccination (13.5%). Overall, we found that most non-responders (n=80) to ITI were related to idiopathic SSHL (83.8%). Interestingly, when examining responders to ITI (n=150), we found that the majority of these individuals reported identifiable causes for their SSHL (67.3%), perhaps indicating that cases with suspected etiologies respond better to ITI. Further, among responders to ITI, a higher percentage received concurrent oral steroid treatment (53.4%), supporting combination therapy as the most efficacious form of SSHL treatment. Finally, when comparing suspected causes for SSHL, we found significant differences in responder status based on suspected SSHL etiologies including idiopathic (p less than 0.001) viral illness/vaccination (p less than 0.001), and other identifiable origins (p less than 0.01).

Conclusions: First-line treatment for SSHL cases is isolated ITI or concurrent oral steroid treatment. Although most cases of SSHL are idiopathic, those with identifiable etiologies respond significantly better to ITI and combination therapy.

M147. Performance on the Triple Digit Test Relates to a Blood Marker of Brain Injury in Patients With Alzheimer's Disease

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Category: Other, Auditory Processing

Background: Hearing loss is considered a risk factor for Alzheimer's disease (AD), but the underlying mechanism remains elusive. A "bottom-up" hypothesis has been suggested, where peripheral (i.e., cochlear) damage at the "bottom" of the auditory pathway accelerates AD pathophysiology by sensory deprivation. This hypothesis suggests interventions to improve hearing might slow or prevent progression towards dementia. Alternatively, a "top-down" relationship may exist where protein deposition within the brain at the "top" of the central auditory processing pathway impairs the brain's ability to process sound. To assess which might predominate, we examined the relationship between primarily peripheral auditory tests (audiometry, highest audible frequency), centrally focused tests (triple digit test, staggered spondaic words, dichotic digits, and the

hearing-in-noise tests), and a marker of brain injury in Alzheimer's disease (glial fibrillary acidic protein (GFAP).

Methods: 21 individuals (8 M, 13 F, 10 cognitively normal, 11 with mild cognitive impairment) from a study of audition and Alzheimer's disease had complete auditory and GFAP data. The relationship between peripheral or central hearing test results and GFAP was examined using lasso regression. Lasso regression was performed 100 times using bootstrapping with replacement. The number of times an individual predictor was selected was expressed as a percentage. If the percentage was greater than 90% we performed multiple linear regression to confirm the association of plasma GFAP with the auditory test result. Regressions were run with the individual auditory tests as outcomes and GFAP, age, and the interaction of age and GFAP as predictors.

Results: For GFAP, both age and performance on the triple digit test (TDT) were strong predictors. Worse TDT performance was strongly related to increased GFAP levels ($p=0.002$) with a significant age interaction ($p=0.01$). As age increased, TDT performance worsened as GFAP levels increased. The peripheral auditory tests did not show any relationship to GFAP. Peripheral auditory tests, however, did influence TDT performance. For the TDT, both GFAP and the highest audible frequency were significant predictors.

Conclusions: These preliminary data provide support for the "top-down" hypothesis for how Alzheimer's disease affects hearing. This blood marker of brain injury was strongly related to performance on a central, rather than any peripheral auditory test. These results also highlight the three-way interaction of central auditory test performance, peripheral hearing ability, and AD-related brain injury that needs to be considered when evaluating the relationship between hearing and Alzheimer's disease. A larger longitudinal dataset, including plasma and CSF samples is needed to explore the interactions between cognitive performance, aging, co-morbidities, and both central and peripheral hearing ability. Such a comprehensive approach will be instrumental in identifying the relationship between hearing and AD.

M148. Sensory Transduction Contributes to Synapse Maturation During Auditory Hair Cell Development

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Category: Hearing Loss: Consequences and Adaptation

Background: Inner hair cells (IHCs) convert sound stimuli into electric signals through activation of mechanosensory transduction channels (TMC1 and TMC2) localized at the tip of the stereocilia (Pan et al., 2013; 2018; Kurima et al. 2015). They are associated with other proteins, notably TMIE and CIB2 (Xiong et al. 2012; Zhao et al. 2014; Giese et al. 2017). Hair cells acquire transduction, progressively, from the basal end of the cochlea to its apical end, during the first postnatal week in mice (Lelli et al., 2009). Before the onset of hearing, IHCs also fire spontaneous action potentials which are believed to play a role in the development and maturation of the auditory system (Kros et al., 1998; Trish et al., 2007; Johnson et al., 2011; 2017). 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 or carrying dominant mutation in TMC proteins and mice carrying a recessive mutation in TMIE (Spinner mice). We performed single cell electrophysiological recordings to assess voltage-dependent calcium currents and exocytosis and examined ribbon synapse with immunostaining and transmission electron microscopy at 3 weeks. We assessed the spontaneous and evoked firing properties of ANF, in vivo, using single fiber recording in anesthetized mice (2-4 months).

Results: Our work demonstrates preservation of synaptic properties and features in Tmc2 KO mice and alterations in fast and sustained exocytosis along with impairment of voltage-dependent calcium currents in Tmie, Tmc1 KO and double Tmc1/Tmc2 KO mice. These changes are also associated with alteration in the morphology of the synapse as we demonstrated previously (Lee et al., 2021) and further validated in this study. Specifically, we observe alterations in the localization of Ca2+ channels clusters near the ribbon

synapse in absence of Tmc1 or Tmc1/Tmc2 and concomitantly reduced ribbon size. We observe reductions in spontaneous firing rates of ANF in absence of Tmc1, Tmc1/2 and Tmc2.
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.

M149. Open-Source Music Playlist for Cochlear Implant Users: An Accessible Tool for Clinicians

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Category: Hearing Loss: Consequences and Adaptation
Background: Traditional clinical practice encourages patients with cochlear implants (CIs) to experience a diverse range of sounds after surgery. In this sense, listening to music is usually recommended by professionals. However, specific guidance on the types of music that would best aid rehabilitation has been absent. Therefore, most patients find it difficult to choose music to listen to, and doctors also struggle to provide guidance on which music would be beneficial for hearing.
Methods: This study introduces a music playlist, specifically designed for the auditory rehabilitation of CI users, based on characteristics of their auditory perception mechanisms. Each piece of music on the playlist has been selected based on findings from previous studies on music and speech perception in CI users. We hypothesized that the progression from simpler to more complex musical pieces enhances CI users' accessibility to musical experiences and appreciation.
Results: While the impact of this list awaits further evaluation, its development represents a significant step forward in providing practical guidelines for clinical sites.
Conclusions: The playlist could be used as an accessible tool for clinicians and therapists, and provide frameworks for rehabilitation. Moreover, it holds the potential to amplify auditory enjoyment and overall quality of life for individuals using cochlear implants.

M150. Machine Learning-Based Prediction of Hearing Recovery Prognosis in Canal Wall Down Mastoidectomy Patients

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Category: Hearing Loss: Consequences and Adaptation
Background: Chronic Otitis Media (COM) is an inflammatory condition affecting the middle ear, marked by symptoms like hearing loss and discharge due to tympanic membrane perforation and middle ear diseases. While conservative medical treatment is an option, surgery is essential to address the root causes. The goal is to prevent recurrence by removing middle ear inflammation and enhancing auditory function through reconstruction. Canal Wall Down mastoidectomy (CWD) offers surgical benefits, including improved visualization and reduced recurrence rates. However, it poses a significant patient burden and complex decision-making due to hearing expectations. Our study proposes a machine learning-based decision support system for medical professionals to aid in this process, using patient data from COM cases who underwent CWD to predict post-surgery auditory outcomes.
Methods: We conducted a retrospective study at Korea University, Ansan Hospital, covering patients diagnosed with chronic otitis media who underwent canal wall down mastoidectomy between March 2007 and August 2020. Data from 261 patients were collected, including underlying conditions, pre-operative status, microbial culture, pathology, surgical technique, MERI score, postoperative tympanic membrane condition, and auditory status categories. The study comprised 31 variables, both continuous and categorical. Patients were categorized based on Pure Tone Audiometry (PTA) results, assessing hearing recovery with criteria such as post-operative PTA ≤ 30, post-operative Air-Bone Gap (ABG) ≤ 20, and a pre-post operative PTA difference ≥ 15. Data preprocessing involved omitting surgical and PTA-related variables to predict pre-operative and post-operative outcomes. Correlation analysis removed insignificant numerical attributes, while categorical variables underwent expert-guided categorization, followed by label and one-hot encoding.

RobustScaler normalized numerical variables, and feature selection used the Forward Floating method from the Sequential Feature Selector. Machine Learning Model Training included Decision Tree, Random Forest, LightGBM, SVM, Logistic Regression, and XGBoost models. The dataset was split into 90% training data and 10% test data.

Results: Utilizing a single-cohort dataset consisting of 261 patients from Korea University, Ansan Hospital, Nested Cross-validation was employed for model training. After the training, model performance evaluation was conducted on test data. 6 different model performances on the test data is examined. Decision Tree / Random Forest / Logistic Regression / XGBoost / LightGBM / and the last is SVM model. During these models, LightGBM model exhibited the highest performance with an accuracy of 68% and a recall of 75%. The three key factors selected by the model are gender, surgical procedure, and smoking intensity (pack-years)."

Conclusions: This study represents the first paper to predict auditory prognosis after surgery by applying machine learning to data from patients who underwent canal wall down mastoidectomy among those diagnosed with chronic otitis media. Subsequent research aims to enhance reliability by employing more advanced model performance evaluations and external validation.

M151. Analysis of Receiver Operating Characteristics to Evaluate the Performance of Predictor Variables for the Diagnosis of Cochlear Synaptopathy

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Category: Hearing Loss: Consequences and Adaptation

Background: Mammalian inner ear pathology resulting from exposure to moderate levels of noise is widely recognized as an unanticipated and alarming disorder generally characterized by a temporary threshold elevation, a permanent reduction of response amplitudes to sound, and a partial loss of ribbon synapses connecting inner hair cells to type I spiral ganglion neurons. A diagnostic test to identify the disorder clinically in humans is currently unavailable, and the only objective method to confirm the condition requires quantitative assessment of inner ear synapses in post-mortem tissues. Therefore, efforts to identify response variables that best distinguish animals with cochlear synaptopathy from normal animals were undertaken using a guinea pig model and an array of noninvasive electrophysiological tests that were designed as bridge metrics for human studies. Ultimately, the goal is to construct a multivariate statistical model that is validated in the non-human animal model and apply it to findings from an identical battery of tests carried out in human subjects to parameterize the model for use as a diagnostic tool.

Methods: Analyses aimed at the determination of the sensitivity and specificity of a limited set of findings from our lab including auditory brainstem responses (ABR) and envelope following responses (EFR), masking studies, and experiments designed to “stress” temporal processing capabilities of the system, are reviewed in this report. To efficiently predict the probability that individual animals have synapse pathology, simple logistic regression analyses were conducted using predictor variables derived from ABRs and EFRs. Each logistic regression model was fitted to a training data set and used to predict the probability of synaptopathy in a test data set. To assess how well the logistic regression models fit the data, Receiver Operating Characteristic (ROC) curves were generated for each predictor, and the area under the curve (AUC), and 95% confidence intervals of sensitivities at given specificities were computed based on bootstrap replicates.

Results: AUC varied across the following metrics: ABR amplitudes elicited by different stimulus levels, slopes of response amplitude-level curves, slope of click-evoked amplitudes vs. repetition rates or paired interstimulus intervals, EFR magnitudes at various carrier levels, modulation depths and frequencies, and in the presence of a broadband noise masker. Numerous predictors produced AUC values in the 90%-96% range, while other variables produced lower values, indicating a range of discrimination abilities between (+)- and (-) synaptopathy profiles.

Conclusions: Overall findings reported here indicate that several variables may serve as effective predictors of cochlear synaptopathy and a selected set will be used in a multivariate statistic model and applied to human studies. Currently, work is ongoing to increase sample size and to analyze all experimental variables to identify those that are the strongest predictors of cochlear synaptopathy.

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M152. Longitudinal Study of Dietary Intake and Risk of Incident Persistent Tinnitus

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Category: Tinnitus

Background: Dietary factors have been suggested as contributors to tinnitus, but longitudinal data in large populations with well-validated and a broad range of dietary information are scarce. Findings from cross-sectional and small longitudinal studies are inconsistent. A large cross-sectional study in the UK Biobank found higher intakes of whole grain bread, fruits and vegetables, and lower intake of fish, were associated with higher prevalence of persistent tinnitus. A small cross-sectional study in Italy found higher intake of legumes was associated with lower odds of tinnitus, but no associations for cereals, red meat, fish, fruit or vegetables were observed. A small longitudinal Australian study found lower fruit and cereal fiber intakes were associated with higher tinnitus risk. To overcome the limitations of previous studies, we comprehensively investigated the relations between specific foods and food groups and the risk of incident persistent tinnitus, using detailed, validated dietary assessments in a large well-characterized cohort of women followed over three decades.

Methods: We conducted a longitudinal cohort study (1991–2021) of 64,209 women in the Nurses' Health Study II, aged 27–44 y at baseline. We assessed diet every 4 years with the use of food frequency questionnaires providing information on over 130 foods. Information on tinnitus and potential confounders was obtained on validated biennial questionnaires. Persistent tinnitus was defined as daily tinnitus lasting ≥5 minutes. Baseline and updated information was used in Cox proportional hazards regression models simultaneously adjusted for dietary factors to examine independent associations between dietary intake and risk of incident persistent tinnitus.

Results: During 1,376,362 person-years of follow-up, 8,210 cases of incident persistent tinnitus were reported. Higher intakes of whole grains and legumes were associated with higher risk of incident tinnitus, while higher intakes of fruit and fish were associated with lower risk. Specifically, compared with women in the lowest quintile of intake, the multivariable-adjusted relative risk (MVRR, 95% CI) of persistent tinnitus among those in the highest quintile was 1.29 (1.19, 1.40) for whole grains and 1.15 (1.07, 1.25) for legumes. In contrast, the corresponding MVRRs among women in the highest quintile of intake were 0.80 (0.73,0.87) for fruit and 0.80 (0.75, 0.87) for fish. Within these groups we also identified individual foods associated with higher risk (e.g. cold cereal, oats, dark bread, beans and lentils), and with lower risk (e.g. citrus fruits, tuna fish, white fish and shellfish/shrimp).

Conclusions: Dietary intake influences the risk of developing persistent tinnitus. Higher intakes of whole grains and legumes are independently associated with greater risk of tinnitus, while higher intake of fruit and fish are independently associated with lower risk. These findings could inform further research to investigate whether specific dietary modifications may help mitigate tinnitus symptoms among those with tinnitus.

M153. Effects of Sound Therapy Using Hearing Aids Fitted With Utsunomiya Method in Unilateral Tinnitus Patients With Acquired Ipsilateral Sensorineural Hearing Loss

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Category: Tinnitus

Background: A large number of patients with acquired unilateral hearing loss experience tinnitus which has a serious impact on quality of life. One major hypothesis of the etiology of tinnitus in patients with hearing loss is that the reduction or shortage of auditory input caused by hearing loss changes the neural activity, which results in the perception of tinnitus. Sound therapy using hearing aids is a treatment aimed to supplement the decrease in peripheral input and suppress the perception of tinnitus. However, no study has investigated the effectiveness of the treatment in patients with unilateral hearing loss. The objective of this study is to

evaluate the effectiveness of sound therapy using hearing aids in patients experiencing tinnitus with acquired unilateral sensorineural hearing loss.
Methods: A total of 97 patients with unilateral tinnitus with acquired ipsilateral sensorineural hearing loss were included. Sound therapy using a hearing aid was initiated against all participants after counselling. Hearing Aids were fitted by audiologists with Utsunomiya Method, which is a specific fitting procedure in Japan. The treatment was started at least 6 months following the onset of hearing loss. The treatment efficacy was evaluated by three questionnaires: the Japanese version of the Tinnitus Handicap Inventory (THI), the visual analogue scale (VAS) for loudness and annoyance, and questionnaires on subjective symptom improvement for tinnitus loudness and annoyance. Evaluation was administered at entry, 3 months after treatment, and 1 year after treatment. For each questionnaire, the scores at entry and those obtained 3 months following the initiation of treatment were compared using the paired t -test. Analysis of variance was performed with post-hoc Bonferroni test for the comparison of the scores at entry and those obtained at 3 months and 1 year.
Results: The mean Tinnitus Handicap Inventory score before treatment decreased significantly (p less than 0.01) at 3 months (50.0 ± 24.5 to 12.7 ± 16.2), and 1 year (53.3 ± 25.5 to 8.79 ± 13.9), after treatment. Despite the degree of hearing loss, the THI score decreased significantly at the following 3 months and 1 year than at treatment initiation (p less than 0.01).Moreover, the visual analogue scale score decreased significantly at 3 months (loudness, 69.6 ± 21.9 to 29.1 ± 27.2; annoyance, 71.1 ± 24.7 to 22.7 ± 25.5) and 1 year (loudness, 69.8 ± 22.0 to 21.1 ± 26.1; annoyance, 72.7 ± 25.6 to 19.4 ± 27.6). Approximately 80-90% of patients noticed improvements in tinnitus annoyance and loudness, as determined by their responses to the questionnaires of subjective symptom improvement.
Conclusions: Sound therapy using hearing aids fitted with Utsunomiya Method is remarkably effective for patients with unilateral tinnitus with acquired ipsilateral sensorineural hearing loss.

M154. Biomarker Profiles of Cochlear Injury in Tinnitus

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Category: Tinnitus
Background: Previous studies have shown that individuals with hearing loss are at increased risk for tinnitus perception. However, the link between hearing loss and tinnitus is not straightforward; many individuals experience tinnitus despite audiometrically normal hearing. Indeed, our own analysis of University of Pittsburgh Medical Center (UPMC) records show that 35% of patients who seek audiological care despite normal audiograms do so with tinnitus as their primary complaint. Furthermore, not all individuals with audiometric threshold elevation experience tinnitus.
Methods: While audiometry can measure outer hair-cell injury, diffuse inner-hair-cell damage and cochlear synaptopathy remain hidden. Our recent body of work has identified individualized, non-invasive biomarkers that are sensitive to these otherwise-hidden components of hearing loss. Accordingly, the present study seeks to clarify the relationship between hearing loss and tinnitus by measuring audiometrically hidden components of sensorineural hearing loss (SNHL). Specifically, we will measure otoacoustic emissions (OAEs), envelope-following response (EFRs), and wideband middle-ear muscle reflexes (WB-MEMR) from individuals with and without tinnitus but with matched audiograms. Furthermore, we will analyze large clinical datasets from CDC NHANES and UPMC Audiology Clinics.
Results: Preliminary data support the hypothesis that, compared to controls with similar audiograms, individuals reporting tinnitus have a greater degree and/or more steeply sloping frequency profiles of audiometrically hidden forms of cochlear injury.
Conclusions: Ongoing work will test whether subclinical forms of hearing loss contribute to tinnitus risk or severity.

M155. Daily Sound Exposure Patterns After Tinnitus Onset

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Category: Tinnitus

Background: Tinnitus, the sensation of sound absent an external stimulus, is often linked to a history of excessive noise exposure from occupational and/or recreational noise exposure. The association between noise exposure and tinnitus has been established using various methods, including experimental animal models of tinnitus and self-reports of noise exposure. Most tinnitus and noise exposure investigations focus on noise exposure before tinnitus onset (Eggermont and Roberts, Cell and Tissue Research, 2015) or globally across the lifespan (Guest et al., Hearing Research, 2017). For instance, a recent study using a structured interview of exposure history found higher levels of lifetime noise exposure in tinnitus participants compared to age-matched controls. Lifetime noise exposure was estimated using sound levels derived from vocal effort (Guest et al., Hearing Research, 2017). To date, little attention has been given to noise exposure patterns and behaviors after the onset of tinnitus. This is surprising given the high comorbidity of tinnitus and hyperacusis.

Methods: Motivated by the above, we led an observational study where we compared the "noise lives" of participants with chronic tinnitus (n=57) to age-matched controls (n=50). "Noise lives" refers to the totality of environmental noise in everyday life (Tufts and Skoe, International Journal of Audiology, 2018). As part of this study, participants (age range, 18-80) wore a personal data-logging noise dosimeter (Etymotic, Inc.) for eight calendar days (168 hours) during all activities.

Results: As a group, tinnitus participants were exposed to significantly less noise during the week compared to controls, consistent with a pattern of hyperacusis. Based on the dosimeter data, neither group was at significant ongoing risk of noise-induced hearing loss, as daily cumulative sound levels did not exceed 75 dB LAeq for either group.

In addition to dosimetry, for a subset of participants, serological measurements of inner ear proteins were taken. Our team's recent collaborations suggest that prestin, the cochlear protein associated with cochlear amplification, is regulated by environmental sound conditions (Parker et al., Scientific Reports, 2021) and decreases nonlinearly with age (Parker et al., Hearing Research, 2022). Planned analyses on the current dataset will investigate whether similar trends are observed in tinnitus participants, predicting that tinnitus may distort typical relations between serum prestin, noise exposure, and aging.

Conclusions: If routine noise exposure was a precursor to tinnitus onset in our dataset, our findings suggest that participants with tinnitus have, on the whole, discontinued regular (weekly) exposure to noise during daily activities.

Dosimetry and serological findings will be discussed within the context of other ongoing work by our group into the noise lives of different demographic and clinical groups. We will summarize the strengths and limitations of wearable technology in its ability to advance mechanistic understanding of the interplay between auditory function and everyday sound exposure.

M156. Reduced Sizes of Efferent Nerve Termini in the Cochleae of Rats With Noise-Induced Tinnitus and Evidence of Therapeutic Mitigation With NHPN-1010

Qunfeng Cai*[1], Xiaoping Du[2], Weihua Cheng[2], Jianzhong Lu[2], Zachary Yokell[2], Wei Li[2], Don Nakmali[2], Richard D. Kopke[2], Matthew West[2]

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Category: Tinnitus

Background: Olivocochlear efferents deliver inhibitory signals to the cochlea to safeguard against noise damage. It has been suggested that changes in efferent innervation are a potential mechanism involved in the development of noise-induced tinnitus (NIT). We developed a temporary threshold shift (TTS) model of NIT in rats and measured the sizes of efferent termini in the cochleae from these animals and evaluated the treatment effects of NHPN-1010, a Phase II-ready combination drug composed of HPN-07 and NAC in this context.

Methods: Rats were exposed to an 8-16 kHz OBN at 108 dB SPL for two hours. Auditory brainstem responses and acoustic startle reflex tests were conducted to assess hearing acuity and tinnitus. One group of rats with established behavioral evidence of NIT at four weeks post noise was administered NHPN-1010 (300 mg/kg twice daily) for 14 days. Immunolabelling of ChAT (choline acetyltransferase), a biomarker for efferent nerve

termini, was conducted, and the sizes of outer hair cell (OHC) ChAT silhouettes were measured. Densities of afferent (inner hair cell) ribbon synapses (RSs) immunolabelled with CtBP2 and GluR2 were also measured.
Results: A TTS animal model of NIT without hair cell loss was successfully established. 61% of rats exhibited tinnitus at four weeks post-noise exposure. Significantly smaller efferent nerve termini were measured at 9.3, 16, 20 and 24 kHz tonotopic frequency positions in all rats exposed to noise, with or without tinnitus, compared to the naïve controls (p less than 0.01 or 0.001) at this time point. However, rats with tinnitus had significantly smaller OHC ChAT silhouettes compared to rats without tinnitus (p less than 0.05 or 0.001) at all frequencies measured. Noise exposure also induced significantly reduced RS densities at 32k and 45.2 kHz compared to naive controls (p less than 0.01 or 0.001), although no differences were measured between noise-exposed rats with or without tinnitus. When NHPN-1010 treatment was initiated at four weeks post-noise exposure in animals with established tinnitus, OHC ChAT silhouette areas at 12 weeks post-noise were significantly than in saline-treated controls at all evaluated tonotopic frequency positions (all p less than 0.001), coinciding reduced numbers of tinnitus-positive animals. These therapeutic effects were also measured among impacted IHC RS populations.
Conclusions: Our TTS noise exposure model significantly and permanently reduced the sizes of OHC efferent termini in rats, and animals with behavioral evidence of tinnitus had significantly smaller ChAT silhouettes than tinnitus-negative animals. These results indicate that reduced feedback inhibition from the central auditory system may be a key mechanism associated with NIT. Furthermore, NHPN-1010 treatment resulted in average increases in OHC efferent silhouette areas and demonstrated efficacy for treating chronic tinnitus in this context.

M157. Distinctive Trends of Masking Release Effect in Frequency Following Response

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Category: Binaural Hearing and Sound Localization
Background: The binaural masking level difference (BMLD) is one of the key psychoacoustical phenomena that listeners perform better signal detection when interaural phase conditions, either signal or noise, are out-of-phase. The frequency following response (FFR) mirrors the phase-locked response evoked by periodic characteristics in the input stimulus and enables us to observe the binaural processing as a function of interaural stimulus difference. An advantage of the FFR is to identify the direct change of binaural activities generated by the binaural cues such as interaural loudness difference (ILD) and interaural time difference (ITD). The current study aimed to examine the changes in neural activities generated by multiple interaural phase configurations in both in-quiet and in-noise environments using the FFR technique. Consequently, the trends in neural activities of the effect of masking release were analyzed how FFR outcomes are distinctively correlated with behavioral records.
Methods: A total of ten young adults with normal hearing voluntarily participated in this study. The acoustic signal was a 400-Hz tone burst presented at 70 dBSPL in all conditions, and white noise was combined to establish the desired signal-to-noise ratio (SNR). The starting phase of the tone presented to the right ear was consistently 0 degrees while the phase of the tone introduced to the left ear was varied in increments of 90 degrees, ranging from 0 to 180 degrees. As a result, the study encompassed three distinct interaural phase conditions: 0, 90, and 180 degrees, coupled with three levels of noise: +5, +10, and +15 SNR. These conditions were selected to ascertain how phase configurations in signal and/or noise contribute to reflecting the FFR outcomes.
Results: The pilot data yielded a reduction in the spectral amplitudes of the FFR as the interaural phase increased. Although this finding did not directly signify the BMLD effect, it reflected the neural correlations related to signals in both ears. Furthermore, the alteration in interaural phase difference within the signal exhibited more pronounced amplitude changes than the phase difference within the noise. This pattern persisted across all SNR conditions. However, the FFR under diotic conditions demonstrated a more substantial decrease in amplitude compared to the results observed in dichotic conditions when the SNR was decreased.
Conclusions: In conclusion, the outcomes of FFR did not directly indicate the advantage of masking release. However, they contained its original characteristics to change of phases as an electrophysiological

measurement. Moreover, the study investigated the distinct contributions of both signal and noise components to neural activities, enabling a comparison with the behavioral BMLD results.

M158. Impact of Acute Impairment on Adaptive Head Movements Individuals With Single-Sided Hearing Loss

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Category: Binaural Hearing and Sound Localization

Background: Single-sided deafness (SSD) is a condition where one ear has no functional hearing while the other ear has normal hearing. Research has shown that individuals with single-sided deafness struggle with localizing sounds and understanding speech in the presence of background noise (Firszt et al 2017; Mondelli et al 2016; Brungart et al 2014; Asp et al 2018;). Though some studies have highlighted the importance of limited auditory deprivation on speech outcomes (Atak et al, 2023; Nassiri et al, 2022; Kurz et al, 2018), it remains poorly understood how duration of deafness or ongoing auditory stimulation may impact these abilities. It is also unclear how adaptive listening strategies may play a role in performance on binaural tasks as most current test paradigms require fixed head positioning and limit an individual's ability to utilize head movement adaptations to assist with listening. Here, we aim to characterize and compare head movement patterns during a combined localization and speech-in-noise task for individuals with longstanding organic SSD to those with acute conductive hearing loss from ear plugging to better understand how adaptive behaviors emerge.

Methods: Normal hearing subjects were tested in an un-occluded (NH-UO) and occluded condition (NH-O), with the latter facilitated by placement of a deeply seated earplug and over-the-ear muff, and SSD subjects were tested in an unaided condition alone. Broadband noise (BBN), narrowband noise (NBN), and speech-in-noise (SIN) stimuli were presented in a hemi-anechoic chamber using 24 speakers, evenly spaced 15o apart, spanning 360 o around the subject. For the localization only tasks (BBN, NBN), subjects indicated the perceived location of the stimuli with a button push, and for the combined task (SIN), subjects repeated the target stimuli (Harvard IEEE sentences) in addition to indicating perceived stimulus location. A head-position tracker captured real-time movement throughout the task.

Results: For localization only and combined tasks, head movement analysis will quantify movement delay (ms), absolute total displacement (degrees), and total response time (ms). Localization accuracy will be calculated as root-mean-square error and linear best-fit characteristics across target locations. Combined task SIN performance will be characterized as psychometric functions of percent correct according to stimulus signal-to-noise ratio. Data from all analyses will be compared between groups (NH-UO, NH-O, and SSD) and stimulus conditions (BBN, NBN, SIN).

Conclusions: Data presented here will provide valuable insight into the adaptation of localization and speech understanding for individuals with SSD. While only an initial investigation of the acute effects of hearing impairment on adaptive head movement, this is a first step towards developing a better understanding of what adaptive strategies are employed and how they are developed in individuals with SSD.

M159. Aging Impairs Binaural Processing and Spatial Hearing, While Increasing Synaptopathy, in the Mongolian Gerbil

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Category: Binaural Hearing and Sound Localization

Background: Aging can lead to problems in spatial hearing abilities and 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), spatial hearing behavior and cochlear histology in the Mongolian Gerbil (Meriones unguiculatus) to examine mechanisms of age-related dysfunction

in the auditory pathway. We performed ABRs in a cohort of young (2-10 month) and aged (greater than 30 month) gerbils to assess physiology of the auditory brainstem and calculate 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 showed thresholds that are considered normal by clinical standards (less than 20 dB shift), indicating these changes are not solely due to peripheral hearing loss. To see if these changes correlated with impaired spatial hearing behaviors, we ran our cohorts of gerbils through spatial hearing tasks utilizing prepulse inhibition of the acoustic startle response (PPI). PPI requires no training and also offers a high-throughput measure such that a large number of animals can be tested in a relatively short timeframe. 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 shorter 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 cochleas from our young and aging cohorts to assess levels of cochlear synaptopathy. Synaptopathy is thought to be involved in impairment of spatial hearing and difficulty in speech in noise. We found that our aging gerbil cohort had reduced synapses on inner hair cells compared to our young cohort.
Conclusions: Collectively, the data shows aged gerbil have impaired auditory brainstem physiology and binaural processing, which leads to impairment of spatial hearing behaviors and may be caused in part by increased synaptopathy. [Supported by R01-DC017924]

M160. Quantifying Anatomical Myelination Changes in Fragile X Syndrome Mice During Development in the Auditory Brainstem

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Category: Binaural Hearing and Sound Localization
Background: Fragile X Syndrome (FXS) and autism are neurodevelopmental and communication disorders. FXS is the most common 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 location computation starts with the auditory brainstem, using interaural timing differences (ITD) and interaural level differences (IID) from both ears. Highly myelinated axons in the medial nucleus of the trapezoid body (MNTB) of the auditory brainstem encode sound information quickly and precisely. Reduced or delayed myelination and altered binaural hearing have both been found in FXS patients. ASD and FXS are neurodevelopmental disorders in which underlying symptoms arise during critical developmental time-points. The study of myelination during critical developmental timepoints will help to establish when during development altered hearing ability arises in Fmr1 knockout (KO) mice.
Methods: We are analyzing 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, P21-23, and P60-90 using immunohistochemistry and electron microscopy. We are measuring both male and female C57BL/6J wildtype, Fmr1 KO and heterozygote female mice.
Results: Preliminary data suggests that there are alterations, such as thinner myelin sheath and increased spacing of Nodes of Ranvier, in the microstructure of myelinated axons in the auditory brainstem.
Conclusions: These findings are important for understanding mechanisms underlying FXS related to myelination and when during development they arise, particularly related to sensory sensitivity common in this condition.

M161. Open Board

M162. Older Adults Rely on the Structural and Functional Connectivity Between Auditory and Visual Cortex When Identifying Audiovisual Speech

James Dias*[1], Jeffrey Rumschlag[1], Carolyn McClaskey[1], Kelly Harris[1]

[1]*Medical University of South Carolina*

Category: Multisensory Processing/Interactions

Background: Older adults have more difficulty than younger adults identifying the speech they hear and see, but they often do not demonstrate such difficulties when identifying audiovisual speech. The neural architecture that supports this preservation of audiovisual speech is unknown. Existing evidence suggests that older adults demonstrate greater auditory-visual enhancement when identifying speech in noise and more cross-sensory neural activity in response to unisensory stimuli than younger adults (e.g., larger auditory-evoked responses measured in visual cortical areas). Older adults may rely more on cross-sensory processing to support audiovisual perception. We examined how functional and structural connectivity between auditory and visual cortex may support audiovisual speech perception using a multi-method-multi-metric approach. We hypothesize that age-related increases in auditory-visual enhancement, previously found to account for the preservation of audiovisual speech perception, is supported by greater reliance on the structural and functional connectivity between auditory and visual cortex.

Methods: A group of younger (18-28 years) and older (55-74 years) participants completed an audiovisual speech identification task, identifying words spoken in noise presented in auditory-alone (AO), visual-alone (VO), and audiovisual (AV) conditions. We calculated audiovisual enhancement from identification performance across conditions [SA=AV-(AO+VO)]. We recorded auditory-evoked potentials (AEPs) elicited by 1 kHz and 4 kHz tone bursts in all participants. Additionally, we performed structural imaging (sMRI) and diffusion imaging on all participants. Each participant's AEP and T1 structural image was used to source-constrain their auditory-evoked response to auditory cortex and visual cortex, measuring the auditory-evoked response generated in each cortical region. For each participant, the average cross-correlation between the response generated in auditory and visual cortex was computed, z-transformed, and used as a measure of functional connectivity between cortical areas. Deterministic streamlines tractography was performed on participants' diffusion images to identify the white matter tract connecting auditory and visual cortex. Metrics of fractional anisotropy (FA), axial diffusivity (AD), and radial diffusivity (RD) were calculated from within these tracts and used as measures of structural connectivity.

Results: Across age-groups, greater functional connectivity between auditory and visual cortex was associated with lower radial diffusivity and higher axial diffusivity in the white-matter connecting cortical areas (even after controlling for whole brain white matter), implying that both myelin and axonal structure support coherent function between sensory cortices. Confirming our past findings, older adults demonstrated more audiovisual enhancement than younger adults. Importantly, path analyses revealed that age-group differences in audiovisual enhancement were mediated by the structural and functional connectivity between auditory and visual cortex.

Conclusions: The results confirm our hypothesis. The enhanced multisensory perception of older adults is (at least partially) supported by cross-sensory neural mechanisms. The results suggest that older adults rely more on cross-sensory processing of speech information than younger adults when identifying audiovisual speech.

M163. Audiovisual Decision-Making Deficits After Hearing Loss

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Category: Multisensory Processing/Interactions

Background: Sensory impairments, including hearing loss, can lead to cognitive processing deficits. For example, hearing-impaired individuals exhibit reduced temporal integration when performing an auditory task (Florentine et al., 1988) and diminished audiovisual integration (Musacchia et al., 2009) compared to age-

matched individuals with normal hearing. Here, we examined how hearing loss impairs the decision-making variables of temporal integration, audiovisual enhancement, and sensory domain-general function.
Methods: Adult gerbils with normal hearing (NH) and those with hearing loss (HL) were trained to perform a single-interval alternative forced-choice audiovisual decision-making task. Gerbils initiated trials by placing their nose in a nose port and were then required to discriminate between slow (less than 6-Hz) versus fast (greater than 6-Hz) presentation rates of amplitude-modulated (AM) noise ("auditory-only" condition), light-emitting diode (LED) flashes ("visual-only" condition), or simultaneous AM and LED flashes ("audiovisual" condition) by approaching the left or right food tray. Temporal integration was quantified as the duration from trial onset to when animals departed the nose port area and approached one of the two food trays (i.e., "integration time"). We induced HL by exposing animals to loud broadband noise (~120 dB SPL) during a single 2-hour session. We measured hearing sensitivity by recording auditory brainstem responses (ABRs) pre-noise exposure and 1-, 7-, and 14-days post-noise exposure. Videos of task performance were captured with a USB camera and pose-tracking software (SLEAP) was used to track gerbil position. Labeled frames were then fed through a residual neural network (RNN) to analyze and predict trial outcomes.
Results: Exposing gerbils to loud broadband noise at ~120 dB SPL permanently decreased hearing sensitivity. Specifically, we found a significant increase in ABR thresholds ranging ~20-35 dB SPL for clicks and tones (1 to 16 kHz) that persisted across the two measured weeks post-noise exposure. Gerbils with HL displayed a slower rate of task acquisition across all trial types compared to NH animals. Following task acquisition in both NH and HL animals, discrimination performance for audiovisual trials was consistently more accurate with faster integration times relative to the single-modality trials. Superior performance for audiovisual trials in NH gerbils was predicted by the optimal cue integration of auditory and visual cues according to Bayesian Decision Theory. After inducing a subset of trained NH gerbils with HL, performance for auditory-only trials was severely impaired, and performances for visual-only and audiovisual trials were modestly altered. However, integration times for all sensory conditions were significantly extended following HL. Given behavioral trajectories, the RNN successfully differentiated between decisions but could not predict them with limited data.
Conclusions: These findings suggest that HL impairs sensory-informed learning and decision-making skill beyond auditory function. We will further examine this by combining in vivo neural recordings with computational (RNN and algorithmic) analysis.

M164. Hearing With Hands: Vibrotactile Stimulation Generates a Frequency-Following Response

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Category: Multisensory Processing/Interactions
Background: Auditory perception is the primary gateway for communicating with others through language and music. But the environment is rarely unisensory, so other senses can support or alter this perception. The interactions between auditory and tactile perception remain understudied to date even though these two modalities are responsive to similar physical phenomena (i.e., vibrations). Prior studies have documented that auditory cortex can respond to vibrotactile input, in both healthy and hearing-impaired populations. However, the nature of this neural response remains unclear; in particular, the degree to which periodicity encoding occurs in the brain in response to vibrotactile input remains unknown.
The frequency-following response (FFR) is a non-invasive evoked brain response measured using electroencephalography (EEG) that can be used to study the fidelity of auditory feature encoding of complex sounds and how they are represented in the central auditory system. Despite being employed in a wide range of auditory research, the FFR has never been used to investigate if a similar neural response that reproduces periodic acoustic features can be found when sounds are presented as vibrations to the tactile modality. The main goal of this study was to investigate if FFR could be a useful tool to investigate the underlying mechanisms of tactile perception of sounds.

Methods: We acquired EEG while healthy young participants were presented with 4000 repetitions of a synthesized speech syllable /da/ (fundamental frequency: 98 Hz), which is a classic stimulus used to elicit

FFR. The stimuli were presented under three conditions: binaural auditory input, vibrotactile input, and the two modalities combined. A technology developed within our laboratory (Sharp et al., 2023), the Multichannel Vibrotactile Glove, was used to present sounds (vibrations) to the back of all fingers of the left hand.
Results: Results reveal that it is possible to measure a FFR using vibrotactile stimulation in the absence of auditory input. The tactile FFR exhibits somewhat different characteristics as compared to unimodal auditory FFRs, including lower amplitude and no sensitivity to harmonics. Combined modality stimulation exhibited different characteristics than either modality by itself.
Conclusions: This study revealed for the first time that FFRs can be elicited solely via tactile stimulation, indicating that periodicity is encoded in the neural temporal firing pattern for both auditory and tactile modalities. These findings underscore the similarities of brain representations of pitch and vibration, and suggest that these modalities interact, opening up new neuroscience questions about the origins and pathways responsible for the phenomenon. More broadly, this research introduces new possibilities for using tactile perception to mitigate the effect of hearing loss in language and music perception.

M165. The Role of Sensory Loss and Plasticity in Auditory and Audiovisual Speech Recognition in Noise in Older Adults

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Category: Multisensory Processing/Interactions
Background: Sensory loss may contribute to changes both within the affected sense and across the cortex, including decreased inhibition (decreases in GABA). These changes have been interpreted as both beneficial and maladaptive. We previously reported that smaller auditory nerve (AN) responses contribute to a loss in cortical GABA, resulting in larger cortical responses, known as hyperexcitability, and decreased auditory speech in noise (SIN) recognition. In the current experiment, we tested the hypothesis that while maladaptive for auditory SIN, sensory-driven decreases in GABA may contribute to increased cross-modal plasticity and a greater reliance on multisensory integration contributing to the relatively preserved audiovisual (AV) SIN often reported in aging adults.
Methods: Participants included groups of older (55+ y/o) and younger (18-30 y/o) adults. AN function was assessed by measuring the amplitude of the N1 of the compound action potential (CAP N1). Cortical activity, P1 and N1, were measured from EEG source-constrained to auditory and visual cortex, elicited in response to simple auditory and visual stimuli. GABA was measured from auditory and visual cortices using MRS spectroscopy. Speech recognition was measured to bi-syllabic words presented binaurally in auditory only (AO), visual only (VO), or audiovisual (AV) trials, presented at 70 dB SPL in speech-shaped noise at 65, 70, or 75 dB SPL.
Results: Older adults exhibited smaller CAP N1 amplitudes than younger adults. These smaller N1 amplitudes were associated with older adults' decreased cortical GABA, and larger auditory cortical responses. Lower levels of GABA also contributed to greater cross-sensory activity in older adults than younger adults, with visual stimuli producing significantly larger responses in auditory cortex. Greater cross-sensory plasticity was associated with better AV SIN in older adults.
Conclusions: Prior research from our lab and others have consistently found that decreased AN function, decreased inhibition, and cortical hyperexcitability result in poorer auditory SIN. Results of the current study build upon this research and suggest the intriguing possibility that in some older adults sensory-driven changes in the cortex that may be 'maladaptive' for auditory processing may contribute to greater cross-modal plasticity and help maintain AV SIN.

M166. Lateral Line Contributes to Locomotion in Depth by Sensing Vertical Drift

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Category: Multisensory Processing/Interactions

Background: The lateral line enables fish and amphibians to sense water flow in their environment. Hearing and balance researchers commonly leverage this system to study hair cells, which transduce stimuli in both the inner ear and lateral line. This orienting system is involved in initial swim bladder inflation and guides navigation into current. However, the role of the lateral line in vertical locomotion remains ambiguous.
Methods: Larval zebrafish were incubated with 10 uM copper sulfate to ablate hair cells of the lateral line. Freely-swimming larvae were then recorded in a predominantly vertical plane in constant darkness and in ambient light as described below.
Results: Following lateral line ablation in the dark, we demonstrate increased sinking during passive movement which is compensated during active locomotion. Acute lateral line loss caused larvae to sink faster and further between swim bouts. Without lateral line input, larvae exhibited greater displacement in depth with altered lift generation in upward swim climbs, indicating that larvae adapt their activity depending on their navigational goals. Light ameliorated many but not all deficits from lateral line loss, implying that additional feedback from the visual system can partially compensate for lateral line input.
Conclusions: These results tie the lateral line to vertical navigation and we hypothesize that this system provides input along with visual feedback to centers of navigation planning. Lateral line information is thus relevant to volitional navigation and related kinematics. Additionally, deprivation of both lateral line and visual signals while leaving the vestibular system intact suggests that the aforementioned effects are driven largely by vestibular information. Given the interaction of lateral line signals with many other sensory systems, this work serves as a prime example of multisensory integration and sensory-motor transformations.

M167. Does the Gender of the Tester and Subject or a Change in Olfactory Function Affect Auditory Function in Mice?

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Category: Otoacoustic Emissions
Background: Gender-based differences in the auditory function of mice have been reported and are attributed to biological and psychological factors affecting auditory behavior. We investigate whether neurobehavioral effects secondary to the gender of the experimenter may additionally alter auditory function in mice. We hypothesize that, in mice, distinct olfactory stimuli from male and female testers affects auditory function, inducing further gender-based differences in hearing between male and female mice. Conversely, when drug-induced smell dysfunction occurs, we expect to observe fewer differences in auditory performance across groups.
Methods: Testers evaluated auditory function in male (n=30) and female (n=30) C57BL/6J mice at ages 6-8 weeks in a series of six experiments. In Experiment 1 and 2, mice were assessed by both male and female testers at baseline. In Experiment 3 and 4, mice were assessed by only male or only female testers at baseline, followed by administration of cyclophosphamide (Experiment 5) or methimazole (Experiment 6) to induce smell dysfunction, and reassessed post-injection.
Auditory function was assessed with ABR (click stimulus, tone pip at 8-, 16-, 24-, and 32kHz) and DPOAE (F2 at 8kHz – 16kHz). Results were compared between male and female mice as well as between the male and female testers. Baseline, 7-day post-injection, and 30-day post-injection values were compared across Experiments 3-6.

Results: Overall, we observed a trend towards better auditory performance in female mice, which had lower ABR thresholds than male mice when tested by male or female experimenters. Male and female mice displayed lower ABR hearing thresholds when tested by a female experimenter. Further, both male and female mice demonstrated better auditory function following the induction of olfactory dysfunction 7 days post-injection, regardless of experimenter gender.
Conclusions: Auditory function in mice shows similarities to human patterns but is also affected by the gender of the tester. When mice are unable to detect experimenter gender, auditory function results show improvement. This is the first study incorporating olfactory dysfunction in mice while examining the gender effects of both experimenter and subject on mouse auditory function and has implications for future auditory research. Gender-based differences should be considered and interpreted with caution with any neurobehavioral experiment.

M168. Case Study: Audible Fluttering Sounds Associated With Eye Movements in a Patient With Palatal and Possible Tensor Tympani Myoclonus

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Category: Otoacoustic Emissions

Background: Information about eye movements is critical for the integration of auditory and visual signals across space. Signals related to eye movements have been identified both in auditory regions of the brain (e.g.Groh et al Neuron 2001) as well as in the ear itself (eye movement-related eardrum oscillations (EMREOs, Gruters et al. PNAS 2018; Lovich et al Phil Trans B 2023; Brohl and Kayser J. Neurosci 2023). Some people report hearing sounds associated with eye movements. The possible connections between these neurophysiological demonstrations of eye movement signals in the auditory pathway and the perception of sounds associated with eye movements in some individuals remains uncertain.

Methods: Here we report findings from a patient with a confirmed diagnosis of palatal myoclonus as well as possible tensor tympani myoclonus. This patient reported hearing a "flutter" associated with large leftward eye displacements. The patient further reported that the fluttering sound could be heard by a third party leaning close to their ear. We invited the patient to visit the lab for further testing involving video eye tracking (Eye Link 1000) and microphone recordings (Etymotics ER10b+) from the ear canal.

Results: The patient was able to replicate the "flutter" sound by making large leftward eye displacements (30+ degrees) in the laboratory. When the microphone recordings were amplified and played back, we (i.e., the patient + members of the laboratory) could all hear the fluttering sound. The patient confirmed that these recordings sounded like what they had perceived in conjunction with eye movements.

Conclusions: How does this fluttering sound compare to the EMREOs observed in normal participants? For smaller eye movements, the EMREOs of this patient appeared to be very similar to those of normal participants, and indeed the patient did not report hearing any sounds in conjunction with those saccades. The anomalous, perceptible "flutter" sounds were associated only with very large (30-40 degree) shifts in eye position. These eye displacements were typically broken into several successive saccades, and the waveform of these signals did not appear to be directly comparable to the previously characterized EMREOs.

M169. Relationship Between Stimulus Frequency Otoacoustic Emission Estimates of Cochlear Tuning and Speech-In-Noise Recognition

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Category: Otoacoustic Emissions

Background: Recent studies have shown that the audiogram, the hearing healthcare gold standard, is insensitive to common auditory deficits. For example, some listeners with normal audiograms have a disproportionately poor understanding of speech in background noise; likewise, hearing-impaired listeners with similar audiograms may demonstrate very different speech-in-noise abilities. It is possible that the differences among these listeners exist in the peripheral auditory system. One of the fundamental mechanisms that contribute to understanding speech-in-noise is peripheral auditory filtering, or frequency selectivity. Cochlear filtering is a critical first step in everyday processing of complex auditory stimuli and is essential for pitch, speech, and music perception. However, it can be challenging and tedious to measure cochlear frequency selectivity using behavioral methods. Stimulus frequency otoacoustic emissions (SFOAEs) have been suggested as a possible indirect measurement method of cochlear frequency selectivity that is noninvasive, objective, and more rapid than behavioral methods. SFOAEs are generated by the active cochlea in response to tonal stimuli presented to the ear. Specifically, the phase delay of the SFOAE response can provide valuable information regarding cochlear mechanics and frequency selectivity. Given that frequency tuning at the level of the cochlea is the initial relay of tuning information to the auditory system, it is highly likely that sharp cochlear tuning is necessary for successful understanding of speech-in-noise. Despite its importance, there are limited studies examining the role of cochlear frequency selectivity and speech-in-noise

ability. The purpose of this study was to explore the relationship between cochlear tuning estimates from SFOAE phase-gradient delays and speech-in-noise recognition in normal-hearing adults.
Methods: Twenty-nine normal hearing adults (mean age = 23.6 yr; SD = 3.2 yr) participated in this study. Swept-tone SFOAEs were recorded from 1-4 kHz using a 40 dB probe and 60 dB suppressor tones. Two separate signal-to-noise ratio (SNR) criteria (6 dB and 9 dB) were applied to the SFOAE recordings to filter out noisy data. Phase-gradient delays were computed using two strategies: (1) peak-picking and (2) energy weighing. Cochlear tuning (Qerb) was estimated at one-half-octave intervals using an established procedure (Shera et al., 2010). Speech recognition thresholds (SRTs) for sentence-in-noise ability were measured using the Hearing in Noise Test, an adaptive speech-in-noise test.
Results: SFOAE estimates of Qerb were consistent with forward masking tuning curves reported in the literature for normal-hearing subjects. SFOAE estimates of tuning were related to SRTs.
Conclusions: These results indicate that sharper cochlear tuning, or smaller cochlear filter bandwidths, may predict speech-in-noise ability for listeners with normal audiograms. These findings contribute to the body of literature investigating potential mechanisms underlying speech-in-noise deficits that are not detected by the audiogram.

M170. Development of Novel Auditory Alerts That Foster Efficient Detection and Discrimination in Complex Auditory Environments

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Category: Psychoacoustics
Background: In complex auditory environments, e.g., operating rooms and airplane cockpits, auditory alerts occur along with distracting masking stimuli. To begin investigating salient acoustic features of alerting stimuli, we designed narrowband and broadband alerts with consonant and dissonant harmonies. The masker consisted of continuous "vehicle noise." For each type of alert, we measured psychometric functions relating performance to signal-to-noise ratio (S/N).
Methods: We developed four alerts: one set had broadband power spectra and one set had narrowband power spectra. Alerts durations ranged between 350 and 715 ms. For each set, we created one "friendly" and one "enemy" alert, which had consonant and dissonant harmonies, respectively. Then, we measured detection and discrimination performance as a function of signal-to-noise ratio (S/N), separately, for each set of alerts embedded in a "vehicle noise" masker. A descending series was employed. Each series spanned a range of 10 dB in 2-dB steps. At each S/N, four instances of the "friendly" and four instances of the "enemy" alert occurred in random order and at randomly determined times before proceeding to the next lower S/N. In isolation, the overall level of the vehicle noise was approximately 70 dB SPL. Stimuli were presented over Sennheiser HD 280 Pro headphones to participants (n=17) who listened in conventional quiet rooms.
Participants were instructed to detect and identify the alerts. Specifically, participants identified the alerts on the computer keyboard by pressing the "f" or "e" key for "friendly" or "enemy," respectively. Participants received on-screen correct-answer feedback. At the beginning of an experimental run, each alert was presented in isolation, along with its identity. Participants were instructed to respond quickly whenever they detected the addition of an alert signal, even despite any uncertainty regarding the identity of the alert.

Results: Best-fitting logistic functions relating performance to S/N were derived for each of the four alerts. For both the "friendly" and "enemy" broadband alarms, the data suggest that, as expected, discrimination required a higher S/N than detection for a given level of performance. That trend, however, was not evident when the narrowband friendly and enemy alerts were employed.
Conclusions: The findings will be discussed within the context of auditory masking and the perceptual salience of customized alerts. This work is a first step toward designing auditory alert stimuli that leverage various acoustic characteristics and parameters to enhance their detectability and identification within complex auditory environments. Follow-up work will compare the detectability and discriminability of the alerts under single and dual-task conditions.

M171. Cross-Frequency Spectral Integration in Electro-Acoustic Stimulation and Bimodal Hearing

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Category: Psychoacoustics

Background: Hearing aids (HA) and cochlear implants (CI) are combined in electro-acoustic stimulation (EAS) within one ear and in bimodal hearing across ears, both merging acoustic and electric signals. However, their performance varies largely and could be because EAS integrates sound in one ear, while bimodal integrates across ears. Our knowledge of auditory information integration in these hearing technologies remains limited. We aimed to compare the spectral integration in EAS and bimodal hearing, which could help develop better fitting methods and improve technology use in the future.

Methods: Twelve adult listeners with normal hearing participated in both EAS and bimodal simulation tasks. Four synthesized vowels with increasing frequency spacings between the first two formants (i.e., F2-F1) (/ɔ/: 270 Hz, /ʊ/: 653 Hz, /œ/: 1040 Hz, and /I/: 1607 Hz) were utilized. For each vowel, F1 was simulated for acoustic stimulation by low-pass filtering with a fixed cutoff frequency of 750 Hz and a 48 dB/octave slope. F2 was simulated for an electric stimulation with an 8-channel sinewave vocoder and with matched input and output frequency ranges (188-7938 Hz). Vowel identification was administered in five listening conditions: Control, EAS, Bimodal, CI alone, and HA alone. For control, acoustic simulations of both F1 and F2 formants were presented to both ears. For EAS, F1 and F2 were presented to the left ear, while for bimodal hearing, they were presented to opposite ears. For CI alone, F2 alone was presented to the left ear, while for HA alone, F1 alone was presented to the left ear.

Results: Frequency spacings and the listening conditions played a significant role in spectral integration. Frequency spacings of 270 Hz and 653 Hz integrated significantly better than 1607 Hz, suggesting challenges while integrating widely spaced frequencies. The control condition produced the best vowel performance followed by EAS, though not statistically significant. EAS and control performed significantly more than the bimodal condition, highlighting the cross-frequency integration deficit in bimodal hearing. Moreover, EAS and bimodal conditions outperformed the CI alone or HA alone. Though non-significant, the bimodal performance was poorer than CI alone for /I/ vowel, demonstrating potential interference when the CI ear perceives a dominant cue and the HA ear perceives the non-dominant cue.

Conclusions: The frequency difference is crucial for spectral integration, with closely spaced frequencies showing better integration than distant ones. Spectral integration in EAS occurring at the peripheral level is more efficient in comparison to bimodal hearing, which rather involves central integration. Under the same conditions, bimodal interference arises when the CI ear is dominant, however, interference does not occur in EAS, indicating that interference does not occur due to peripheral monaural interactions rather at or above the site of binaural interaction.

M172. Multiple Pitch Perception With Rate-Place Metamers

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Category: Psychoacoustics

Background: Pitch is a key perceptual attribute of natural sounds, but the neural basis of pitch perception remains under active debate. Some models of pitch perception rely on analyzing patterns of neural activity over the tonotopic axis, or the rate-place code. For many common pitch stimuli, such as harmonic complex tones (HCTs) with low-rank components, human auditory filtering is likely sharp enough to resolve spectral details, but spectrally denser stimuli like combinations of multiple simultaneous HCTs (i.e., chords) may exceed this resolution limit and have limited rate-place information. Some pitch tasks remain feasible under such conditions, but it is unclear whether listeners rely on weak residual rate-place cues or turn to other cues. To adjudicate between these possibilities, we tested listeners on pitch tasks using "rate-place metamers", synthetic stimuli with rate-place representations matching those of pitch-evoking stimuli, but otherwise unconstrained.

Methods: We generated rate-place metamers by iteratively filtering samples of Gaussian noise to minimize differences between simulated auditory-nerve average-rate (ANAR) responses to the HCT stimuli and to the

filtered noise. The procedure was based on the humanized variant of an established low-spontaneous-rate auditory-nerve-fiber model (Zilany, Bruce, and Carney, 2014; JASA, 135:283). We then measured F0 difference limens and chord discrimination performance for original HCT stimuli (ORIG) and metamers (META). Both ORIG and META conditions included stimuli with a range of fundamental frequencies (F0s) and harmonic ranks.

Results: Preliminary F0DL measurements for single complex tones and their metamers followed similar patterns, suggesting that metamerization faithfully preserved the cues needed for single-pitch perception. However, preliminary results for multiple-pitch stimuli revealed some differences in performance between ORIG and META conditions, suggesting that listeners may rely on cues in multiple-pitch perception that are not captured by the ANAR. Temporal cues have been postulated to underlie performance in these cases, and post-hoc evaluation of autocorrelation functions (ACFs) revealed that some META stimuli contained significant timing information. However, the amount of preserved timing information varied across conditions in a way that did not systematically explain differences between behavior for ORIG and META.

Conclusions: Our preliminary results suggest that although pitch discrimination for single HCTs may be accounted for by traditional models of pitch perception, more work remains to identify the neural mechanisms of pitch perception with multiple HCTs. Where metamers showed degraded timing representation, impaired performance suggests that timing cues may play a role in multiple pitch perception. In other conditions, neither rate-place ANARs nor summary ACFs can explain the impairment. [NIH grant R00DC017472 (AHM)]

M173. Influences of Pitch and Spatial Trajectories on Relative Timing Judgments in Auditory Sequences

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Category: Psychoacoustics

Background: Pitch, timing, and space are important cues for auditory streaming. These cues interact perceptually such that changes in one stimulus dimension can distort judgments within other feature dimensions (Boltz, 1998; Henry et al., 2009; Jones et al., 2006). One example of interactions between pitch and timing information is the auditory kappa effect, an illusion whereby larger pitch changes lead to the perception of larger inter-stimulus time intervals in tone sequences (Cohen, et al., 1954; Henry and McAuley, 2009). The kappa effect has been typically studied using 3-tone sequences with a consistent (e.g., increasing/upward) pitch trajectory, where the first and third tones had fixed pitch and timing separations. Varying the pitch of the second tone biased timing judgments of the second tone relative to the first and third; larger pitch changes between tones were judged as longer time intervals. The prevailing explanation for this effect is an auditory motion hypothesis (Henry and McAuley, 2013), which proposes that participants perceive auditory motion with an inferred velocity across sequential tones due to spatialized representations of pitch (i.e., "up and down"). We propose an alternative auditory grouping hypothesis, which posits that similar auditory events are grouped as part of an auditory object. Thus, the second tone is grouped with the tone more similar in pitch and perceived as closer in time to that tone. The present study aims to 1) test these competing explanations for the auditory kappa effect, 2) establish a novel kappa effect for spatial hearing, and 3) evaluate the relative contributions of pitch and spatial trajectory to the kappa effect.

Methods: Three sets of experiments were conducted using variations of the original kappa task. In all experiments, participants heard 3-tone sequences with fixed timing and pitch or spatial separation between the first and third tone, varying the second tone trial-to-trial. Participants made timing judgments by responding "short-long" to indicate that the second tone was closer in time to the first or "long-short" to indicate that the second tone was closer to the third. The first experiment manipulated the predictability of pitch trajectories, the second experiment manipulated only spatial trajectories using a 360-degree speaker array, and the third experiment manipulated both pitch and spatial trajectories together.

Results: Preliminary results suggest that the kappa effect can be observed even when the auditory motion trajectory is unpredictable, indicating support for the auditory grouping hypothesis as well as highlighting the effects of pitch and spatial manipulations on timing judgments.

Conclusions: Results of these experiments inform our understanding of how listeners use and group multiple auditory features to form perceptual objects and how contrasting features bias auditory perception.

M174. CAT-Egorical Perceptual Similarity Does Not Explain Memory Organization of Auditory Sequences

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Category: Psychoacoustics

Background: Sound sequences perceived as a single stream vs. as discrete events may be stored differently in working memory. In a past study, listeners heard a sound sequence and, in two different tasks, judged whether 1) a subsequent sequence of the same elements was ordered identically or had two adjacent elements exchanged ("same/different," SD task) and 2) an element was in the original sequence ("present/absent," PA task). Listeners were relatively good at the SD task for tones compared to everyday sounds, but relatively good at the PA task for everyday sounds compared to tones. Here, we ask whether the perceptual similarity of elements in a sequence explains this reversal of perception. We conducted online experiments replicating the previous experiment, including a third stimulus set of sounds perceived as similar to one another, but heard as independent events when presented in a sequence.

Methods: Three stimulus sets were tested: 1) broadband, complex tones differing only in pitch (semitones spanning C4 to D#5), everyday sounds (spectrotemporally dissimilar items from the ESC-50 dataset), and cat meows (from Noyce et al. 2017, J. Neuro.).

Study 1 included three experiments exploring the perceptual similarity across pairs of stimulus sets (e.g., tones and meows). Listeners (N=20 in each experiment) judged the perceptual similarity of all possible pairings of elements across the two tested sets. We used multidimensional scaling to compute the average within-set similarity of elements, then quantified the relative similarity across sets in each experiment.

Study 2 comprised two separate experiments in which listeners performed both SD and PA tasks for sequences (length four) from two stimulus sets: tones and everyday sounds (N=40, Experiment 2A) and tones and meows (N=41, Experiment 2B).

Results: Study 1 established that complex tones were most perceptually similar to one another, everyday sounds the least similar, and meows in between.

Both experiments in Study 2 revealed a significant interaction between stimulus set and task. Specifically, Experiment 2A replicated previous results: performance in the SD task was better for tones than everyday sounds, but performance in the PA task was better for everyday sounds than tones. In Experiment 2B, performance was similarly worse for meows than tones in the SD task, but better for meows than tones in the PA task.

Conclusions: We replicated previous results: it is easier to recall the order of a sequence of tones than of everyday sounds (SD task), but harder to access individual items for tones than everyday sounds (PA task). The fact that meows (which are quite similar to each other but heard as discrete events) produce results similar to everyday sounds suggests that perceptual similarity alone does not explain how items are organized and stored in memory.

M175. Modeling Auditory Attention With Machine Learning

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Category: Psychoacoustics

Background: Attentional selection allows human listeners to successfully recognize speech in noisy environments (the "cocktail party problem"). Although attentional listening abilities have been characterized to some extent in humans, we lack quantitative models of auditory attention that are capable of explaining attention-mediated behavior. We also lack normative models of attention that reveal how attention should influence neural representations to enable selective listening. Inspired by known neurophysiological effects of attention, we introduce a model of auditory attention by adding feature-based attentional gain parameters

to an artificial neural network, optimizing both the network and the gain parameters to perform a spatialized word recognition task in the presence of competing sounds.
Methods: We built a deep neural network optimized to perform an attentional word recognition task using stereo audio signals, reporting words spoken by a cued "target" talker in a multi-source spatialized scene. Audio signals were spatialized by rendering sounds at locations sampled from a set of simulated reverberant rooms using head-related transfer functions. The model implemented feature-based attentional gain at each network stage via learnable logistic functions operating on the time-averaged feature activations of a cued talker. Features of the cued talker could thus generate high gains, while those absent from the cued talker could generate low gains, to an extent 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.

M176. Phasic Pupillary Response Traces Salient Auditory Events in a Multidimensional Feature Space

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Category: Psychoacoustics
Background: The eyes' pupil responds not only to visual stimuli but also to salient sounds – louder sounds evoke stronger pupil dilation responses (PDR). The sound-induced PDR is speculated to reflect the orienting response based on neural activities in the superior colliculus (see a review by Strauch et al., 2022). However, when it comes to auditory salience, this concept becomes quite intricate, involving interactions among various acoustic features. Kaya and Elhilali (2014) developed an auditory salience model based on the predictive coding theory using sounds containing multidimensional features and verified the model by psychoacoustic evaluations. The current study examined whether PDR reflects the deviant salient auditory events in such multidimensional feature streams and the extent to which behavioral and pupillary responses reflect similar or divergent trends of integration across acoustic dimensions.
Methods: Participants listened to short sound clips (5 or 6 s) containing temporally overlapping musical tokens (Experiment 1) or bird singing calls (Experiment 2). The salient event was defined by the deviation of acoustic features (intensity, pitch, or timbre) from the background (the same stimuli used in Kaya and Elhilali, 2014). The deviant token appeared at the timing uniform distributed within each quadrant of the total stimulus length in half of the trials. Participants listened to the sound clip while an infrared-based eye camera measured their pupillary responses. They reported whether they heard the deviant token after the sound presentation.
Results: Behavioral results showed similar interactive patterns among the acoustic features as in Kaya and Elhilali (2014). Moreover, participants detected the deviant token better at a later quadrant of the total stimulus length. This could be explained as the brain could build a more stable model of the scene, allowing the contrast to be more prominent. Correspondingly, pupil results showed a clear phasic dilation response following the deviant token: when segregating the trial by the quadrant of the deviant event's presentation timing, pupil diameter peaked around 1-1.5 s after the median timing within each quadrant. We further examined how intensity affected behavioral performance and PDR. When segregating the trials by the deviant event's intensity in each quadrant condition, the larger pupil diameter responding to the higher intensity deviant token (i.e., intensity-induced PDR) was observed only in the first quadrant but not in the rest of the periods. The result is unexpected, given the weaker behavioral response to early deviants. It suggests that it underlies automatic mechanisms of buildup of scene statistics that are easier to discern for higher intensity tokens.

Conclusions: Phasic pupillary dilation responses trace bottom-up auditory attention over time. The intensity-induced PDR, presumably reflecting the orienting response, tracks deviant events early before a stable model is built. The brain has multiple systems to monitor auditory salience.

M177. Cues for Tone-In-Noise Detection: Evidence From Constant-Stimuli and Adaptive Approaches

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Category: Psychoacoustics

Background: Tone-in-noise detection has been used to study frequency selectivity for almost a century. However, exactly how a tone is detected has recently become a topic of controversy, with potential cues including an increase in level and a change in the modulation spectrum. The present study investigated performance in conditions that limited access to either level or modulation cues, with the hypothesis that performance would worsen when the cues normally used by listeners in a classic tone-in-noise task were limited. Performance was measured for both narrowband and broadband noise conditions. As an adaptive procedure may allow listeners to learn different cues within each run, listeners were also tested using a constant-stimuli procedure, where performance was measured for randomly interspersed trials involving primarily level cues, primarily modulation cues, or both.

Methods: Listeners detected a 2-kHz tone in noise that was either narrowband (60-Hz bandwidth centered at 2 kHz) or broadband (threshold equalizing noise, TEN, extending from 125 to 15000 Hz). For narrowband noise (n=24), there were three main stimulus conditions: (1) tone added to noise (classic); (2) the tone and noise combination were rescaled to have the same overall level as the noise alone (rms-equalized); (3) a 60-Hz bandwidth noise signal added to the noise (noise-in-noise). For broadband noise, similar manipulations were performed to affect only the output of the estimated human equivalent rectangular bandwidth (ERB) surrounding 2 kHz. Thresholds were initially estimated via an adaptive procedure for the classic condition. This was followed by the constant-stimuli procedure with the signal-to-noise ratio (SNR) individually set to threshold from the classic condition. Finally, adaptive thresholds were measured in counter-balanced order for all conditions.

Results: For the narrowband conditions, thresholds worsened significantly when modulation cues were removed and even more when level cues were removed. Constant-stimuli performance was significantly worse than in the classic condition when level cues were removed, but not when modulation cues were removed. However, mean performance remained above chance in all conditions. Preliminary results suggested similar trends for the broadband experiment.

Conclusions: In general, results show a greater dependence on level cues than modulation cues for tone-in-noise detection; however, individual differences were apparent and several aspects of the results support the integration of both cue types.

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M178. Noise Schemas Aid Hearing in Noise

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Category: Psychoacoustics

Background: Human hearing is robust to noise, but the basis of this robustness is poorly understood.

Methods: We conducted a series of psychophysical and computational experiments to explore whether internal models of noise aid the ability to hear foreground sounds in real-world noise.

Results: One prediction of this hypothesis is that hearing should improve with exposure to a noise source, since noise properties can be better estimated with more samples. Consistent with this idea, we found that detection, recognition, and localization in background noise improved with exposure to the background. An observer model designed to detect outliers from a distribution of background noise accounted for this pattern of human behavioral performance. A second prediction of the internal model hypothesis is that listeners should

retain a benefit for familiar noises over time. Consistent with this second prediction, detection performance was enhanced for recurring backgrounds and was robust to interruptions in the background, suggesting listeners build up and maintain representations of noise properties over time.
Conclusions: The results suggest noise robustness is supported by internal models—"noise schemas"—that capture the structure of noise and facilitate estimation of other concurrent sounds.

M179. Hearing Impairment Causes Different Saccade Errors Than Normal Hearing in Multisensory Localization Tasks

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Category: Psychoacoustics
Background: In the real-world, we often rely on both sight and hearing to localize objects. Visual information provides a reference to guide action, with visual stimuli (Pick et al., 1969) and the visual environment itself (Platt and Warren, 1972) influencing the accuracy and variability of performance. Our previous work showed that uncertainty in auditory-alone localization led to increased visual bias in auditory-visual localization for both normal-hearing and hearing-impaired individuals (Montagne and Zhou, 2016; Venskytis et al. 2019) using button-pushing as response. In this study, we measured eye saccades, a natural orienting behavior, in response to auditory and visual stimuli, similar to those presented in our previous work. The experiments were designed to obtain evidence for the effects of hearing impairment on eye saccade behavior during a spatial task.
Methods: Experiments used stereophony techniques to render brief 15-ms noise bursts in a horizontal range of ±30°. Two visual environmental texture effects were evaluated: (1) No visual references and (2) Seven square (1 in2) visual references placed 5° apart and spanning ±15°. Listeners were tasked with localizing a sound source by making a saccadic eye movement toward the perceived sound source location. We measured response accuracy, saccade trajectory, and saccade response time. The results were compared between timing- and level-based stereo conditions, between auditory-alone and auditory-visual conditions in two different visual environments, and between normal hearing (N=22) and bilaterally hearing impaired (N=6) listeners.
Results: Results from saccade responses confirmed our previous findings that (1) light stimulation can alter auditory localization towards the direction of light (i.e., visual capture); (2) visual bias is significantly correlated with the uncertainty in auditory-alone responses (i.e., cue saliency
principle). We also found that hearing-impaired individuals showed increased response errors due to visual bias and that their saccade trajectories differed from those of normal-hearing individuals. Hearing-impaired individuals tended to make swift saccades following the light direction, whereas normal hearing individuals often executed small double-step saccades in the correct sound direction. These differences were more prominent when light and sound came from opposite sides.

Conclusions: Saccade patterns offer rich information about auditory decision making. Saccade accuracy and saccade trajectory can reveal how visual information of a sound source and its visual environment are integrated into the sensorimotor loop in everyday listening tasks. Hearing impairment deteriorates sensory evidence and decreases a listener's confidence in making prompt decisions about sound information. Saccade behaviors offer a promising direction for new research in auditory decision making related to hearing impairment.

M180. Cortical and Subjective Measures of Individual Noise Tolerance Predict Noise-Reduction Outcomes

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Category: Speech Perception
Background: One of the chief complaints of hearing aid users is increased difficulty when trying to understand speech in locations with background noise. Although the noise reduction (NR) algorithms are

implemented in most digital hearing aids to attenuate background noise, the NR feature involves some speech distortions which lead to mixed reviews for NR. However, little is known about the potential factors that drive these individual differences in perceptual benefits from NR. We recently documented that cortical measures of individual noise tolerance (coined neural SNR) may predict NR outcomes. In this study, we aimed to investigate if the relationship between neural SNR and perceptual benefits differs with different NR strengths. Further, given that differences in noise-evoked potentials mainly drive the variance in the neural SNR, we investigated whether the subjective measures of noise tolerance could correlate with the neural SNR.
Methods: Thirty subjects between 18 and 35 years of age who were native speakers of American English were recruited from a population of students at Montclair State University. Their hearing sensitivity was assessed and found to be no worse than 20 dB HL at octave frequencies from 250 to 8000 Hz, as well as at 12,000 Hz. Cortical evoked responses and behavioral performance were simultaneously recorded during the speech-in-noise task with a couple of variations of the Ephraim-Malah NR algorithms involving different levels of noise attenuation and speech distortions. The neural SNR was calculated for each individual using the amplitude ratios of auditory-cortical responses to target words and noise. Each participant also completed an abbreviated Weinstein's Noise Sensitivity Scale that included 10 statements regarding levels of tolerance to noise.
Results: The findings from the experiment showed that the cortical measures of individual noise tolerance (i.e., neural SNR) predicted NR outcomes with different strengths. When stronger noise attenuation was used, which is accompanied by more speech distortions, the correlation between neural SNR and NR outcomes increased. Subjective measures of noise tolerance also showed a significant correlation with NR outcomes. A multiple linear regression analysis was conducted in a stepwise manner to investigate the joint effects of cortical and subjective measures. When the subjective noise sensitivity scale was added to the initial model that only included neural SNR, the final model explained additional variance in NR outcomes. However, it became less significant with both measures. Those cortical and subjective measures were not correlated.
Conclusions: Our results indicated that cortical measures of individual noise tolerance can predict NR outcomes with different strengths. It should be noted that subjective measures of noise tolerance explained additional variance in NR outcomes. These findings suggest that cortical and subjective measures capture different aspects of individual noise tolerance that may determine an individual's perceptual benefits from NR.

M181. The Effect of Second Language Acquisition on Central Auditory Processing Abilities

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Category: Speech Perception
Background: Comprehending and speaking a second language is a multifaceted task that offers benefits across numerous neurocognitive domains. Existing cross-sectional and longitudinal data show that performance on central auditory tests (CATs) correlates with neurocognitive test results in HIV-positive individuals. Second language learning may modify this relationship. To understand this, the present study compares performance on CATs among children with and without HIV taking into consideration if they are learning English as a second language.
Methods: This study includes 372 native Swahili-speaking children aged three to ten years old, including 196 HIV-negative and 176 HIV-positive children, in Dar es Salaam, Tanzania. Participants completed socio-demographic questionnaires providing information about English language learning, socio-economic status (SES), and health history. Three central auditory tests—the Triple Digit Test (TDT), the Staggered Spondaic Word Test (SSW), and the Hearing In Noise Test (HINT)—were used to assess central auditory processing. Data from the sociodemographic questionnaire and the central auditory tests were stored in a Research Electronic Data Capture (REDCap) database. These data were analyzed using MATLAB. Multiple linear regression was used to assess the effect of second language learning on CATs with age, HIV-status, and SES included in each model.
Results: Children actively learning spoken and written English at home have significantly increased central auditory processing abilities on the TDT compared to children not actively learning English at home (p less than 0.01). Children learning spoken and written English at school also performed significantly better on the HINT (p less than 0.05) than their counterparts not actively learning English at school. The SSW showed a

similar trend with children learning English at home or in school performing better on the test, however, these findings were not statistically significant ($p = 0.06$). Second language learning effects were independent of HIV status, age, and socio-economic status, which were all significant predictors of all three central auditory tests being examined.
Conclusions: This study found differences in central auditory processing between children exposed to English at home and in school, suggesting that second language learning mediates central auditory processing outcomes along with HIV, SES, and age. These findings support literature suggesting that second language learners have dynamic brains, with increased cognitive flexibility, and these changes can occur during the early stages of second language acquisition (ages 3-10 years). These findings underscore the significance of second language acquisition, as a potential influential factor affecting central auditory function within a Swahili-speaking cohort. Given that HIV plays a significant role in central auditory function, these findings suggest that second language learning can be used as an intervention to mitigate HIV effects.

M182. Pupillometry and Eye Movements Indicate Listening Effort Differently in Both Younger and Older Adults

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Category: Speech Perception
Background: Age-related hearing loss is associated with speech comprehension difficulties and listening effort in the presence of background noise. Measuring listening effort objectively has increasingly become the focus within the hearing sciences. Pupillometry has long been used to measure listening effort, but has some disadvantages (e.g., being light sensitive). Recent work indicates eye movements may provide an additional measure of listening effort: eye movements decrease as speech-masking increases. However, any measure of listening effort must be sensitive to different levels of listening effort, such as low effort due to easy listening condition or 'giving-up' listening, and high effort during challenging listening. The current study investigated the sensitivity of eye movements to the different facets of listening effort.
Methods: Pupillometry and eye movements were measured in younger and older adults (18-35; 55-75 years; N greater than 110 datasets). Participants listened to sentences masked by babble at +9 dB SNR (easy), -3 dB SNR (difficult), or -17 dB SNR (impossible); SNR was +2 dB more favorable for older adults. We expected listening effort to be low for easy and impossible (give-up) SNRs, but high for the difficult SNR. We examined pupil area and eye movements (fixation duration, gaze dispersion) in three experiments that manipulated prior knowledge about the upcoming SNR. In Experiment 1, listeners did not know whether a sentence was easy, difficult, or impossible. In Experiment 2, a cue prior to sentences indicated the difficulty level. In Experiment 3, participants were also cued and SNR order was blocked.
Results: In all three experiments, pupillometry indicated listening effort as expected, such that the pupil area was larger for difficult SNRs compared to easy and impossible SNRs, although this effect appeared more reliable in older adults. For eye-movement metrics, fixation duration increased and gaze dispersion decreased for difficult compared to easy SNRs for the duration a sentence was presented in noise for both age groups. However, eye movements also decreased for the impossible relative to the easy SNR, and this continued throughout a trial. That eye movements remained reduced throughout a trial, even after a sentence ended, shows that the eye-movement reduction is not driven by the SNR decrease per se, but rather by the listening experience. Eye-movements thus appear to index a different process during difficult speech listening compared to pupillometry.
Conclusions: In a series of experiments, the current study shows that pupillometry indexes listening effort as expected, i.e., only for difficult SNRs, but not when listening is easy or impossible (i.e., when people give up). In contrast, eye movements decrease for difficult and impossible SNR, suggesting different processes underlying changes in pupil area and eye movements under difficult listening situations. We speculate that the former may reflect more involuntary, whereas the latter more voluntary processes.

M183. Perceptual Learning of Temporally Degraded and Spectrally Degraded Speech in Children and Adults

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Category: Speech Perception

Background: The perception of different forms of degraded speech improves rapidly with practice, a process known as perceptual learning. The perception of different forms of speech also develops with age. For example, the recognition of spectrally degraded speech is still not mature by early adolescence (Huyck, 2018). Whether the same is true for perceptual learning remains uncertain. The aim of the study was to compare perceptual learning patterns between children and adults for two different types of degraded speech.

Methods: Fifty typically developing children (ages 8-10) and fifty young adults (ages 18-35) were assessed on their ability to learn to recognize accelerated (time-compressed) speech and spectrally reduced (noise-vocoded) speech. The participants listened to 30 sentences of each type of degradation (in counterbalanced order) and repeated what they heard. They also completed tests to assess working memory, verbal skills and non-verbal reasoning. Learning was modeled as a non-linear function of sentence number and compared between children and adults.

Results: The data suggests that learning developed with age, but development depended on the type of degradation. Both children and adults exhibited rapid learning of both degradations. However, whereas the degree of learning time-compressed speech was similar in children and adults, children learned noise-vocoded speech to a lesser extent. Furthermore, working memory capacity predicted learning, with greater perceptual learning for those with higher scores of digit span test.

Conclusions: The findings suggest that maturational processes affect the magnitude and rate of perceptual learning. Moreover, the development of perceptual learning might depend on the type of degradation, similar to the development of speech perception. Working memory may be a predictor of learning across age groups.

M184. Event Processing During Story Listening in Background Noise

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Category: Speech Perception

Background: Investigations into speech-comprehension difficulties often focus on the intelligibility of short, disconnected sentences, possibly limiting generalization to everyday listening. Novel approaches to understanding naturalistic speech listening are critical to gaining insight into impaired speech processing. Advances in memory research have demonstrated that, while environmental information evolves in a continuous stream, individuals perceive, encode, and recall information as temporally extended discrete events (e.g., I baked cookies). Segmenting the continuous environment into discrete events helps in predicting upcoming information and recalling experiences; however, this work has not been leveraged to understand challenging speech comprehension. Here, we investigate how individuals segment continuous speech into events and how event segmentation patterns are affected by background noise.

Methods: Participants (n=20, 18-38 years) were tasked to either listen to or read three 10-minute stories. Spoken stories were overlaid with a twelve-talker babble in three conditions ranging from easy to difficult speech intelligibility: clear, +2 dB SNR, and -4 dB SNR. Participants were asked to identify event boundaries during listening/reading and subsequently recall the narrative. Speech intelligibility for sentences extracted from the stories were assessed and analyzed as the proportion of correctly heard words. Story recall data were analyzed using novel artificial intelligence-based analytics, including GPT-4 and Universal Sentence Encoder. Analysis of event boundary patterns involved examining the consistency of event boundary placement (i.e., individual-to-group agreement).

Results: Speech intelligibility decreased with decreasing SNR, as expected, whereas recall only decreased for the -4 dB SNR condition, relative to the clear and +2 dB SNR conditions. The number of identified events was not affected by background noise, whereas a higher number of boundaries were recorded during reading. Event boundary placement was more consistent during reading in comparison to listening under all speech intelligibility conditions, suggesting that listening, generally, makes segmenting speech into events more challenging. Critically, event boundaries were more consistently placed for the +2 dB SNR than -4 dB SNR

and clear conditions, potentially because participants had to listen most attentively during moderate speech masking (+2 dB).
Conclusions: The current study investigates event segmentation during the comprehension of continuous speech in noise. More consistent event boundary identification during reading than listening suggests that the stories are processed differently across modalities. This may, in part, be related to the ever-progressing nature of spoken speech, whereas reading allows for the revisiting of material. Critically, we also show that individuals exhibit diminished proficiency in segmenting continuous spoken speech and have reduced recall for narrative events under challenging speech-masking conditions. This suggests that background noise may impede how listeners encode naturalistic speech, with longer-term consequences for later recall. Our results reveal a crucial link between background noise and event processing during speech listening and provide a foundation for novel recall metrics for various research contexts.

M185. Source Analysis of the Neural Response at the Fundamental Frequency of Speech

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Category: Speech Perception
Background: The human auditory system extracts information from complex sounds at different processing rates and stages. In speech, early neural processing tracks the fundamental frequency (f0) of the voiced parts of the speech signal. Previous EEG studies have found a subcortical contribution to this speech-FFR at a latency of about 10 ms. MEG studies identified later contributions from the auditory cortex, at a latency of around 35 ms. EEG studies with short speech segments have confirmed that both subcortical and cortical contributions can be measured. However, the employed short speech tokens did not allow a precise estimate of the latencies of the different responses. Here we therefore investigate the sources of the speech-FFR evoked by continuous speech.
Methods: We analyzed EEG recordings from 13 subjects who listened to audiobooks with a total length of about 40 min. We extracted two features from the stimuli: (1) a fundamental waveform that oscillates at f0, and (2) the envelope modulation at f0 in the high-frequency spectrum between 200 – 4,000 Hz. For the first feature, we applied a bandpass filter to the audio signal around the estimated f0 boundaries (approx. 75 – 150 Hz). For the second feature, we used different methods to compute the high-frequency spectra. In the first method, we employed a bandpass filter with constant bandwidth applied over the range of the high-frequency spectrum. Second, we also investigated a gammatone filterbank. As a third method, we utilized a model of the auditory periphery reflecting early stages of auditory processing. We subsequently computed temporal response functions (TRFs) to relate the two acoustic features to the EEG data. We finally applied source reconstruction to determine the origin of the evoked neural activity.
Results: We found an early response to the fundamental-waveform feature with a broad range of latencies centered at about 18 ms. Source analysis revealed a major midbrain activity as well as smaller contributions from brainstem sources. Similar latencies and neural patterns were identified for the envelope-modulation feature in the gammatone and in the auditory-periphery model. For the auditory-periphery and the bandpass model, a later response at latencies of about 35 ms occurred in the auditory cortex.
Conclusions: Our results show that TRF-based source analysis allows for a relatively precise assessment of the subcortical and cortical contributors to the speech-FFR elicited by continuous speech. They further show that the cortical contribution emerges for some but not all models related to the envelope-modulation feature.

M186. Decoding Cortical and Frequency-Following Responses to Speech Using Deep Neural Networks

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Category: Speech Perception
Background: During speech perception, a listener's electroencephalogram (EEG) reflects low-level acoustic processing as well as higher-level variables such as speech comprehension and attention. However, relating EEG signals to speech remains challenging, owing in part to the low signal-to-noise ratios of EEG signals, as

well as the fact that EEG signals are highly specific to individuals. Advances in auditory EEG decoding could lead to basic insights into how the auditory system processes sound, or lead to applications in diagnosing hearing disorders or controlling cognitively-steered hearing aids.
Methods: Our decoders were originally developed for the match-mismatch task of the ICASSP 2023 Auditory EEG Signal Processing Grand Challenge (SPGC). In the match-mismatch task, the goal is to determine which of two candidate speech segments is temporally aligned, or matched, with a given temporal segment of EEG recordings. The mismatched segment is selected to be temporally misaligned with the EEG segment, and it does not overlap with the matched segment.
Inspired by two well-known speech-related auditory responses – the cortical response to the speech envelope, and the frequency-following response at the fundamental frequency (speech-FFR) – we selected two acoustic features from the speech segments. These were the temporal envelope of a speech signal, as well as its high-frequency envelope modulation at the fundamental frequency, yielding the speech-FFR. The latter feature primarily encodes the glottal pitch envelope of the higher harmonics of the fundamental frequency. Two distinct deep neural networks were trained to relate the EEG signals to these two respective features. The decoders were then combined via a linear classifier.

Results: The proposed decoding system was the top-performing submission to the SPGC. In our subsequent analysis, we show that the accuracy of the speech-FFR decoder is lower for higher-pitched speakers, although the effect size is smaller than would be expected based on the existing literature, which mainly employs linear models. Both decoders generalise remarkably well between participants, and they even generalise to other datasets that were not seen during the SPGC. Furthermore, both the speech-FFR and envelope-based decoders can reliably detect the focus of a listener's auditory attention.
Conclusions: The results show that the speech-FFR carries additional information about speech processing which, when combined with the temporal envelope feature, leads to an enhanced match-mismatch classification accuracy. The decoders generalised remarkably well between participants, indicating that the decoding system is robust to between-participants variability. In line with recent research, our results show that the speech-FFR decoder can be used to detect the focus of a listener's auditory attention. Since the system can robustly detect the focus of a listener's auditory attention with a high accuracy, it may have applications in cognitively-steered hearing aids.

M187. Extending Subcortical EEG Responses to Continuous Speech to the Sound-Field

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Category: Speech Perception
Background: The auditory brainstem response (ABR) to short, repetitive stimuli is a valuable clinical tool for objective hearing assessment. Recent work has shown that brainstem responses similar to click-ABR wave V could be estimated from electroencephalography (EEG) recorded while test persons listen to continuous speech presented via earphones. Here, we replicate and extend earlier studies by measuring subcortical responses to continuous speech presented via both earphones and in the sound-field.
Methods: EEG was recorded from 24 normal hearing participants while they listened to clicks and short stories presented in an insert earphone and a sound-field condition. Subcortical linear temporal response functions (TRFs) were computed after accounting for non-linear processing in the auditory periphery by using predictors formed by simple rectification of the speech waveform or generated from an auditory nerve model. Responses for different stimuli (clicks, continuous speech), conditions (earphones, sound-field), and predictors (rectification, auditory nerve model) were compared, and the minimal amount of data needed for obtaining wave V responses above the noise floor was determined by calculating responses to an increasing number of data in 4 minute steps (8, 12, 16, 20, 24, 28, 32 minutes of data).
Results: Our results demonstrate that subcortical responses to continuous speech can be measured in the sound-field. Neural responses to continuous speech presented in the sound-field showed a wave V-like peak which was highly correlated in both amplitude and latency with response peaks obtained to clicks in the sound-field and clicks and speech presented via earphones. Model fits and response peak SNRs improved significantly when using models of auditory nerve activity instead of simple rectification as the predictor. For

all participants, subcortical TRFs to speech presented in the sound-field and analyzed incorporating an auditory nerve model exhibited significant wave V peaks based on only a 16 minute portion of the data.
Conclusions: This work shows that subcortical responses to continuous speech presented in the sound-field are highly consistent with both responses to clicks, and to speech presented through earphones. Critically for clinical applications, clear responses were obtained for all participants. Extending the measurement of subcortical neural responses using an ecologically relevant, complex stimulus to the sound-field lays the groundwork for objective hearing assessment in a more realistic setting. This might enable us to gain a better representation of people's hearing ability in daily life. Even though we demonstrate that subcortical responses can be obtained to speech presented in the sound-field, our work shows that they may have been affected by room acoustics, and points towards the possibility of improved brainstem responses when estimated using sound recorded close to the ear. Combined with the information it may reveal about hearing status, this might pave the way towards smart assistive hearing technologies in the future.

M188. Simulated Bimodal Speech Perception Among Bilingual Speakers

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Category: Speech Perception
Background: Approximately 77% of the potential cochlear implant (CI) adult candidates can benefit from a bimodal hearing configuration, which uses a hearing aid in the non-implanted ear in combination with the CI. Bimodal hearing benefits multiple auditory domains, such as speech perception in quiet and noise, music appreciation, and sound localization. These benefits are also observed in simulated bimodal hearing with normal-hearing listeners. Most published data on bimodal benefits were reported in monolingual speakers. However, there is a paucity of research on the bimodal benefits in bilinguals who need to understand both their native and non-native language. The purpose of this study is thus to address three questions: 1) Does simulated bimodal hearing provide benefits in speech perception for Mandarin-English bilingual speakers? 2) If so, do the bimodal benefits differ for native and non-native speech? 3) In noise conditions, are there effects of signal-to-noise ratio (SNR) and temporal modulation of the noise on the bimodal benefits for native and non-native speech?
Methods: In this study, 27 Mandarin-English bilingual participants with normal hearing were recruited. These participants were native speakers of Mandarin Chinese with English as their second language. Speech recognition was completed using sentences from Coordinate Response Measures (CRM) in both Mandarin and English. The performance was measured as the percent correctness of target word recognition in a sentence, i.e., the combination of color and digit. All target sentences were processed to simulate bimodal hearing by using an 8-channel noise vocoder to simulate CI in the right ear and a low-pass filter with a cutoff frequency at 750 Hz to simulate high-frequency hearing loss with a hearing aid in the left ear. Steady-state noise and temporally modulated noise at -3 and -6 dB SNR were used as the maskers.
Results: Results suggested that bimodal benefits were only observed in noise but not in quiet, probably due to the ceiling effect occurring in speech recognition in quiet. Larger bimodal benefits in noise were observed for Mandarin than for English. These results may be related to the advantage of native speech processing and the availability of F0 information by acoustic stimulation, which may contribute to Mandarin lexical tone recognition. Moreover, a significantly positive correlation was found between English bimodal benefit in noise and the length of living experience in the U.S., i.e., longer residency history in the US, greater bimodal benefits for bilingual speakers with simulated hearing loss.
Conclusions: In conclusion, bimodal benefits in noise were observed in native and non-native speech perception of Mandarin-English speakers with simulated hearing loss, indicating a need for dual-language clinical support for bilingual bimodal patients.

M189. Speech-Perception Training For Older Adults With Hearing Loss Increases the Consistency Of Cortical Activity During A Speech-In-Noise Task

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Category: Speech Perception

Background: Older adults often struggle to understand speech in noisy environments. Speech training can improve word recognition for older adults with age-related hearing loss [Humes et al., 2009, Ear and Hearing]. This study sought to identify changes in brain activity that underlie this speech training benefit. We hypothesized that speech training would alter the activity of cortical regions that support word recognition in challenging listening conditions.

Methods: 29 older adults completed either an 8-12-week training program [N = 14, mean age = 71.5 years, average pure-tone thresholds (PTA, 0.5-8kHz) = 30.4 dB HL] or control program [N = 15, mean age = 71.1 years, PTA = 34.8 dB HL]. During baseline and follow-up visits, participants completed behavioral assessments to measure word-, phrase-, and sentence-in-noise recognition performance, and underwent fMRI scanning during a words-in-noise recognition task. Inter-subject correlation (ISC) was performed on preprocessed BOLD timeseries at both baseline and follow-up to identify common patterns of brain activity within each group and visit. ISC quantifies the temporal consistency of neural responses within a group and can identify regions where the strength of within-group consistency differs between two groups/timepoints. To assess training effects, ISC permutation testing (n=1000) and a conjunction analysis with cluster-extent thresholding was used to identify brain regions where within-group consistency was significantly greater both (1) in the training group at follow-up compared to baseline and (2) in the training group at follow-up compared to the control group at follow-up.

Results: Behavioral: In both the behavioral assessment and fMRI task, speech-in-noise recognition significantly improved in the training group at follow-up compared to baseline, while the control group showed no improvement. Neural: Reflecting the auditory demands of the task, ISC identified within-group correlated activity in the bilateral auditory cortices that was significant in both groups at both timepoints, but which did not become more consistent with training. ISC conjunction analyses identified clusters in the left parietal, left inferior frontal sulcus (IFS), and rostral cingulate where activity became significantly more consistent across participants as a result of training. Post-hoc analysis revealed that, for all participants, BOLD activity in the left parietal region was positively associated with activity in the left IFS, suggesting a common network activation.

Conclusions: Results indicate that speech training over 8-12 weeks increased the consistency of brain activity across participants during a words-in-noise recognition task. Training effects were most pronounced within a left-lateralized cortical distribution consistent with a frontoparietal attention network. Follow-up tests indicated that these correlations were not specific to auditory stimulus features, suggesting that speech training increased the similarity of brain activity between participants performing a challenging listening task rather than increasing or decreasing cortical responses to specific stimuli. Future work will determine if these effects are specific to listening task conditions.

M190. Impact of Hearing Protection Devices on Speech Perception in Noise

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Category: Speech Perception

Background: Hearing protection devices (HPDs) are critical in preventing noise-induced hearing loss, but they distort sound according to their individual attenuation curves leading to distinct alterations in perception. While passive and active (electronic) HPDs have different attenuation mechanisms, any alteration could affect speech perception. Previous research reports mixed findings on the effects of HPDs on speech intelligibility. The literature overall suggests that HPDs reduce speech intelligibility in noise at low sound levels, but some findings suggest improvement in intelligibility at higher levels. Others suggest HPDs have no effect on speech intelligibility for normal hearing listeners. The goal of this study is to determine the effect different HPDs have on speech intelligibility for normal hearing listeners using two speech-in-noise-tasks presented in sound field. This study is part of a larger effort to quantify perceptual effects of hearing protectors.

Methods: Subjects, 127 audiometrically normal-hearing adults aged 18-45 (43 male, 84 female) across two sites, were administered the Quick Speech-in-Noise (QuickSIN) and Modified Rhyme Test (MRT). Seven listening conditions were tested, including open ear and 6 HPDs (active and passive). QuickSIN presents a list of 6 sentences with co-located multi-talker babble. The signal-to-noise ratio (SNR) decreases by 5 dB per sentence, with the sixth presented at 0 dB SNR. During MRT testing, 60 target words were presented with omnidirectional pink noise at varied SNRs (spanning -12 to 0 dB across sites). Participants selected the target word via keypad input amongst 6 rhyming words displayed on a screen. Performance for both tasks was characterized as percent correct as a function of dB SNR for each listening condition.
Results: QuickSIN results demonstrated the worst performance overall for a high-attenuation passive HPD (EAR Classic). At the lowest SNR, performance was better than open-ear for two active HPDs (ComTac and Invisio). Other HPDs did not yield noticeable differences in performance compared to the open condition. Similarly, MRT results showed the worst performance using a high-attenuation passive device (EAR Classic) at low SNRs (-9 and -6). Performance was better than open-ear at the low SNRs (-12, -9, -6) for an active HPD (Comtac) and a low-attenuation passive HPD (Combat Arms Earplug). Other HPDs had no noticeable differences in performance compared to the open condition.
Conclusions: Overall, there appears to be a higher likelihood of degradation of speech comprehension in noise when an individual is using passive than active HPDs. Active devices tend to produce better speech understanding, especially as the SNR becomes less favorable. Findings are interpreted in the context of target audibility and direction-dependent HPD acoustic impacts on transmitted spectra. These findings have implications for HPD selection in military and industrial settings, toward the goal of providing for both communication and protection from hazardous noise.

M191. Ossicular Chain Mobility Evaluation by OCT Vibrometry in Normal Ears

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Category: Middle and External Ear
Background: Optical coherence tomography (OCT) is a non-invasive, high-resolution imaging modality that provides three-dimensional imaging of the middle ear within seconds. In addition to structural imaging capabilities, OCT enables functional measurements, such as vibrometry (OCTV). OCTV measures the vibrational motion (amplitude, frequency and relative phase) of the middle ear constituents, such as the tympanic membrane (TM) and the ossicles. OCTV provides information on the mobility of the ossicular chain. In this study, we evaluated the amplitude at different locations of the ossicular chain as measured by a novel OCTV device, the correlation between the motion at these different locations and the correlation between both ears.

Methods: The OCT device utilizes a swept source to rapidly acquire cross-sectional images of the middle ear cavity. The sensitivity was 99.5 dB close to the zero-delay line with a roll-off of 0.17 dB/mm. The laser sweep-rate was set 90 kHz. The frequency of the emitted acoustic wave was set to 1000 Hz; its power was below 95 dB(A). The scan protocol consisted of 10.000 repeated A-lines at each location of interest, which took 110 ms. Amplitudes were subsequently calculated by custom-built software.
Vibrations were measured at four locations: On the umbo, on the center of the arm of the malleus (MA), on the superior part of the malleus (MS) and on the long crus of the incus. All measurements were repeated between 3 and 5 times.

Both ears of two normal hearing subjects were measured in-vivo. To assess the reproducibility, mean values, standard deviations (SD) and coefficients of variation (CoV) were calculated. The differences between left and right ears were compared to the differences between the individuals.

Results: The mean (SD) vibrational amplitude was 28.3 (2.5), 21.9 (4.1), 10.8 (1.6) and 9.2 (1.2) nm for the umbo, MA, MS and incus, respectively. The corresponding CoVs were 0.09, 0.19, 0.15 and 0.13.
The differences in vibrational amplitude between the two participants were 13.5, 0.6, 4.6 and 6.1 nm for the umbo, MA, MS and incus, respectively. The corresponding mean differences between the two ears of each

participant were 5.2, 4.8, 3.3 and 0.9 nm. The ratios of the difference between ears over the difference between individuals were therefore 0.38, 8.6, 0.71 and 0.15.

Conclusions: The vibrational measurements were largest at the umbo, and reduced towards the malleus' superior part, consistent with a pivoting motion around the malleus' head. The reproducibility of the vibrational measurements, as expressed by the CoV, ranged between 9 and 19%. Converting the vibrational amplitudes to a dB scale showed a reproducibility between 0.8 and 1.8 dB.
Except for the measurements at the malleus arm center, the difference between each participant's two ears was considerably smaller than the difference between participants, suggesting substantial symmetry between ears.

M192. Association Between Earwax-Determinant Genotypes and Acquired Middle Ear Cholesteatoma in a Japanese Population

Satoshi Hara*[1], Satoshi Hara[2], Takeshi Kusunoki[2], Yu Toyoda[3], Hiroshi Nakagawa[4], Shuko Nojiri[2], Kazusaku Kamiya[2], Masayuki Furukawa[2], Yusuke Takata[2], Hiroko Okada[2], Takashi Anzai[2], Fumihiko Matsumoto[2], Katsuhisa Ikeda[2]

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Category: Middle and External Ear
Background: A single-nucleotide polymorphism 538G.A in the human ABCC11 gene is a determinant of the earwax morphotype. ABCC11 538GG and GA correspond to wet earwax and 538AA to dry earwax. Despite a putative positive correlation between the frequency of the 538G allele and the prevalence of cholesteatoma, minimal clinical information is currently avail- able. We aimed to evaluate this association between the ABCC11 genotypes and acquired middle ear cholesteatoma.
Methods: We recruited 67 Japanese patients with acquired middle ear cholesteatoma (cholesteatoma group) and 100 Japanese controls with no history of middle ear cholesteatoma. We assessed the ABCC11 genotypes for all participants. Clinical information was collected from the cholesteatoma group. The genotype data of 104 Japanese people from the 1000 Genomes Project who represent the general population were used.
Results: The proportion of participants with ABCC11 538GG or GA was significantly higher in the cholesteatoma group than in the control group or general Japanese population (P \.001). The ABCC11 538G allele frequency was also significantly higher in the cholesteatoma group than in the control group or general Japanese population (P less than .001). Multivariate logistic regression analyses revealed a significant association between the ABCC11 genotype and acquired middle ear cholesteatoma (odds ratio, 5.49; 95% CI, 2.61-11.5; P less than .001).
Conclusions: Our results suggest that the ABCC11 genotypes could be associated with the development of acquired middle ear cholesteatoma among Japanese people.

M193. Development of an Intensimetric Probe for Wideband Acoustic Immittance

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Category: Middle and External Ear
Background: Wideband acoustic immittance (WAI) is an emerging tool for the assessment of middle-ear function. A common form of WAI, absorbance, has been shown to be sensitive to abnormal middle-ear states such as positive and negative pressure in the middle-ear cavity, a fluid-filled middle ear, malleus or stapes fixation, incudostapedial-joint disarticulation, tympanic-membrane perforations, and superior semicircular canal dehiscence. Because of its sensitivity to these pathologies, and its superior sensitivity compared to the standard 226-Hz tympanometry, absorbance has been recommended for clinical diagnosis of various middle-ear disorders. However, direct calculation of absorbance, and thus impedance, requires measurement of both acoustic pressure and velocity. As current measurement probes only sense acoustic pressure, WAI measurements require regular Thévenin calibration to estimate impedance. The need for Thévenin calibration may limit clinical utility of these measures and can introduce errors and variability into these measurements.

The goal of this work is to develop and validate an intensimetric probe system that has a microphone for sensing acoustic pressure and a velocimeter for sensing acoustic velocity, allowing for the direct measurement of acoustic impedance without the need for Thévenin calibration.
Methods: Prototype: A prototype was developed by mounting a probe head to a commercial off-the-shelf headset. The probe head consists of a 3D printed enclosure that contains a Microflown velocimeter and pressure sensor, two miniature speakers, a signal conditioning board, and analog-to-digital conversion boards. The contralateral ear cup is used to hold a battery and processing electronics. The device is controlled wirelessly through MATLAB.
Human Subject Testing: WAI measurements were made in participants with varying hearing and middle-ear status. Measurements were made with both the intensimetric probe and a commercial device for comparison (the Interacoustics Titan, which measures WAI using a Thévenin calibration). Accuracy and reliability of the probe were evaluated. Two measurements were made with each probe with different probe insertions on the same day, and a third measurement was made in each ear from each participant with both probes on a different day.

Results: Preliminary data analyses suggest that the intensimetric probe produces measurements in reasonable agreement with the Interacoustics Titan and demonstrates increased reliability both within and across test sessions in the same ears. Increased reliability may be because the intensimetric probe is measuring impedance directly as opposed to estimating it through Thévenin calibration. Limitations of the current prototype will also be discussed, including increased noise at low frequencies.
Conclusions: Overall, preliminary data suggest that the intensimetric probe is both accurate and reliable without the need for Thévenin calibration, and in fact, may result in increased reliability due to the direct measurement of impedance.

M194. Open Board

M195. Clinical Assessment of Eustachian Tube Dysfunction Through Questionnaire and Immittance Audiometry

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Category: Middle and External Ear
Background: Eustachian tubes equalize the atmospheric pressure between the middle ear cavity and the ear canal. Eustachian tube dysfunction (ETD) is a prevalent and bothersome condition caused either by inadequate or persistent opening of the tube and presents in patients as feelings of aural fullness, hearing impairment, tinnitus, and many other disabling features. Given the clinical prevalence of ETD, the Eustachian Tube Dysfunction Questionnaire (ETDQ-7) was created to help clinicians diagnose patients with ETD and help understand the effects symptoms have on a patient's daily life. Past research has demonstrated this tool has the potential for evaluating ETD symptoms but despite its wide clinical use, it has yet to demonstrate consistent validity in all patient groups. In this study, we review ETDQ-7 results in a diverse group of patients, and compare them to an objective test of ETD (modified immittance audiometry). We include patients with any degree or type of hearing loss, and with a variety of tinnitus symptoms to ensure our sample is an accurate representation of a true clinical population. As the ETDQ-7 includes questions about “ringing in the ear”, it is important to establish the co-occurrence of ETD with tinnitus that may be caused by other issues (e.g., sensorineural hearing loss).
Methods: This retrospective study analyzes results of ETDQ-7, custom tinnitus quality questionnaire, pure-tone audiometry, and tympanometry with a pressure change in clinical sample. A lack of considerable change in the tympanometric peak pressure during swallowing or performing valsalva maneuver was taken as an objective indicator of ETD positive cases.
Results: Preliminary analyses were performed on data from 100 clinical patients. Data for 96 patients were considered viable with 51% cases consistent with positive ETD diagnosis. Between the ETD positive and negative cases, the total ETDQ-7 scores spanned similar ranges and were not significantly different. The

prevalence of tinnitus in ETD positive and negative cases was also similar with over 50% of patients in each group indicating they experience ringing in one or both ears.
Conclusions: Preliminary analyses suggest limited utility of total ETDQ-7 score as an indicator of Eustchian tube dysfunction. In the next step, we aim to evaluate the co-occurrence of positive ETD cases with tinnitus, and whether the tinnitus description differs between groups with and without ETD.

M196. Open Board

M197. Music Listening Habits and Auditory Function in Young Adults

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Category: Other, Hearing loss: human studies
Background: The World Health Organization reported that over one billion young adults worldwide are at risks of permanent, yet avoidable hearing loss due to prolonged and excessive exposure to loud sounds from personal listening devices and attendance at loud music venues. Therefore, early detection of hearing impairment in young adults and raising social awareness about preventive measures are essential to mitigate the negative impact on individuals' quality of life and reduce societal costs associated with hearing impairment. In this study, we investigated the music listening habits of college students through audiological function tests, including extended high-frequency (EHF) audiometry, uncomfortable loudness level (UCL) test, and measurements of music listening volumes, to assess the effects of the music listening habits on hearing functions of young adults.
Methods: We recruited 57 healthy college student volunteers in their 20s who underwent a comprehensive assessment, including a questionnaire on music listening habits, along with pure-tone audiometry, tympanometry, distortion product otoacoustic emissions, EHF audiometry, and UCL tests. Furthermore, we measured the usual listening sound pressure levels using an iPod touch, EarPods, and the Health Care App (Apple Inc.). The association between music listening habits and listening sound pressure levels, and the results of various auditory function tests were examined.
Results: Among the 57 participants, 42 were men and 15 were women, with a median age of 23 [interquartile range: 22–24] years. All participants exhibited a mean hearing threshold (500, 1,000, 2,000, and 4,000 Hz) of less than 25 dB HL on pure-tone audiometry and had a type A tympanogram. The mean UCL (500, 1,000, 2,000, and 4,000 Hz) had a median of 102.5 [91.9–112.5] dB HL, and the mean EHF threshold (10,000, 12,000, 14,000, and16,000 Hz) had a median of 5 [1.6–10] dB HL. Notably, 48 (84.2%) participants used earphones/headphones daily, with a median of 2 [1–3] hours of use. The median listening sound pressure level was 52 [44–57] dB HL. The correlations between the duration of music device use (earphones, headphones, and speakers), listening sound pressure levels, EHF thresholds, and UCL were not significantly different. The correlation between the duration of acoustic device use and listening sound pressure levels was weakly positive (r=0.3239). Notably, a stronger positive correlation (r=0.4978) was observed when we focused on 19 participants with high UCL values (110 dB HL or higher).
Conclusions: Herein, we assessed the association between detailed auditory functions and music listening habits of young adults. The positive correlation between the duration of sound equipment use and listening sound pressure levels suggested that inappropriate music listening habits, including prolonged listening time and elevated listening volume, might synergistically act as risk factors for hearing impairment in young adults.

M198. Protocol for Supervised Automation of Cell Counting in Confocal Microscopic Cochlear Imaging Datasets Using Macro and Batch Processing

Muhammad Rahman*[1], Nashwaan Khan[1], Mobin Mokbul[2], Cristina Garcia[1], Ibrahim Razu[1], Peter Eckard[1], Bryce Hunger[1], Shakila Fatima[1], Marlan Hansen[1]

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Category: Other, Image Analysis and Automation

Background: Regular image analysis derived from confocal microscopic data of biological tissue samples involves a significant amount of repetition on the part of the researchers for each image. Image analysis is commonly used in biomedical research, and is a necessary, time-consuming, labor-intensive task for labs working with confocal microscopy. It shifts the laboratory workforce from intellectually challenging tasks reducing both productivity and innovation. We have developed a supervised automation protocol in our lab to execute our everyday cell and spot-counting tasks (e.g., neuron, nucleus, macrophages) on images obtained from mouse cochlear samples.
Methods: 10-12-week-old CX3CR1+/GFP Thy1+/YFP mice on C57Bl6 background with normal hearing were used in this experiment. Under Intraperitonel Ketamine (100 mg/kg) and Xylazine (12.5 mg/kg) anesthesia, mice were exsanguinated, and perfused with transcardiac 4% PFA. Harvested and processed were cryosectioned at 30 μm and were labeled with DAPI. Sections were imaged using 20X objective with Stellaris V confocal system. Using Imaris 9.9.0, outlines of modiolus and scala tympani, Rosenthal canal, and lateral wall in the base, middle, and apical turn of the cochlea were traced, and volumetric surfaces were created. Masks were generated for individual channels for every surface. 'Spots' were created based on every mask. Volumes were measured for each surface. The protocol was recorded as 'macros' using a "Macro Scheduler 15". This recorded macro can then be utilized to automate the analysis of other images. Finally, a 'master macro' was developed to re-iterate the process of image analysis using the 'macros' enabling batch processing of multiple images.
Results: Once recorded, the 'macros' can efficiently create surfaces, masks, and spots. Tracing the areas and setting the threshold has to be done manually. The "Master Macro" was able to call the "macros" on every file in a folder. In our experiments, we have tested a folder size of a maximum of 100 images for which the 'master macro' is capable of creating surfaces and masks. The maximum number of images on which the 'master macro' can create spots is 20. This semi-automated, supervised image analysis protocol significantly decreases the number of manual steps, the time spent on analysis and training, and analysis-related fatigue while maintaining comparable quality of image analysis.
Conclusions: The proposed semi-automated protocol can be reproduced and customized by scientists working on basic and experimental neuro-immunology and neuroanatomy research and in other disciplines as well. Though it has some limitations and challenges, the protocol can be further enhanced with the integration of machine learning. We believe our developed protocol may enlighten the scientific community on the controlled automation of image analysis tasks, may translate to a substantial reduction of the manual labor of the researchers, and can increase laboratory efficiency and productivity.

M199. Stimulus-Specific Adaptation in the Hippocampus of the Anesthetized Rat

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Category: Other, Auditory electrophysiology
Background: The hippocampus is well known for its role in spatial and episodic memory. A broader function has been proposed including aspects of perception and relational processing. Recently the hippocampus has also been associated with the detection of auditory novelty. Neural bases of sound analysis have been thoroughly described along the auditory pathway up to the auditory cortex (AC), and beyond in the prefrontal cortex (PFC). However, wider networks supporting auditory cognition are still elusive.
The human brain can automatically detect auditory changes, as indexed by the mismatch negativity (MMN) event related potential. MMN is a key biomarker of automatic deviance detection, thought to emerge from the AC and PFC. Stimulus-specific adaptation (SSA), is considered to be the cellular version of MMN at a neuronal level. It is well established that the hippocampus has pathways that interconnect with the AC and PFC. The existence of these pathways further emphasizes the potential role of the hippocampus in the cortical manifestation of MMN. Recent studies showing similarities between neuronal SSA and behavioral habituation hint at the possibility that SSA interacts with, and may be part of, the brain's memory systems.

Methods: In the current study, we used the classical oddball paradigm and two control sequences (many-standards and cascade). Stimuli were pure tones 75 ms long, presented at a rate of 1 Hz. We recorded neuronal spiking activity to mismatch responses, in the hippocampal regions CA1, dentate gyrus and subiculum. We

found that the hippocampus shows long-lasting auditory responses with latencies ranging from 125-325 ms after stimulus onset. We also found stimulus-specific adaptation to pure tones in CA1, as well as distinct auditory responses in dentate gyrus and subiculum.
Results: Since it has been shown in visual and spatial recognition studies that the hippocampus responds better to complex stimuli. We also decided to analyze the response to the same oddball but presented periodically. As expected, the SSA indexes obtained and the response to the deviant are lower than when the deviant sound was presented randomly.
Conclusions: There is compelling evidence that shows hippocampal alterations in auditory disorders, including hearing loss, tinnitus, and neurodegenerative diseases. The present results open new avenues in our understanding of the interactions between the hippocampus and auditory areas. Understanding the role that the hippocampus plays in sound processing and predictive coding, in conjunction with other works, will help us to get a step closer to find applications to increase life quality of people with neurodegenerative diseases and other conditions mentioned above.

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M200. An Integrated System for Comprehensive Mouse Vestibular Function Evaluation Based on Vestibulo-Ocular Reflexes

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Category: Vestibular: Basic Research and Clinical
Background: Vestibulo-ocular reflexes (VORs) are the compensatory ocular reflexes that ensure stable vision during head motion. VORs, including aVOR (angular VOR) or OVAR (off-vertical axis rotation) test, have been used by various groups to evaluate the mouse vestibular function, of the semicircular canal and otolith organ, respectively. However, the effectiveness of these two tests has not been systematically evaluated, and the lack of commercial equipment has also restricted its accessibility. Here, we developed an integrated instrument system with both aVOR and OVAR modes. To demonstrate its efficacy, the vestibular function of classical vestibular animal models, drug-induced and inherited, was evaluated with comprehensive tests. The streamlined design of the system enables efficient daily testing on a large number of animals.
Methods: The instrument includes several key components: a computerized motor platform that generates a comprehensive stimulus pattern for both aVOR and OVAR, a compact eye-tracking high-speed video recording system integrated with the motorized platform, and eye-tracking software based on deep learning algorithms for analyzing eye movements. Vestibular deficit mouse models including 1) Vestibulotoxicity drugs 3,3'-iminodiproprionitrile (IDPN, 1 mg/g, 2 mg/g and 4 mg/g) induced; 2) Critical MET-related mutant (Cdh23v2J/v2J and TMC1-/-); 3) Vestibulo-specific mutant (Zpld1-/- for semicircular canal dysfunction and Otop1tlt/tlt for otoconia deficient; 4) Unilateral vestibular lesion (UVL) model by injecting gentamicin into horizontal semicircular canals; were used to test the efficacy of the system.
Results: (1) For the IDPN models, daily test results of aVOR and OVAR over an 8-week period were recorded and demonstrated: a) dose-dependent amplitude decreases; b) recovery of the amplitude after 11 days in low-to-mid dose group; c) interesting aVOR gain increased at 1 mg/g and 2 mg/g groups from day 2 to day 4 compared with the control group. (2) Cdh23v2J/v2J mice have no obvious eye movements under all stimuli; but TMC1-/- mice exhibited normal aVOR and OVAR responses. (3) Zpld1-/- mice showed decreased aVOR response but normal OVAR response, whereas Otop1tlt/tlt mice exhibited normal aVOR responses but impaired OVAR responses. (4) The unilateral vestibular loss (UVL) mouse model showed asymmetric fast-phase nystagmus during OVAR tests.
Conclusions: We developed a comprehensive system for testing vestibular function in animal models with vestibular dysfunction. Based on the test results, we can draw the following conclusions: 1) Accurate quantification of vestibular function is achieved, specifically with a dosage of 1 mg/g of IDPN. 2) The integrated OVAR and aVOR tests allow for the assessment of both the otolith system and semicircular canals,

respectively. 3)The presence of asymmetric fast-phase nystagmus during OVAR tests enables the identification of unilateral vestibular loss (UVL).

M201. Could Stimulus-Evoked Changes in Extracellular [K+] and Electrical Potential Beyond the Synaptic Cleft Affect Encoding by Vestibular Calyx Afferents?

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Category: Vestibular: Basic Research and Clinical

Background: Vestibular Type-I hair cells, which detect head motion, sit within and transmit to cup-shaped terminals (calyces) of afferent neurons. These neurons guide motor reflexes that maintain gaze, balance, and our sense of orientation. In addition to glutamate release (quantal transmission), ions flow through the basolateral hair cell (pre-synaptic) membrane into the synaptic cleft (SC) and through the inner calyx (post-synaptic) membrane (nonquantal transmission-NQT). Using a computational model of the vestibular hair cell calyx synapse, we previously presented the sequence of events underlying NQT and how changes in potassium ion concentration '[K+]' and electrical potential 'ϕ', in the SC , drive post-synaptic currents during hair cell stimulation (Govindaraju et al. 2023). There exists however a discrepancy between action potential (AP) latencies and high-frequency responses recorded in excised preparations and in vivo – the latencies are smaller in the latter and phase-locked AP firing is observed for high frequency stimuli (greater than 100 Hz) delivered as bone conducted vibration or air conducted sound. Multiple differences between the in vitro and in vivo experiments are likely to contribute by reducing the input to the afferent spike initiation zone. Here we consider the possibility that [K+] and ϕ are, in vivo, also elevated in the extra-synaptic intercellular space (EIS) of an intact sensory epithelium. This could reduce AP generation latency by dynamically aiding the depolarization of the afferent.

Methods: To simulate transmission between hair cell and afferent neuron, our model uses Hodgkin-Huxley-style ion currents based on whole-cell recordings, continuity equations to describe changes in electric potential within hair cell, SC, afferent fiber, and the EIS. Electro-diffusion equations were used to model [K+] and [Na+] in the SC and EIS. In our previous study, boundary conditions (ion concentrations and electrical potential) were fixed to present a perilymph bath at the apical tip of the calyx terminal; in this study the bath is presented where the afferent fiber would exit the basement membrane of the epithelium. Hair bundle deflection or voltage step protocols are used as input.

Results: We present simulations of [K+] and ϕ gradients surrounding the calyx and their effect on AP generation by the afferent. AP latencies were reduced for both step and sinusoidal stimuli and AP generation was extended to higher frequencies. Phase-locking was also observed. The rate of K+ clearance from the EIS was varied to study the influence of KCC transporters present in adjacent supporting cells; greater rates diminish enhancement of AP generation in the afferent.

Conclusions: Our results show that conditions in the EIS are likely to facilitate fast vestibular encoding. Further studies of supporting cell activity in the epithelium are required to determine the extent to which K+ clearance is through transporters as opposed to passive diffusion to perilymph underlying the basement membrane.

M202. Development of a Reliable and Portable Pediatric Oculomotor and Vestibular Tool for Objective Evaluation of Concussion

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Category: Vestibular: Basic Research and Clinical

Background: Current diagnostics for mild traumatic brain injury (mTBI) rely on self-reported symptoms, subjective clinical exams, and comparison of pre/post-injury neurocognitive assessments, which are rarely available. Inappropriate management leads to negative outcomes, including extended periods of

symptomology and associated psychosocial sequelae, and in the most severe cases, death. In this study, we piloted a novel oculomotor, vestibular, and reaction time test battery (OVRT-c) with a portable, head mounted virtual reality display (Neurolign Dx 100 system-Children) I- for use in children.
Methods: Healthy participants (n=40) between the ages of 6-16 completed the OVRT-c test battery, demographic questionnaires, and a satisfaction questionnaire to assess goggle comfort and instruction comprehension. OVRT test results were stratified by age (Middle Childhood [6-10 years]; Young Teenagers [10-14 years]; Older Teenagers [14-18 years]). Linear mixed effects models and binomial generalized linear mixed models were generated to examine differences in individual OVRT-c tests.
Results: Auditory and visual reaction times decreased with age, though only differences between the youngest children and teenagers were significant. Saccades and motor response times were shorter in teenagers during combined saccade and reaction time testing, as was latency during horizontal/vertical saccade testing. Teenagers also exhibited higher saccades per second and eye velocity during self-paced saccades. In the youngest children, velocity saccade percentages were higher when compared to the other groups examined during both smooth pursuit horizontal and smooth pursuit vertical assessments. Inward time constants (i.e., convergence) were also significantly larger in the youngest aged children compared to both young and older teenagers. All participants completed the OVRT-c test battery without incident. Nearly all participants wore the goggle headset for the entirety of the test battery and reported minimum discomfort during the test battery.
Conclusions: Given the age-related differences observed herein, it is imperative to collect large-scale normative data for middle childhood, young teenagers, and older teenagers in order to account for within-subjects and between-subjects differences. This work broadly supports the viability and continued development of this diagnostic platform. Further development of a pediatric normative database will improve the standard of care and return to school/play protocols.

M203. Multiple Noise Exposures to Intense Noise (110dB) Cause Persistent Vestibular Deficits

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Category: Vestibular: Basic Research and Clinical
Background: Previous studies showed that rats exposed to 6 hours of 120dB SPL noise had attenuation of VsEP responses to jerks and reduced numbers of calretinin positive (CR+) calyces in the sacculus (Stewart et al 2020). However, when the noise intensity was less (110dB), VsEP responses recovered in most animals within one week (Stewart et al 2021) as did the number of CR+ calyces. We have now examined if multiple exposures (which can occur commonly) to 110dB noise produce persistent attenuation of the VsEP and fewer CR+ calyces.
Methods: Experiments were conducted in male and female Long-Evans rats. Surgical procedures to implant a head post were described in Stewart, et al 2020. Rats were exposed to 4 hours of 110dB SPL band-limited noise (Stewart et al 2021) three times at either 3- or 7-day intervals. VsEP measurements were obtained 7, 14, 21 and 28 days after the final noise exposure. We assessed latency and amplitude of evoked VsEP waveforms (P1-3) to determine if noise-induces changes in individual components of the VsEP response after multiple exposures. We assessed behavioral metrics that were altered by 120dB single exposures such as head stability and head orientation (Niwa M et al. ARO 2024). Behavioral data were collected 2 days after each noise exposure and up to 4 weeks after the final exposure. Rats were euthanized 28 days after the final noise exposure and the striolar regions of the saccular and utricular maculae were immunostained with calretinin antibody to visualize terminations of calyx-only vestibular afferents.
Results: Multiple exposures to 110dB noise produced persistent changes in VsEP responses comparable to those previously reported for single exposures to 120dB noise. In particular, the later waves of the VsEP (vestibular nerve and vestibular nuclear complex synchronized responses to jerk) exhibited longer latencies and reduced amplitudes when compared to pre-noise values. Although there were quantitative differences, both the 3- or 7-day exposure interval produced significant changes in VsEP responses that persisted during the 4-week post exposure period. Preliminary data show significantly fewer CR+ calyces in both 3-and 7-day exposure interval animals than in unexposed rats.
Conclusions: These findings suggest that multiple exposures to loud noise can produce persistent VsEP deficits and changes in CR+ calyces that are comparable to those produced by a single exposure to a much

louder (120dB) noise. This result is significant because it suggests that repeated exposure to less intense but relatively common environmental noises (e.g., 110dB at concerts, bars, sports venues) may in time lead to balance disorders and increased propensity to fall.
This work was supported by R01 DC018003-01 (King), I01RX003250-02 (Altschuler)

M204. Vestibular Function After Simultaneous Bilateral Cochlear Implantation in Adults

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Category: Vestibular: Basic Research and Clinical
Background: Compared to sequential surgery, simultaneous bilateral cochlear implantation (CI) is thought to produce binaural effects at an earlier stage and facilitate the integration of the bilateral auditory cortex at the central level. However, CIs have been reported to be associated with dizziness and vestibular dysfunction, raising concerns regarding the risk of bilateral postoperative vestibular dysfunction with simultaneous CI. In this study, we investigated changes in vestibular function over time in adult patients who had undergone simultaneous bilateral CI using the latest minimally invasive electrodes and surgical techniques.
Methods: A retrospective review was conducted on 10 patients who underwent simultaneous bilateral CI at our hospital. Vertigo symptoms and vestibular function test results were examined preoperatively, one month, 3 months, 6 months, and one year after surgery. Nystagmus tests, caloric reflex tests, vestibular evoked myogenic potentials (VEMP) measurements, and static stabilometry were performed as vestibular function tests.
Results: In the caloric reflex tests, 7 patients (13 ears) who preoperatively had response (mean 23.8°/s) showed a significant decrease in response one month after surgery (mean 14.1°/s, P less than 0.05). However, no significant deteriorations were observed in caloric reflex test, 3 months (mean: 16.7°/s), 6 months (mean 22.4°/s), and one year after surgery (mean: 21.3°/s), compared with preoperative value.In 6 patients (11 ears) who had normal oVEMP before CI (mean 12.6 μV), oVEMP was significantly worse at 3 months (8.3 μV, P less than 0.05), and 6 months (7.3 μV, P less than 0.01) after surgery. However, there was no significant difference at one year after surgery (mean 11.5 μV). As for cVEMP, 6 patients (10 ears) who had normal results preoperatively (mean 337.0 μV) showed significant decrease in response at one month (mean: 212.9 μV, P less than 0.01), 3 months (204.9 μV, P less than 0.001), and 6 months after surgery (162.4 μV, P less than 0.0001). On the hand, cVEMP at one year after surgery (mean 275.7 μV) was not significantly different from preoperative value. Of note, regardless of the presence or absence of abnormalities in caloric reflex, oVEMP, or cVEMP, no significant deterioration was detected in the static stabilometer test. No patient reported subjective vertigo.
Conclusions: Although simultaneous bilateral CI may lead to a temporal decrease in vestibular function, this decline is not sustained in the long term. In addition, no patients complained of dizziness at one year after srugery, and no worsening were observed in the static stabilometer test which integrates vestibular function with other senses in postural maintenance. These findings suggest that minimally invasive surgery successfully avoided equilibrium dysfunction, even in simultaneous bilateral CI. Given the patient's needs and burden, simultaneous bilateral CI surgery should be considered.

M205. Investigating Gait Kinematic Variability in Patients With Chronic Vestibulopathy

Angelica Perez Fornos*[1], Gautier Grouvel[1], Anissa Boutabla[2], Stéphane Armand[1], Julie Corre[1], Rebecca Revol[1], Samuel Cavuscens[2], Maurizio Ranieri[2], Raymond van de Berg[3], Nils Guinand[1]

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Category: Vestibular: Basic Research and Clinical
Background: The vestibular system's sensory inputs are crucial for maintaining gait stability. However, although it is generally accepted that vestibular disorders have a notable impact on the daily lives of the affected patients, the effect of these deficits on gait characteristics, and especially on stability, remains still in investigation. This study explores the variability of gait kinematics during walking in patients with chronic

vestibulopathy to understand the impact of these disorders on gait stability. We hypothesize that kinematic variability will be greater in patients with vestibular deficits than in healthy subjects.
Methods: Thirty subjects were enrolled in the study: 10 patients with bilateral vestibulopathy (BV) (5 female; 64.4 ± 9.6 years), 10 patients with unilateral vestibulopathy (UV) (5 female; 63.4 ± 6.2 years), and 10 healthy subjects (HS) (6 female; 64.6 ± 10.0 years). A motion capture system, composed of 12 optoelectronic cameras (Oqus7+, Qualisys), was used to track the subjects' body movements. Subjects were equipped with 35 reflective markers placed according to body landmarks and they were asked to perform gait trials at slow, comfortable, and fast self-selected walking speeds. Joint kinematics were then computed from marker trajectories using a custom Matlab script. The Convention Gait Model was used as the kinematics model. Finally, the gait standard deviation (gaitSD) was calculated as the square root of the average variance over 9 kinematic variables. GaitSD results were compared between the three groups using a non-parametric Wilcoxon test.
Results: For the analysis, the left side was randomly selected for the BV and HS groups, and the affected side for the UV group. We observed that there were significant differences in the gaitSD between the BV and HS groups, at each walking speed (slow: p=0.036; comfortable: p=0.016; fast: p=0.005). No significant differences were observed with the other pathlogical group (UV). Additionally, within group comparisons showed that GaitSD was not significantly different at slow and fast walking speeds compared to comfortable speed. This result applies for all 3 groups.
Conclusions: GaitSD can be a relevant outcome to show how a pathology impacts gait. Despite a small number of participants per group, BV patients show the highest variability in gait kinematics. These results could explain why balance during walking is a difficult task for BV, even on flat floors and with functional vision. This imbalance can also be related with the risk of falling. Future research should focus on treatment to achieve gait consistency to achieve a better stability in patients with vestibulopathy.

M206. Constant "Baseline" Electrical Stimulation of Vestibular Nerve Afferents Improves Function in Human Patients Implanted With Cochleo-Vestibular Implants

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Category: Vestibular: Basic Research and Clinical
Background: Prototypes of vestibular implants are under development to rehabilitate patients with bilateral vestibulopathy. The approach consists in using motion sensors to modulate electrical currents delivered to the vestibular nerve branches according to head movements, mimicking the physiology of the vestibular system. Another "simplified" approach consists in delivering constant electrical stimulation to restore some kind of "baseline" activity in the vestibular system. This approach has shown some benefit in galvanic stimulation research and in vestibular implant trials where the otolith organs were the main stimulation targets. The aim of this study was to objectively quantify the benefits of a "baseline" stimulation delivered to semicircular canal afferents during a long period of activation.
Methods: Study participants are two female patients with bilateral vestibulopathy fitted with a modified cochlear implant providing 3 additional extra-cochlear electrodes implanted in the semicircular canals (P01: 73 years old, left ear implanted; P02: 68 years old, right ear implanted). The participants' speech processor was programmed with their regular cochlear implant map while the electrode in the posterior semicircular canal was programmed to deliver constant "baseline" stimulation for a period of 2.5 months. Objective measurements, i.e., gait analyses, and subjective measurements, i.e., questionnaires, were acquired throughout the testing period and afterwards over 6 sessions (S1: activation day – system off; S2: 1 month – system on; S3: 2.5 months – system on; S4: 2.5 months – system off; S5: post 1 week – system off; S6: post 1 month – system off). Dizziness-Handicap-Inventory (DHI) scores and number of steps for a tandem walk task were analyzed for each session.
Results: For P01, DHI scores improved from 52 (S1) to 42 (S2) and 30 (S3), reflecting clinically relevant changes (minimal-detectable-change = 17.18). When "baseline" vestibular stimulation was deactivated, the DHI score increased to 48 (S5) and 36 (S6), representing a decline from a mild to a moderate handicap. The number of steps in the tandem walk task increased from 2 steps (S1) to 10 steps (S2 and S3) before falling

back to 2 steps for the post-deactivation sessions (S5 and S6). Results for P02 will be presented once the trial for this patient is completed (ongoing at the time of abstract submission).
Conclusions: Results in at least one patient demonstrate that vestibular deficits were reduced during the activation period, complemented by positive feedback from the patient (e.g., "the implant makes it much easier to follow a line when walking"). Objective/subjective gains disappeared when "baseline" stimulation was deactivated (e.g., "the effect of stimulation diminished day by day"). This method might be relevant while waiting for devices allowing stimulation methods that are closer to human physiology to be clinically available.

M207. Na/K-ATPase Alpha Subunit Heterogeneity in the Glial Cells of the Mammalian Vestibular Ganglion

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Category: Vestibular: Basic Research and Clinical
Background: Na pump, or the Na,K-ATPase (NKA) plays an important role in maintaining the electrolyte composition such as endolymph and cerebrospinal fluid. The expression of NKA alpha subunit isoforms varied among different cell regions of the inner ear especially in the auditory and vestibular sensory epithelium. However, the expression of those isoforms was not elucidated in the vestibular ganglion. Because the expression of different molecular variants or isozymes enables distinct functional capabilities, we hypothesized that NKA alpha subunit heterogeneity in the glial cells of the vestibular ganglia might contribute to diversity of afferent nerve fibers.
Methods: Cryosections of the whole head of B/6N male mice including the inner ear and the vestibular ganglion were analyzed for immunofluorescence, between embryonic day 13.5 and postnatal day 28. We examined the expression of NKA alpha1, NKA alpha2, beta-3 tubulin in the vestibular ganglion. We also evaluated the susceptibility to ouabain, which inhibits NKA, of the vestibular ganglion as well as of the spiral ganglion. 1 ul of ouabain solution (1 mM in normal saline) was injected into the posterior semicircular canal of B/6N male mice aged at postnatal day 28. Whole heads including the vestibular ganglion was collected 1 week after the ouabain application.
Results: Between embryonic day 13.5 and postnatal day 1, glial cells in the vestibular ganglion weakly expressed both NKA alpha1 and NKA alpha 2. At postnatal day 14 on, glial cells demonstrated mutually exclusive expression of NKA alpha1 and NKA alpha2. NKA alpha1 positive glial cells in the superior vestibular ganglion were significantly more than those in the inferior vestibular ganglion. The cluster of NKA alpha2 existed especially in the inferior vestibular ganglion. Neurons in the superior vestibular ganglion, surrounded by NKA alpha1 positive glial cells, were significantly damaged by ouabain. In contrast, neurons in the inferior vestibular ganglion, surrounded by NKA alpha2 positive glial cells, were significantly damaged by ouabain. Those results indicated that NKA alpha isoforms in the glial cells do not fully account for the susceptibility to ouabain. While vestibular ganglion neurons are diversified to VGN-1 and VGN-2 as early as embryonic day 13.5 (Sun, 2022), our data demonstrated that glial cells are not diversified until postnatal day 14, especially regarding the expression of NKA alpha subunits.
Conclusions: NKA alpha subunit heterogeneity existed in the mature mammalian vestibular ganglion. Future studies are needed to elucidate whether heterogeneity of glial cells and neurons might be associated with each other.

M208. In Vivo Calcium Imaging of Efferent Vestibular Nucleus Neurons Reveals Activity During Self-Generated Movements but Not During Moderate Passive Motion

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Category: Vestibular: Basic Research and Clinical

Background: The Efferent Vestibular System (EVS), with its bilateral origin the Efferent Vestibular Nucleus (EVN), provides extensive cholinergic input to the vestibular neuroepithelium, directly innervating type II hair cells, afferent nerve fibers and bouton and calyx afferent nerve terminals. Since its discovery, nearly 70 years ago, numerous studies have attempted to determine EVS function in balance behavior, VOR adaptation and compensation, and development. Here, we present in vivo calcium imaging data from EVN neurons in awake, freely moving, and behaving mice during natural active head movements as well as during moderate passive motion (provocative motion, PM).
Methods: We used a miniaturized brain-implanted microscope ('miniscope') system to record real-time gCaMP calcium activity from a transgenic ChAT-gCaMP6 mouse strain. These mice express the fast calcium sensor protein, GCaMP6f, in cholinergic EVN neurons with high accuracy. A miniscope Gradient-Index lens was implanted into the brainstem, just dorsal to the EVN. In all cases, to confirm the precise location of the implanted lens, brain tissue was immunolabelled and imaged using confocal microscopy. In addition to calcium activity, we collected time-locked data from an Inertial Measurement Unit (IMU), which detected changes in x, y, z axes and yaw, pitch, roll orientations, as well as time-locked videos of mouse behavior. This comprehensive data set allowed us to accurately relate neuronal activity to behavior and specific head movements. Furthermore, our recordings suggested that EVN neuronal activity appeared to be synchronized. Therefore, we used fluorescent immunolabelling to reveal the presence of connexin proteins, markers of electrical gap junctions between EVN neurons.
Results: Our in vivo calcium imaging data in these GCaMP6f transgenic mice revealed increased EVN activity during large, high-acceleration, self-generated head movements, but little or no calcium activity during moderate passive motion. In some cases, the calcium transients anticipated head movements by ~120 ms, suggesting potential input of intentional movement-coding from higher-order brain centers. Impulse signal cross-correlation showed EVN activity responded preferentially to head movements in the x (fore-aft), z (up-down), and pitch axes. These observations support a recent study in alpha-9 knock out mice. EVN activity was significantly reduced during PM-induced motion sickness due to the cessation of active head movements. Furthermore, immunohistochemical labelling of gap junction proteins connexin-32 and connexin-36 supports the notion that EVN neurons are electrically interconnected, and their activity is potentially synchronized.
Conclusions: Our data agrees with previous studies from the toadfish and mice which supports the idea that the EVS exerts its effects on the vestibular periphery during large active head movements modifying afferent discharge rates on a short timescale, and rapidly responding to volitional movements.

M209. Simulation of Semicircular Canal Cupulae Deflections Evoked by Head Impulse Tests and Orientation Relative to Gravity in Control and BPPV Conditions

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Category: Vestibular: Basic Research and Clinical
Background: Benign Paroxysmal Positional Vertigo (BPPV) is the most common cause of vertigo and typically arises from dense otolithic debris in the endolymph (canalithiasis) or adhered to the cupula (cupulolithiasis). The most common form of BPPV is canalithiasis in the long-arm of the posterior canal (PC), but case reports of atypical forms of PC-BPPV including cupulolithiasis, canalithiasis in the short arm, Type 2 BPPV and canal-jam are increasing. The primary diagnostic tools for BPPV are evocative tests that change the orientation of the head and plane of the canal relative to gravity (e.g. Dix-Hallpike, supine head roll), but evidence is emerging that the video Head Impulse Test (HIT) can also be useful in differential diagnosis of BPPV types. The purpose of this descriptive study is to compare cupulae deflections in response to HITs, evocative maneuvers, and canalith repositioning maneuvers (CRMs) in health vs. disease.
Methods: Endolymph and cupulae displacements were simulated within a rigid 3D reconstructed model of a human membranous labyrinth. The endolymph was modeled as a Newtonian fluid and the cupulae were modeled as poro-elastic solids. BPPV was modeled using heavy debris either suspended in the endolymph or adhered to a cupula. Cupuloperfluo was modeled by allowing endolymph leakage through or around the cupula, and canal-jam was modeled as a reduction in canal cross-sectional area at the location of the debris. Equations of motion were solved numerically.
Results: Simulation of the Dix-Hallpike test predict distinct differences in the time course and direction of PC cupula displacement for BPPV including long and short arm canalithiasis, and cupulolithiasis, consistent

with recent case studies. Analysis of various CRMs predict a modified Epley maneuver (Epley-U) and a modified Barreto maneuver (Barreto-U) are "universal" CRMs capable of clearing canaliths from all three canals at the same time in a single movement. Results for the Semont-Plus CRM predict a higher probability of failure relative to the Epley-U CRM. HIT simulations in the control condition predict cupulae displacements that closely follow angular velocity of the head, are insensitive to offsets in the axis of rotation, and obey "cosine" spatial tuning (directional coding) in 3D space.
Conclusions: Results identify universal CRMs that clear all 3 canals based on the morphology of an individual subject, and suggest it might be possible to design a single universal CRM with high probability of success across a population of patients. In the absence of central pathology, simulations of atypical forms of BPPV suggest the direction and time course and direction of nystagmus arises from the mechanics and can be used to imply locations and properties of otolithic debris. (Support: NIH R01 DC006685)

M210. Infant Vestibular Evoked Myogenic Potentials: A Scoping Review

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Category: Vestibular: Basic Research and Clinical
Background: Children diagnosed with hearing loss typically demonstrate increased rates of vestibular loss as compared to their peers, with hearing within normal limits (Dhondt et al., 2021; Janky et al., 2018; Selz et al., 1996). Decreased vestibular function is linked with delays in gross motor development, acquisition of gross motor skills, and academic challenges (Braswell and Rine, 2006; Franco and Panhoca, 2008; Janky et al., 2018; Maes et al., 2014). Timely development of sitting and walking gross motor skills aids in the progress of environmental exploratory activities, which have been tied to cognitive, language, and vocabulary development (Franchak, 2019). Considering the time-sensitive development of gross motor skills and cognitive, language, and vocabulary development, identifying vestibular loss in infancy can support early intervention. This scoping review analyzes stimulus, recording, and participant factors relevant to assessing cervical vestibular evoked myogenic potentials (cVEMPs) in the infant population.
Methods: The systematic literature review was conducted on literature published between 2000-2023, focusing on articles assessing cVEMPs in infants. Two authors independently followed Preferred Reporting Items for Systematic and Meta-Analysis (PRISMA) guidelines for title and abstract screening, full-text review, data extraction, and quality assessments. Eighteen articles meeting the inclusion criteria were included in the analysis.
Results: The existing literature lacks consensus regarding stimulus and recording parameters for measuring infant cVEMPs. Additionally, the review reveals a decrease in cVEMP responses with the severity of hearing loss, especially in cases of severe to profound hearing loss, compared to mild to moderate sensorineural hearing loss (SNHL) in infants.
Conclusions: This scoping review demonstrates the increasing use of cVEMP as a reliable tool for objectively assessing infant vestibular function. The lack of consensus in stimulus and recording parameters emphasizes the need for systematic research to establish an evidence-based protocol for cVEMP measurements in infants. Such a protocol will ensure the reliable measurement of cVEMPs in infants and enhance the effectiveness of cVEMP as part of the infant vestibular test battery. Additionally, there is a necessity for a comprehensive large-scale study to evaluate the practicality and feasibility of implementing vestibular screening protocols for infants diagnosed with sensorineural hearing loss in the United States.

M211. Synaptic Activity in Embryonic Vestibulo-Ocular Reflex (VOR) Neurons in a Chick Model for Congenital Vestibular Disorders

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Category: Vestibular: Basic Research and Clinical
Background: Children with syndromic, congenital vestibular disorders (CVDs) form a sac-like inner ear with missing or truncated semicircular canals and undergo delayed motor development with challenges in

maintaining posture and balance throughout life. This lab has implemented an animal model to investigate CVDs, the ARO chick, by Anteroposterior Rotation of the Otocyst surgically on one side so that a sac-like inner ear develops.
Methods: Whole-cell patch-clamp recordings were performed on brain slices from 16-day-old embryonic ARO chicks and age-matched normal controls to study synaptic events in a subset of VOR neurons, the principal cells (PCs) of the tangential nucleus. Spontaneous excitatory (sEPSCs) and inhibitory (sIPSCs) postsynaptic events were recorded in the same cell using cesium gluconate pipette solution to distinguish events based on voltage dependencies at -60 mV and +10 mV, respectively.
Results: PC input resistance increased on the rotated side (156 ± 26 MΩ (SEM); n=7), but less on the intact side (128 ± 21 MΩ; n=5) compared to controls (100 ± 9 MΩ; n=7). The frequency of sEPSCs decreased on the rotated side (1.1 ± 0.5 Hz; n=7) and increased on the intact side (4.1 ±1.4 Hz; n=5) compared to controls (1.4 ± 0.6 Hz; n=7). The frequency of sIPSCs increased on the rotated (3.4 ± 2.1 Hz; n=7) and intact sides (3.4 ± 1.4 Hz; n=5) compared to controls (2.5 ± 0.8 Hz; n=7). The sEPSC/sIPSC ratio was about 1:3 on the rotated side, 1:1 on the intact side, and 1:2 in controls. On exposure to TTX, EPSC frequency decreased 45% on the rotated side, 80% on the intact side, and 50% in controls, whereas IPSC frequency decreased 79% on the rotated side, 76% on the intact side, and 36% in controls. Altogether, these preliminary data suggest that a larger proportion of postsynaptic activity in ARO chicks is action-potential dependent, especially inhibitory events. Confocal imaging of biocytin-injected and recorded PCs revealed that the primary dendrites were longer in ARO chicks than in controls, but the exuberant secondary and tertiary dendritic branching observed in controls was lacking in PCs on the rotated side.
Conclusions: In conclusion, major increases in action-potential dependent synaptic events and decreased dendritic outgrowth in embryonic VOR neurons may contribute to the developmental delays and neural circuitry dysfunction found in CVDs.

M212. The Role of Glutamatergic Input in Shaping the Biophysical Properties of Developing Vestibular Ganglion Neurons

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Category: Vestibular: Basic Research and Clinical
Background: In the vestibular system, hair cells transmit information about changes in head positioning and movement via synaptic transmission with vestibular ganglion neurons (VGN). In vitro firing patterns of VGN become more phasic with decreased excitability during the first two post-natal weeks. These changes coincide with increases in low-voltage activated potassium currents (IKL) conducted in-part by KCNQ channels. Studies in spiral ganglion neurons show that glutamatergic input from auditory hair cells is necessary for normal afferent maturation and diversification. Here we tested if the maturation of VGN is similarly dependent on glutamatergic inputs from hair cells. The results presented here update last year's abstract by including new data over a wider age range and with new control data from wild-type mice as opposed to Long-Evans rat.
Methods: We compared the biophysical maturation of vestibular-ganglion neurons and their patterns of innervation within developing vestibular epithelia in VGlut3-KO mice (whose hair-cell synapses lack glutamate) to wild-type mice and Long-Evans rats using in vitro patch-clamp recordings and immunohistochemistry. Patch-clamp recordings were made from dissociated, cultured neurons at the end of the first, second, and third postnatal weeks (P9, P15 and P22, ± 1 day). Immunohistochemistry for KCNQ4 and Calretinin expression was performed on vestibular epithelia at P15 and P30.
Results: Despite lacking glutamatergic input, VGN in VGlut3-KO mice are still diverse in their firing patterns, with both phasic and tonic firing populations present at the end of the first, second, and third postnatal week. We found no difference between VGlut3-KO and wild-type mice in the proportions of VGN that are tonic firing (N=31/48 and N=32/48, respectively). Similar to wild-type mice, VGlut3-KO express KCNQ4 in the epithelia, which is highly concentrated in the striolar zone by P30. We previously reported that VGN in VGlut3-KO mice were more excitable compared to VGN in Long-Evans rats by the end of the second post-natal week, presumably due to a delay in KCNQ4 upregulation in the Vglut3-KO mice. Our updated results with age-matched mouse controls show no significant difference between the KO and wild-type mice but

rather that VGN from mice are more excitable than VGN from age-matched rats (N=21/29 (72.4%) in P15±1 mice vs N=28/59 (47.5%) tonic firing in P15±1 rats).
Conclusions: Our data suggest that the lack of glutamatergic input in the VGlut3-KO does not affect the firing patterns and excitability of VGN, but VGN in mice are more excitable than in age-matched rats. Ongoing work aims to determine whether this difference reflects a transient maturational difference between mice and rats or whether a persistent difference exists between species. Future directions include investigating whether nonquantal transmission could be compensating for the lack of glutamatergic signaling in this transgenic model.

M213. Exploring Age-Related Balance Dysfunction in Rodent Peripheral Vestibular System

Mohammad Al-Amin*[1], Tiffany Vu[1], Brandon Gehrke[1], Anna Dondzillo[1], Frances Meredith[1], Nesrien Mohamed[1], Anthony Peng[1], Katie Rennie[1]

[1]*University of Colorado Anschutz Medical Campus*

Category: Vestibular: Basic Research and Clinical
Background: The vestibular system is important for maintaining balance and head orientation. Vestibular function declines with age, leading to balance disorders, falls and impaired quality of life. However, the underlying causes of vestibular dysfunction with age are not well understood. Here, we investigated vestibular properties in young and aging gerbils using a variety of approaches.
Methods: Whole cell patch clamp recordings were obtained from type-I and -II hair cells from Mongolian gerbil crista in two age groups: young (postnatal day (P)30-60) and advanced age (P265-531). Electrode solution contained: 115 mM KF, 10 mM KCl, 2 mM MgCl2, 2 mM NaCl, 10 mM HEPES, 10 mM EGTA, 3 mM Glucose, pH 7.4 and extracellular solution was L-15. Pharmacological blockers were applied extracellularly. Balance function was tested by placing gerbils on an accelerating Rotarod and recording time to fall. In addition, immunohistochemical staining for hair cells and afferents was performed at different ages.
Results: Advanced age gerbils (8-10 months) performed significantly worse on Rotarod trials compared to 1-2 month old animals, suggesting a decline in vestibular function. At all ages studied, we found that type-I cells exhibited low and high voltage-activated K+ currents that did not inactivate in response to standard voltage protocols. Type-II hair cells demonstrated outward K+ currents that inactivated at depolarized membrane potentials and inward slowly activating hyperpolarization-activated currents at potentials negative to the resting potential. Peak outward K+ currents in type-I hair cells at +20 mV were 3,885 ± 449 pA (mean ± SEM, n = 16) in young adults and 4,904 ± 613 pA (n = 8) in advanced aged gerbils. In type-II hair cells, peak outward K+ currents were 2,044 ± 299 pA (n = 14) and 1,775 ± 257 pA (n = 4) at young and old ages respectively. Extracellular application of the K+ channel blocker 4-aminopyridine (4-AP, 1 mM) blocked greater than 55% of the outward current at P30-60 in both cell types at young and old ages. Extracellular TetraEthylAmmonium (TEA, 20 mM) also blocked ~30% of outward K+ currents in type-II cells at different ages. Tubulin, Myosin VIIa and Calretinin specific antibodies labeled nerve fibers, hair cells and calyx-only afferents respectively at different ages.
Conclusions: Aged gerbils showed impaired performance on the accelerating rotarod task compared to younger gerbils. Voltage-gated K+ currents showed distinct differences between hair cell types in both young and aged cohorts. 4-AP (1 mM) blocked a large component of K+ currents in both hair cell types in young and aging gerbils. Further investigations will examine how age-related changes in hair cell and afferent properties may influence vestibular function. Supported by NIDCD DC018786 (Rennie) and NIA AG073997 (Rennie/Peng)

M214. Hair Bundle Measurement During Mouse Utricle Development and Maturation

Ahmad Mahmoudi[1], Tian Wang[1], Nathan Mohit[1], Kimberly Giffen[1], Alan Cheng[1], Ahmad Mahmoudi*[1]

[1]*Stanford University*

Category: Vestibular: Basic Research and Clinical
Background: Utricular hair cells are mechanoreceptors required for vestibular function, and their degeneration leads to balance disorders in humans. Vestibular hair cells are crowned by kinocilium and hair

bundles (HB). While previous studies have measured dimensions of HBs in non-mammalian and mammalian utricles, recent imaging techniques have enabled more accurate 3D reconstructed images to assess HB dimensions. Moreover, HB dimensions during vestibular hair cell development have not been systematically examined. In this study, we assessed the dimensions of utricular hair bundles using 3D reconstructed images of the developing and mature mouse utricles.

Methods: e examined utricular hair cells from mice at 6 different developmental time points: embryonic (E) 13.5, E15.5, E18.5, postnatal day (P) 0, P37, and P180. The utricles were dissected, and stained for Myosin7a, α-Tubulin, and F-actin at each time point. The samples were then imaged using confocal microscopy and processed using Imaris 3D imaging software. HBs were measured only when they displayed visible Myo7a+ cell bodies. Dimensions including height of kinocilium, height of shortest and tallest stereocilium, and length of the surface array of hair cells were evaluated. Lastly, hair cell subtypes and hair cells in both the striolar and extrastriolar regions were separately analyzed.

Results: We analyzed hair cells from the striolar and extrastriolar regions for every developmental time point. We observed different morphologies of kinocilium- straight, bent, and twisted- with the percentage of bent and twisted kinocilia increasing with age. We also observed different shapes of stereocilia-aligned, spread, and segmented-with the number of aligned stereocilia bundles decreasing with age. Based on the HB measurements, we noted increases in the height of kinocilium and tallest stereocilium from E13.5 to P37 in both the striolar and extrastriolar regions, while the height of shortest stereocilium increased only from E13.5 to P0. Interestingly, the height ranges of kinocilium and tallest stereocilium widened with increasing age, showing a bimodal distribution. Ongoing experiments include analyzing more postnatally added hair cells using lineage tracing, and analyzing hair cell subtypes.

Conclusions: Utricular hair cells elongate their kinocilium and tallest stereocilium as they mature in the late embryonic and early postnatal period. The shortest stereocilium only modestly lengthened in the embryonic period. The wider distribution of kinocilium and tallest stereocilium may be attributed to newly added hair cells in the postnatal utricle.

M215. Are the Factors Driving Expression of Calcium Binding Proteins Conserved in Mammalian Otolithic Epithelia?

Tyler Kramer*[1], Larry Hoffman[2]

[1]*University of California - Los Angeles,* [2]*Geffen School of Medicine at UCLA*

Category: Vestibular: Basic Research and Clinical

Background: Within the mammalian utricle and the saccule, calcium-binding proteins have unique expression patterns in both afferent structures and hair cells. Preceding investigations (Desai et al., 2005) have determined that calretinin-positive (CALB2+) calyces enveloping Type I hair cells define the borders of the striola in both the utricle and the saccule, with limited afferent expression of calretinin outside of this region. These calretinin-positive calyces are bordered laterally by the line of polarity reversal (LPR) in the utricle, and follow a similar pattern in the saccule. Oncomodulin (OCM), also known as β-parvalbumin, follows an expression signature in striolar hair cells similar to calyceal calretinin; however, deviations surface as OCM+ hair cells have been found lateral to the line of polarity reversal and the striola of the utricle. Building upon previous investigations, our study aims to elucidate the topographical distribution of OCM-positivity within the saccule, providing further insight into the factors governing oncomodulin expression and the shared mechanisms between the developing otolithic organs.

Methods: Sacculi from wild-type mice were harvested and processed using immunohistochemical methods routine for our laboratory. Primary antibodies targeting OCM, TUBB3, and calretinin (CALB2) to label OCM+ hair cells, calyces and parent axons, and striolar calyces were selected. Intact sacculi were left to incubate in the primary solution for 72 hours, washed in PBS, then immunolabeled with appropriate fluorophore-conjugated secondary antibodies diluted in blocking solution. Specimens were imaged using a 63X Plan-apochromat objective on a LSM 880 confocal microscope. Raw Airyscan image stacks from the saccular striola and juxtastriolar regions analyzed using custom MATLAB scripts.

Results: Oncomodulin immunoreactivity was observed in 61% of the striola of the saccule and 50.8% of the striola of the utricle. In the saccular striola, 82.6% of Type I hair cells and in 29.2% of Type II hair cells expressed oncomodulin, while in the utricular striola OCM expression was observed in 82.9% of Type I hair cells and 24.1% of Type II hair cells. OCM+ hair cells were observed throughout the juxtastriolar regions with

increased expression in the dorsal regions of the extrastriola. These findings are consistent with previous studies regarding oncomodulin topology in the utricle, highlighting important similarities between the factors responsible for calcium-binding protein expression in the utricle and saccule.
Conclusions: While the role of calcium-binding proteins in vestibular hair cells and afferents remains unclear, this study provides further evidence for the differential expression of calcium-binding proteins in the vestibular periphery and the remarkable consistency of the topological organization of the otolithic organs. Moreover, this data demonstrates the differences in factors driving the polarity of hair cells and expression of these proteins. Further investigation is required to understand the role OCM and other calcium binding proteins play in the vestibular periphery.

Symposium 7 - What Can We Learn From Chicken, Fish, and Stem Cells for Regenerating Hair Cells?

2:15 p.m. - 4:15 p.m.

Platinum Salon 6

What can we Learn From Chicken, Fish, and Stem Cells for Regenerating Hair Cells?

Chair: Takayuki Nakagawa*, Graduate School of Medicine, Kyoto University*
Co:Chair: Stefan Heller*, Stanford University School of Medicine*

Session Description: The ability of avian auditory supporting cells to act as somatic stem cells that replace lost hair cells and functionally restore hearing was discovered more than 30 years ago. Numerous additional discoveries were made that resulted in the first clinical trials aimed at regenerating lost hair cells in humans. We do not share the hype about such trials, and we believe that the existing scientific evidence for efficient regenerative strategies in mammals does not justify rogue experiments with human patients. The path to drug-based human hair cell regeneration is not an easy one – road bumps exist. Nowadays, we are learning more and more about the barriers that prevent regenerative programs of mammalian supporting cells. Simultaneously, recent research advanced towards fundamentally understanding the molecular steps that activate the avian regenerative programs. There are reasons for careful optimism that the field will make sufficient progress for novel human treatment strategies in the future. Here, we propose a symposium to highlight the exciting scientific achievements and strategies, starting with talks on systematic approaches towards identifying the mechanisms of avian auditory hair cell regeneration (Nakagawa and Heller labs), leading to approaches in the zebrafish lateral line and inner ear (Piotrowski and Burgess labs). Finally, we have presentations on human cell guidance (guest speaker Kyle Loh) and on generating auditory hair cell types from human embryonic stem cells (Eri Hashino). The program topic will be introduced by John Brigande and moderated by Stefan Heller and Takayuki Nakagawa.

Quo Vadis, Hair Cell Regeneration?
John Brigande

Oregon Hearing Research Center, Oregon National Primate Research Center, Oregon Health and Science University

Individual Abstract: We understand quite well the mechanisms by which vertebrate hair cells develop and how hair-cell-like cells are generated from stem cells or by reprogramming somatic inner ear cells. This introductory presentation will review selective aspects of the discovery of cell regeneration in non-mammalian vertebrates as well as the limited regeneration in the mammalian utricle. It will touch on the use of Atoh1 and other genes essential for hair cell development for coaxing non-sensory cells into adopting a hair cell fate. Despite these spectacular advances, I will present existing roadblocks and open questions we must address

before advancing hair cell regeneration strategies in patients with hearing impairment and vestibular dysfunction.

A Roadmap for Human Ectoderm Development From Pluripotent Stem Cells
Kyle Loh

Stanford University

Individual Abstract: Stem cell research endeavors to efficiently differentiate human pluripotent stem cells (hPSCs) into specific cell-types. Fundamental challenges including an incomplete understanding of the branching lineage decisions, and the extracellular signals that control them, at each developmental step. We have now mapped hPSC differentiation into a variety of ectoderm progenitors. This revealed multiple surprises, including two separate neural ectoderm progenitors to the forebrain/midbrain vs. hindbrain, which was confirmed in vivo by genetic lineage-tracing. This ectoderm roadmap also revealed the point when neural vs. non-neural fates become segregated, thus enabling efficient production of border and surface ectoderm within 2 days of hPSC differentiation. This could provide a starting point for subsequent optimization of preplacodal ectoderm differentiation, thereby availing efforts to generate hair cells.

Generating Cochlear Hair Cells From Human Pluripotent Stem Cells
Yoshitomo Ueda

Indiana University School of Medicine

Individual Abstract: Loss of mechanosensitive hair cells in the cochlea causes irreversible hearing loss in humans. However, progress in research for realizing biological restoration of hearing has been hampered due to the paucity of human cochlear tissues. To address this unmet need, we sought to develop an in vitro model that recapitulates human cochlear development using aggregates of human pluripotent stem cells. Timed modulations of Sonic Hedgehog and WNT signaling lead to upregulated expression of ventral otic progenitor markers. These progenitors give rise to elaborately patterned epithelia containing hair cells with morphology, marker expression, and functional properties consistent with outer or inner hair cells in the human cochlea. These results suggest that early morphogenic cues are sufficient to drive cochlear induction and establish an unprecedented system to model the human auditory organ.

Mapping and Functionally Testing the Gene Regulatory Network During Zebrafish Inner Ear Regeneration
Shawn Burgess

National Human Genome Research Institute

Individual Abstract: Using adult zebrafish inner ears as a model for sensorineural regeneration, we performed a targeted ablation of the mechanosensory receptors in the saccule and utricle and characterized the single-cell epigenome and transcriptome at consecutive time-points following hair cell ablation. Using deep learning on the regeneration-induced open chromatin sequences, we were able to identify cell-specific transcription factor (TF) motif patterns enriched in the raw data. We correlated enhancer activity with gene expression to identify potential gene regulatory networks. A clear pattern of overlapping Sox- and Six-family transcription factor gene expression and binding motifs was detected, suggesting a combinatorial program of TFs driving regeneration and cell identity. Pseudo-time analysis of single-cell transcriptomic data suggested that the support cells within the sensory epithelium changed cell identity to a multipotent “progenitor” cell population that could differentiate into hair cells. We showed that sox2 became enriched in the progenitor cells and was reduced again when the cells differentiated. Analysis of the scATAC-seq data identified a 2.6 kb DNA sequence element upstream of the sox2 promoter that dynamically changed in accessibility during hair cell regeneration. When deleted, the upstream regulator showed a dominant phenotype that resulted in a hair cell regeneration-specific deficit in both the lateral line and adult inner ear. We are now broadly testing

predicted regeneration responsive elements (RRE) for enhancer activity specifically impacting the regeneration response.

Building a Gene Regulatory Network of Zebrafish Hair Cell Regeneration

Jeremy Sandler

Stowers Institute for Medical Research

Individual Abstract: Zebrafish and mammalian sensory hair cells are functionally and genetically homologous, however zebrafish hair cells rapidly regenerate after damage and constantly turnover during homeostasis. Previous studies in our lab have characterized the transcriptional changes in the lateral line in a fine time scale using scRNA-seq, and we identified three core modules that drive the regeneration of hair cells. Regeneration depends on three subsequently activated gene modules that can serve as a blueprint to trigger regeneration in mammals. A key missing perspective however, is the epigenetic and regulatory landscape during regeneration and which genes directly regulate each other. To understand the gene regulatory program driving hair cell regeneration, we completed an ATAC-seq and ChIP-seq time course of the epigenetic regulatory environment and combined this information with the existing scRNA-seq data. We found that chromatin accessibility and regulatory histone marks rapidly change matching the expression dynamics of genes during regeneration time points. Using a hierarchical clustering method, we found that co-regulated enhancers form ten "regulatory groups" across time. Motif enrichment analysis reveals that each enhancer group is defined by a unique core set of transcription factors necessary at steps of regeneration/regulatory modules in the time series. Early injury/stress response genes, such as fos and jun have enriched binding sites in enhancers of hair cell regeneration genes. Thus, there is a direct regulatory link between the injury response form dying hair cells and the genes necessary to regenerate new hair cells. We identified specific enhancers from the ten regulatory groups and show that they drive reporter gene expression. Functional analyses show that their deletion causes hair cell regeneration defects, demonstrating that these regulatory links identified are essential for hair cells. We further mutated key genes identified in the GRN, such as cbx7a and prdm1a, and observed drastic hair cell development and regeneration deficiencies in these mutants. By understanding the gene regulatory landscape and how links and binding motifs have broken or evolved between zebrafish and mammals, our data provide key insight and gene targets for restoring hair cell regeneration in mammals.

Direct Conversion From Supporting Cells to Hair Cells in the Chicken Auditory Epithelium

Mami Matsunaga

Graduate School of Medicine, Kyoto University

Individual Abstract: Hair cell regeneration spontaneously occurs in response to HC injury through direct conversion or mitosis of supporting cells (SCs) in the avian cochlea, namely basilar papillae (BP). SC direct conversion is also inducible in neonatal murine cochleae by manipulation of signaling pathways. However, the molecular mechanisms of direct conversion from SCs to HCs have not fully been elucidated. To understand molecular mechanisms for SC-to-HC conversion in chick BP, we performed single-cell RNA sequencing during HC regeneration using an explant culture model of chick BP, in which SC direct conversion is a predominant path for replacement of missing HCs. According to the expression of SC marker genes, we extracted the SC subset and performed unsupervised clustering. Based on differentially expressed genes and histological expression patterns, we identified clusters during the process of SC-to-HC conversion. A pseudotime trajectory analysis demonstrated six trajectory patterns. Alteration patterns of signature genes along the pseudotime trajectory indicated stepwise conversion from quiescent SCs to nascent HCs, which consisted of four stages: priming, initiating, intermediate, and differentiating stages. In the initial and intermediate stages, temporal upregulation of unique genes related to the precursor state was identified. In addition, EDNRB2 was specifically expressed in the initial and intermediate stage. Elucidation of the function and associated signaling cascades of EDNRB2 may contribute to understanding mechanisms for SC-to-HC conversion.

An Essential Signaling Cascade for Auditory Hair Cell Regeneration

Nesrine Benkafadar
Stanford University School of Medicine

Individual Abstract: Hearing loss is a prevalent and often disabling condition caused by hair cell loss, which is irreversible in mammals. Lost mammalian hair cells are not regenerated, unlike in non-mammals such as chickens, because supporting cells act as facultative stem cells that can proliferate and regenerate hair cells. Despite extensive research spanning over three decades, the exact mechanism underlying avian hair cell regeneration remains unknown. In this study, we induced ototoxic damage in the chicken basilar papilla and investigated the signaling effector genes responsible for initiating and executing mitotic hair cell regeneration. **Methods:** A single injection of sisomicin into the posterior semicircular canal of 7-days-old chickens triggers complete hair cell loss in the basilar papilla. We performed single-cell RNA sequencing of supporting cells at specific time points post-sisomicin. The time points were based on the time course of hair cell damage and the supporting cells' regenerative responses. We detangled the temporal sequence of gene expression changes, which allowed us to focus on the earliest detectable changes in responding supporting cells. The major constituents of the identified candidate signaling pathway that potentially initiates hair cell regeneration were functionally assessed in vivo using pharmacological approaches combined with qRT PCR, in situ mRNA detection, and detection of pathway protein phosphorylation by Western blotting.

Results: We identified early changes in gene expression in responding supporting cells when the first sign of DNA fragmentation in hair cell nuclei is detected. Through in situ validation, we confirmed the upregulation of distinct genes in these early responding supporting cells. Furthermore, our in vivo assessment of the candidate signaling pathway confirmed the crucial role of a cascade involving the proteolytic activation of F2RL1, followed by matrix-metalloprotease-mediated HBEGF shedding and EGFR-mediated ERK signaling. Additionally, we found that STAT3 phosphorylation converges with this signaling pathway, resulting in the upregulation of transcription factors ATF3, FOSL2, and CREM.

Podium Session 13 - Emerging Gene Therapies in Mice, Monkeys, Men, and Women

2:15 p.m. - 4:15 p.m.

Platinum Salon 5

First-In-Human Cochlear Implant Neurotrophin Gene Therapy Clinical Trial Directed to Close the 'Neural Gap'

Gary D Housley*[1], Catherine S Birman[2], Fadwa Alnafjan[3], Jeremy L Pinyon[4], Georg von Jonquieres[1], Mayryl Duxbury[1], WaiKong Lai[5], Catherine McMahon[6], David McAlpine[6], Rachelle Hassarati[5], Jaime Undurraga[6], Edward N Crawford[1], Cherylea J Browne[7], Halit Sanli[5], Daniel Scherman[8], Corinne Marie[9], James B Fallon[10], Andrew K Wise[10], Robert Shepherd[10], Ya Lang Enke[11], Robert D Gay[11], Paul M Carter[11], James F Patrick[11], Amr Al Abed[12], Matthias Klugmann[1], NIgel H Lovell[12]

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Melbourne; University of Melbourne, [11]*Cochlear Ltd,* [12]*Tyree Institute of Health Engineering (IHealthE), UNSW Sydney; Graduate School of Biomedical Engineering, UNSW Sydney*

Category: Gene Therapy

Background: This first-in-human study 'A phase I/II non-randomized, controlled trial, evaluating the safety and efficacy of neurotrophin gene therapy delivered during cochlear implant surgery' (ACTRN12618001556235; www.cingt.info) utilized a clinical 'Bionic array - Directed Gene Electrotransfer' (BaDGE®) system for delivery of 'naked' DNA encoding BDNF and NT3 to the cochlea. This followed from gene electrotransfer (GET) studies in deafened animal models that achieved rapid directed regeneration of the spiral ganglion neurites towards the cochlear implant (CI) electrodes, closing the 'neural gap', and improving cochlear implant performance (Pinyon et al., Sci. Transl. Med. 2014).

Methods: With Australian Therapeutic Goods Administration regulatory approval, and Royal Prince Alfred Hospital ethics oversight, this unblinded study comprised a CI Neurotrophin Gene Therapy Group (CINGT; 18 patients with neurotrophin GET, then Cochlear® Nucleus™ CI622 cochlear implant), and Control Group (6 patients CI622 implant; 1 subject DNA delivery without GET, then CI622 implant). All subjects had severe – profound hearing loss (PTA greater than 85 dB HL; 1 – 54 years hearing loss; Subject age 27 – 79 years). The BaDGE® probe utilized the apical 16 electrodes of a derivative of a Cochlear® Nucleus™ CI622 array, configured for electric field focusing, and incorporating microfluidics for scala tympani DNA delivery. The clinical DNA comprised a bicistronic construct encoding human BDNF and NT3 in a pFAR4 miniplasmid backbone (Pinyon et al., Hear. Res. 2019). Following instantaneous GET via a pulsed electric field (Digitimer DS5 clinical stimulator), the BaDGE® device was removed and a CI622 array implanted. Hearing performance was assessed across 8 visits from 2 weeks post- CI surgery out to 1 year (on-going).

Results: There were no adverse reactions across the 12 month patient follow-up for the fully-subscribed CINGT arm. One gene therapy patient was non-compliant and excluded. The pre-operative CUNY sentence and CNC word scores for the majority of the treatment group were in the lower quartile pre-operatively, and exceeded speech performance at 3 months post-CI for the equivalent cohort from historical data (Birman and Sanli, Otol. Neurootol. 2020). Three of these subjects achieved greater than 90% CUNY scores within one month. Psychophysical assessment indicated improved hearing sensitivity (greater dynamic range, with lower T levels) in the apical (e19) cochlear region targeted by the gene electrotransfer (ANOVA; P less than 0.05 for both DR and T levels relative to basal e7 reference). Supporting this, at one month, eABR indicated statistically significantly shorter wave V latencies (inferior colliculus) at e19-e22 for the CINGT group. The data are suggestive of rapid hearing acquisition following neurotrophin gene augmentation.

Conclusions: Phase I safety objective measures were satisfied. The phase IIa subjective and objective measures indicate a strong likelihood of accelerated progression in hearing performance in CI patients receiving this neurotrophin gene augmentation treatment.

Funding: NHMRC, Passe and Williams, Cochlear Ltd

Assessment of an Adeno Associated Vector-Based Gene Therapy (GJB2-GT) for the Non-Syndromic Deafness 1 (DFNB1) in Cynomolgus Monkeys

Guillaume Olivier[1], Lise Barrot[1], Christophe Tran van Ba[1], Rafik Boudra[1], Julie Duron Dos Reis[1], Pierre Rambeau[1], Charlene Vaux[1], Sandra Pierredon[1], Anais Pages[1], Pauline Liaudet[1], Audrey Broussy[1], Anne-Gabrielle Harrus[1], Selma Dadak[1], Jérôme Nevoux[2], Arnaud Giese[1], Laurent Desire*[1]

[1]*Sensorion,* [2]*Genetics and Physiology of Hearing Laboratory, Institut de l'Audition/Pasteur*

Category: Gene Therapy

Background: In the world, the estimated prevalence of severe or profound deafness in human is 1/1000 neonates, and genetic factors account for half of the cases. Pathogenic variants of GJB2, the gene encoding for Connexin 26, 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. Here, we identified a capsid that transduces a vast majority of Gjb2-expressing cells of the inner ear, as well as regulatory sequences that avoid expression of GJB2 in inner hair cells, when injected in non-human primates (NHP).
Methods: Round window membrane injections of two AAV prototypes bearing either GFP or a flagged hGJB2 transgene were performed in the inner ear of NHP. Hearing function assessment using audiometric recordings (ABRs and DPOAEs) were analyzed. Analysis of the tropism in the inner ear was performed by immunohistochemistry.
Results: Three weeks post-surgery, ABR measurements and DPOAE amplitudes remained contained within the normal hearing threshold range of NHPs indicating that both vectors were well tolerated. Whole mounts and cryosections of injected inner ears were analyzed to assess the AAV tropism. For both products, a vast majority of SCs that naturally express GJB2, including the great epithelial ridge cells, the lateral epithelial ridge cells, border cells, phalangeal cells and pillar cells, were transduced along the tonopic axis. Cells of the stria vascularis, fibrocytes of the lateral wall and spiral limbus, the spiral ganglion neuron were often transduced. No transduction was found in inner hair cells.
Conclusions: The selected AAV vector components for our GJB2-GT program allow to efficiently and safely target the cells that naturally express GJB2 in the cochlea with both tropism and levels compatible with therapeutic intervention in human. This constitutes a major step toward our future clinical trials to restore physiological hearing in DFNB1 patients.

Initial Characterization of a Naturally-Arising Otoferlin Frameshift Mutation in the Rhesus Macaque

John Brigande*[1], Samuel Peterson[2], Santhosh Verghese[3], Junghyun Ryu[2], Edward Porsov[3], Beth Kempton[3], Fernanda Burch[2], Emily Mishler[2], Nadine Piekarski[2], Carol Hanna[2], Jon Hennebold[2], Betsy Ferguson[2], Meghan Drummond[4]

[1]*Oregon Hearing Research Center,* [2]*Oregon National Primate Research Center, Oregon Health and Science University,* [3]*Oregon Hearing Research Center, Oregon Health and Science University,* [4]*Regeneron Pharmaceuticals, Inc.*

Category: Gene Therapy
Background: The anatomy, physiology, and development of the rhesus macaque inner ear closely models the human inner ear. Notably, hearing onset occurs during the second trimester of pregnancy as it does in humans, and mature auditory function is present at birth. Critically, rhesus macaque neonates have sensitive hearing across the frequency range essential for human oral communication. Our long-term goal is to define genetic mutations in the rhesus macaque that recapitulate human auditory and vestibular disease phenotypes and then evaluate gene therapies to restore hearing and balance. We anticipate that therapeutics efficacious in rhesus macaque models of inner ear disease will persistently restore a clinically-relevant degree of sensory function in human patients. Otoferlin (OTOF) is a multivalent scaffolding protein with C2 domains and a single transmembrane domain that inserts into synaptic vesicles. Calcium-mediated conformational changes in OTOF facilitate the interaction of the synaptic vesicle with the presynaptic membrane fusion complex, which supports indefatigable neurotransmitter release from inner hair cells. Hearing loss in patients with recessive mutations in OTOF (DFNB9) is detected by elevated pure-tone audiometry thresholds with outer hair cell-mediated distortion product otoacoustic emissions (DPOAE) unaffected.
Methods: The Macaque Genotype and Phenotype Resource (mGAP) is an open access bioinformatics database that facilitates the identification of novel disease-associated variants using data sets from the National Primate Research Centers and other NHP Colonies (https://mgap.ohsu.edu/). We used mGAP to identify a four base pair insertion in exon 44 of OTOF that encodes the C2F domain. The insertion creates a frameshift that truncates the protein before the OTOF transmembrane domain. We created a similar exon 44 frameshift mutation in the mouse using CRISPR/Cas9.
Results: Postnatal day 28 (P28) mice homozygous for the frameshift mutation have no auditory brainstem responses (ABR) from 8-32 kHz but retain sensitive DPOAE responses. P28 heterozygotes have wildtype ABR and DPOAE responses. The data suggest that the absence of the OTOF transmembrane domain perturbs wild type function. A male and a female rhesus macaque heterozygous for the OTOF frameshift mutation

were obtained from the Tulane National Primate Research Center. Blastocyst stage embryos were generated following in vitro fertilization and embryo culture. Embryos were genotyped by sequencing DNA obtained from a laser-assisted trophectoderm biopsy. Five homozygous mutant blastocysts were individually transferred to surrogate dams resulting in two pregnancies. OTOF mutants were born on the 21st and 25th of September, 2023 and both neonates appear grossly healthy. An Initial Behavioral Assessment Score that includes acoustic startle reflex testing will occur at P14 and otoscopy, tympanometry, ABR, and DPOAE are scheduled for P36.
Conclusions: Validation of the deleteriousness of this OTOF frameshift mutation may enable rigorous evaluation of gene replacement strategies in the first nonhuman primate model of DFNB9. [Funding: Regeneron Pharmaceuticals, Inc.; NIH P51OD011092; NIDCD 5R21DC018126]

Cerebrospinal Fluid Delivered Cochlear AAV Gene Therapy Enabled by Hair-Cell-Specific Payload

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Category: Gene Therapy
Background: The leading approaches to cochlear gene therapy rely on intracochlear infusion of AAV, an inner ear surgical approach. In humans, this approach necessitates sedation, perforation of the sealed inner ear space, and a patient of sufficient age to tolerate these interventions. Our group recently reported that AAV administered into the cerebrospinal fluid (CSF) yielded robust cochlear inner hair cell transduction in non-human primates. This observation, and recent papers evaluating the patency of both the murine and human cochlear aqueduct suggest the feasibility of "CSF-mediated" AAV delivery as an alternative method for gene delivery to the inner ear. Here, we report on the development of the CSF-mediated gene delivery approach in NHPs using novel AAV capsids, and on creation of a splicing-based cell-type-specific regulatory element which restricts payload expression to auditory hair cells.
Methods: In this work we utilized both intracerebroventricular (ICV) or round window membrane + canalostomy (RWM) infusion of WT and modified AAVs into non-human primates. We utilized whole mount microdissection and fluorescence imaging to quantify capsid distribution throughout all cochlear turns. Using single-cell RNA-Seq data generated from murine auditory hair cells, we selected a hair-cell-specific splice event with 100% sequence conservation between mouse and human and assess its utility to confer hair-cell-specific expression.
We utilized ICV administration of this engineered payload to mouse CSF and assessed payload specificity for hair-cells by fluorescence imaging of brain slabs and cochlear whole mounts.

Results: CSF-mediated delivery of modified AAV variants yield nearly complete IHC transduction across all cochlear turns in the non-human primate cochlea. CSF-mediated delivery yields greater IHC transduction compared to direct-intra cochlear infusion, particularly in the cochlear base. Our splicing-based regulatory element confers auditory hair cell specific gene expression after ICV delivery, thus minimizing gene expression throughout the CNS.
Conclusions: In summary we have developed alternative methods for gene delivery to the inner ear, as well as the tools to limit gene expression to a target cell-type of interest, both steps towards improving gene therapies for hearing loss.

The Effects of Epigenetic Modifications on the Efficacy of Inner Ear Gene Therapy

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Category: Gene Therapy

Background: The Whrnwi/wi mouse does not express whirlin and exhibits abnormally short stereocilia bundles on the cochlear and vestibular hair cells, leading to profound hearing loss and vestibular dysfunction. In a previous study, we showed that neonatal treatment (P0-P5) with AAV8-whirlin (long isoform) was effective at lengthening the stereocilia bundles and partially restoring hearing and vestibular function. However, when AAV8-whirlin was delivered to mature Whrnwi/wi ears (P30), the bundles remained abnormally short, despite the restoration of whirlin expression at the stereocilia tips. One possible explanation for this phenomenon is the difference in epigenetics between the neonatal and adult mouse inner ears. In this study, we explore whether epigenetic modification using the histone deacetylase (HDAC) inhibitor SAHA can help to make the adult Whrnwi/wi mouse cochlea more amenable to the effects of inner ear gene therapy.
Methods: For in vitro testing, utricle cultures from 1–2 month old Whrnwi/wi mice were harvested and treated with three different conditions: culture media alone, culture media + AAV8-whirlin (long isoform, 6 × 1013 GC), and culture media + AAV8-whirlin + SAHA (5μM). Viral transduction and stereocilia elongation were assessed using confocal microcopy.
For in vivo testing, adult Whrnwi/wi mice (1 month old) were injected bilaterally with either AAV8-whirlin alone, SAHA (5μM) alone, or AAV8-whirlin + SAHA (5μM) through the posterior semicircular canal (PSC). One month after the treatment, auditory brain stem responses (ABRs) and vestibular evoked potentials (VsEPs) were performed to assess auditory and vestibular function. Confocal microscopy was used to examine cellular morphology.

Results: Using these three different culture treatment conditions, we observed the most prominent lengthening of Whrnwi/wi utricular hair cell stereocilia when AAV8-whirlin was used together with SAHA. Lengthening of cochlear and vestibular stereocilia bundles was also observed when AAV8-whirlin + SAHA was administered into the adult Whrnwi/wi mice through the PSC in vivo. The length of the stereocilia bundles expressing whirlin from the AAV8-whirlin + SAHA group was longer than the stereocilia expressing whirlin from just AAV8-whirlin alone. Morphologically, hair cell stereocilia architecture is partially restored, and there is a reduction in the supernumerary rows of stereocilia in the transduced hair cells in Whrnwi/wi mice treated with AAV8-whirlin + SAHA. No improvement in the auditory function was observed, yet some Whrnwi/wi mice treated with AAV8-whirlin + SAHA exhibited partially restored vestibular function when compared to groups treated with AAV8-whirlin alone, SAHA alone, and untreated Whrnwi/wi mice.
Conclusions: AAV8-whirlin gene therapy in combination with SAHA resulted in a more effective restoration of stereocilia length in adult Whrnwi/wi hair cells in vitro and in vivo along with partial vestibular functional recovery. Our data suggest that epigenetic modifications may be effective at alleviating structural abnormalities of stereocilia in adult mouse inner ears after the application of gene therapy.

PIK3CD Overexpression Boosts Neurite Outgrowth and Excitability in Spiral Ganglion Neurons

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Category: Gene Therapy

Background: Spiral Ganglion Neuron (SGN) regeneration is critical for hearing restoration, particularly for cochlear Implant (CI) users who rely on intact SGNs for auditory signal transmission. The overexpression of phosphoinositide 3-kinase delta (PIK3CD), encoding phosphatidylinositol 3-kinase (PI3K), has demonstrated its regenerative potential by inducing axonal regeneration in dorsal root ganglion neurons and retinal ganglion cells. However, its influence on SGNs remains unexplored. This research aims to uncover the regenerative potential of PIK3CD in SGNs and the molecular mechanisms underlying these effects. Exploring its influence

on SGN survival, neurite outgrowth, and excitability holds great promise for auditory nerve regeneration and hearing loss treatment, particularly benefiting CI users reliant on SGN viability and excitability.
Methods: Primary SGN cultures were prepared from postnatal rats. Lentiviral transduction enabled PIK3CD overexpression, while a control group received only a GFP-expressing vector. Transduced SGNs were cultured without growth factors, enabling a focused assessment of PIK3CD's influence on cell survival and neurite outgrowth. A positive control group without transduction was maintained in a culture medium with GFs.
Survival rates, soma size, and neurite outgrowth of SGNs were assessed at 3 and 7 days in vitro, under all experimental conditions. We analyzed the downstream elements within the PI3K/AKT/mTOR pathway to elucidate the underlying mechanisms.

To assess the functional preservation of PIK3CD-overexpressing SGNs, we performed patch-clamp assays, enabling a comparison of electrophysiological features across different experimental groups, including spike count, interspike interval, and latency.

Results: We observed a correlation between PIK3CD expression and survival as well as neurite length as early as 3 days in vitro. Even in the absence of exogenous GFs, a clear link emerged. Neurite length in PIK3CD-overexpressing SGNs was comparable to that of SGNs exposed to GFs. Moreover, our mechanistic investigations revealed that the enhanced neurite outgrowth and survival were mediated through the PI3K/Akt/mTOR pathway. Furthermore, our patch-clamp electrophysiology examinations illustrated preserved functionality in the PIK3CD-overexpressing SGNs. They exhibited the highest spike count and shorter latencies across a range of peak amplitudes.
Conclusions: In summary, our study illustrates the promising potential of PIK3CD activation in enhancing the survival and neurite outgrowth of SGNs. These findings hold particular significance, as they suggest that PIK3CD overexpression can compensate for the absence of essential neurotrophic factors, thereby offering a new avenue for the treatment of auditory neuropathy and hearing loss. Moreover, our patch-clamp electrophysiology assessments demonstrated preserved functionality and enhanced excitability of PIK3CD-overexpressing SGNs, likely through ion channel and calcium signalling modulation. Additionally, our exploration into the underlying mechanisms highlights the critical role of the PI3K/AKT/mTOR pathway in mediating these regenerative effects. This study lays the foundation for future therapeutic interventions aimed at auditory nerve regeneration, holding promise for improving treatments for hearing-related disorders and ultimately leading to improved outcomes for CI users.

Virally-Mediated Enhancement of MOC Feedback Reduces Noise-Induced Damage in Wild Type Cochleas

Eleftheria Slika*[1]

[1]*Johns Hopkins University*

Category: Gene Therapy
Background: The medial olivocochlear system provides efferent feedback to the organ of Corti. Efferent neurons from the medial superior olive synapse with the base of the outer hair cells and release acetylcholine to hyperpolarize and shunt the cell to reduce sound-evoked activation. This acts as a protective mechanism against noise- induced hearing loss (NIHL). The nicotinic (α9)2(α10)3 receptor on the OHC membrane mediates the efferent effect. Transgenic knock-in mice with a gain of function nAChR (α9L9'T) suffer less NIHL. α9KO mice lack efferent inhibition and are more vulnerable to NIHL but can be 'rescued' by viral transduction of the α9L9'T subunit.
In the present work we attempt viral transduction of the α9L9'T subunit of the nAChR in wild type C57Bl/J6 mice to enhance the medial olivocochlear reflex and protect them from NIHL.

Methods: The α9L9'T DNA sequence was incorporated into a modified AAV (AAV2.7m8), shown to provide efficient transduction of cochlear hair cells. 700-1000 nl of the viral vector were injected in the left posterior semicircular canal of P2-P5 C57BL/J6 mouse pups. Uninjected littermates served as a control group. 5 week old mice were exposed to 100 dB octave band noise, 8-16 kHz, for 1 hour. ABRs were recorded before (baseline) and after (1, 7 and 14 days) noise exposure. ABR thresholds were measured for clicks and pure tones at 8, 12, 16, 24, 32, 40 and 46 kHz.

To visualize the virally-encoded nAChR, an HA peptide was attached to the α9L9'T sequence. Postsynaptic expression of the transgene was imaged in fixed cochlear tissue by immunofluorescent labeling of anti-HA antibodies.

Results: There were 2 experimental groups: the α9L9'T AAV injected (n=8) and the α9L9'T-HA AAV injected (n=15). A group of uninjected littermates (n=10) served as control. Both of the injected groups showed a significantly smaller threshold shift from baseline at 1, 7 and 14 days postexposure compared to the uninjected group. The absolute ABR thresholds for the α9L9'T injected group were significantly lower than in the control group at 7 and 14 days post-exposure. The absolute threshold values for the α9L9'T-HA injected group also were significantly lower than in controls at 1, 7 and 14 days post-exposure. At 14 days, click wave1 amplitudes were significantly higher for the α9L9'T-HA injected group compared to controls.
HA- α9L9'T immunolabel occurred in dense plaques postsynaptic to efferent terminal contacts on outer hair cells throughout the cochlea.

Conclusions: Viral transduction of the α9L9'T subunit in wild type animals results in expression of the gain-of-function nAChR on the postsynaptic membrane of the outer hair cells, as visualized through HA labelling. The transfected cochleas show partial protection against acoustic trauma compared with uninjected controls. Virally-mediated enhancement of MOC feedback reduces noise-induced damage in wild type cochleas.

Cellular and Circuit Mechanisms of Auditory Cortex Recovery After TOMT Gene Therapy for Hearing Loss

Laura Marinos*[1], Alicia M. Frank[2], Thanos Tzounopoulos[1], Christopher L. Cunningham[1]

[1]*Pittsburgh Hearing Research Center, University of Pittsburgh, Center for Neuroscience at the University of Pittsburgh,* [2]*Pittsburgh Hearing Research Center, University of Pittsburgh*

Category: Gene Therapy
Background: Hearing loss is the most prevalent sensory deficit in the world, affecting over 400 million people. Genetic mutations account for 50-80% of all cases of congenital hearing loss. Although a significant health problem, treatment for hearing loss is limited to devices, such as cochlear implants and hearing aids. Recent preclinical animal studies have provided evidence for the potential utility of AAV-mediated gene therapy for those impacted by genetic hearing loss. The vast majority of these studies focus solely on functional restoration of the cochlea and measure restored responses with peripheral hearing assays (e.g. ABRs and DPOAEs). For proper auditory function and perception, central processing is necessary. Although restoration of peripheral responses in models of genetic hearing loss with gene therapy is evident, it is unclear whether peripheral restoration has the capacity to restore central auditory processing. Specifically, it is unclear the impact that functional peripheral restoration has on auditory cortical responses and the capacity for restoring perception. Thus, the precise auditory cortical cellular and circuit dynamics after peripheral gene therapy are largely unknown. For our deaf mutant we will utilize the TOMT knockout since mutations in the gene LRTOMT (humans) or its homolog TOMT (mice) cause profound nonsyndromic deafness and expression of TOMT is restricted to hair cells in the cochlea.
Methods: To further understand the impacts of peripheral gene replacement within auditory cortex, we employ in vivo calcium imaging in awake, unanesthetized mice to assess sound-evoked and spontaneous neuronal responses of principal excitatory neurons (PN) and interneuron subtypes, including somatostatin- (SOM), parvalbumin- (PV), and vasopressin-expressing interneurons (VIP) in hearing, deaf TOMT knockout mice, and TOMT knockout mice treated with gene therapies that exhibit rescued peripheral hearing. We will rescue TOMT using local inner ear AAV-mediated gene delivery of wildtype TOMT to TOMT knockout mice via the posterior semicircular canal and compare ABR responses to cortical responses.
Results: TOMT mutant mice lack all peripheral responses to sound, and within auditory cortex, we find that TOMT mutant mice lack all sound-evoked responses and have elevated amplitudes of spontaneous events. Furthermore, we demonstrate substantial rescue of peripheral responses and significantly lowered ABR thresholds in TOMT mutant mice, especially in the lower frequencies. Interestingly, when observing cortical widefield responses, PNs are highly responsive across a range of frequencies. Additionally, normal patterns of spontaneous activity are rescued after treatment.

Conclusions: These findings suggest that auditory cortex exhibits a compensatory mechanism to enhance auditory perception following peripheral recovery. Future experiments will continue to investigate widefield sound-evoked and spontaneous responses across cell-types, single-cell tuning characteristics and spontaneous activity across cell-types, and the extent of perceptual threshold response recovery following treatment.

This work was supported with funding from NIH-5RO1- DC019618 and NIH-DC019195.

Podium Session 14 - Auditory Cortex: Human and Animal Studies

2:15 p.m. - 4:15 p.m.

Grand Ballroom Salon E

How Does the Brain of Sleeping Infants Respond to Auditory Stimuli? - The Influence of Stimulus-Induced Brain Arousal

Colette McKay*[1], Onn Wah Lee[1], Demi Gao[1], Tommy Peng[1], Julia Wunderlich[1], Darren Mao[1], Gautam Balasubramanian[1], Linty McDonald[1]

[1]*The Bionics Institute of Australia*

Category: Auditory Cortex and Thalamus: Human Studies
Background: Objective functional hearing assessment is a crucial necessity for infants who are born with a hearing loss, as early intervention is critical for oral language development. In the early months following birth, auditory brainstem responses (ABR) usually provide estimates of hearing thresholds needed for hearing aid fitting. However, ABR is absent or unreliable in infants who have auditory neuropathy (AN). Cortical evoked responses require the infant to be awake, quiet and still, and are unreliable in infants with AN. Functional near-infrared spectroscopy (fNIRS) is a technology that can provide alternative solutions for objective testing in infants. It measures cortical haemodynamic responses that are easily obtained in the sleep state, and are not degraded by the neural dys-synchrony associated with AN. The haemodynamic response morphology in infants has been shown to be variable across different studies, and the variability has been suggested to be due to factors such as early developmental factors, and different stimuli and test protocols.
Methods: fNIRS responses from sleeping infants with various stimulation protocols were analysed.
Results: In this presentation, we show that the infant response is a sum of two independent mechanisms: an auditory response (increase in oxygenated haemoglobin after stimulus onset in auditory areas of the brain) and a brain arousal response. Brain arousal response (defined as a sudden increase in alpha brain activity) is followed by a dip in oxygenated haemoglobin that reaches its minimum around 12 s after stimulus onset and takes up to 24 s to return to baseline levels. Since this response is long-lasting, is simultaneously activated with the auditory response, and rapidly habituates with repeated stimulus block presentations, its effect on fNIRS waveform morphology depends on factors such as duration of the experiment, epoch length and the nature of auditory stimuli used.
Conclusions: The influence of the brain arousal response means that standard statistical techniques used in fNIRS experiments, such as general linear modelling or epoch averaging, are not appropriate for these measurements, since they assume that the response is a certain shape and/or is stationary throughout the experiment. In our lab, we have developed alternative signal processing techniques to overcome these analysis problems, so that fNIRS measurements of hearing function in sleeping infants can be reliably and accurately assessed.

Implicit Learning of Statistical Structure of Music in Ferret Auditory Cortex

Rupesh Chillale*[1], Guilhem Maron[2], Claire Pleofi[3], Shihab Shamma[1]

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Category: Auditory Cortex and Thalamus: Structure and Function
Background: Predictive coding theories posit that predictions are a fundamental aspect of cognition (Clark, 2013). For example, it is established that humans generate predictions as they listen to music and speech, even when portions (e.g., musical notes) are omitted (Di Liberto, 2021). It is also thought that the strength of these predictive signals reflects the statistical structure of the music, and it interacts with the incoming sound to modulate the expression of music in the auditory brain (Di Liberto, 2019). Behavioral studies in humans suggest that the statistics those predictions are based on could be learned through passive implicit learning (Loui, 2010). In this study we investigate the neural underpinnings and to what extent auditory cortex is involved in the learning processes of musical predictions.
Methods: We exposed two ferrets to melodies from Bach chorales for 30 days and recorded neural activity (ECoG) from auditory cortex before and after the music exposure. We also used one ferret as a control (not exposed to music). ECoG activity was contrasted between before and after exposure and compared to that of control ferret. To demonstrate the relevance of the statistical learning of the musical material, we used the IDyOM model (Pearce, 2005), a statistical model of music, and the mTRF model (Cross, 2016), a ridge regression model, to predict the ECoG responses using the computed likelihood of each musical note.
Results: We observed that after 30 days of exposure, the ERPs evoked by the musical notes in Bach exhibit progressively smaller amplitudes in post-exposure sessions. The same is not observed in a control ferret. We measured and contrasted the response changes over the time-duration of the note-ERPs between the two ferrets. The test ferret exhibited a clear negativity at about 150ms relative to the note onset that is absent in the control ferret. Also, the music exposure indues an increase in the correlation between IDyOM model predictions and the ECoG activity only in the test ferrets.
Conclusions: We conclude that ferret auditory cortex can implicitly learn (by exposure) the complex structure of music much as has been behaviorally observed in humans.

Neural Tracking of Different Cognitive Tasks in Human EEG

Moira-Phoebe Huet*[1], Mounya Elhilali[1]

[1]*Johns Hopkins University*

Category: Auditory Cortex and Thalamus: Human Studies
Background: Auditory processing in humans is highly influenced by the task at hand, showcasing a range of objective and attentional demands that affect how we perceive and interact with sound stimuli. Whether it's attempting to comprehend speech in a noisy environment or merely identifying the presence of speech amidst noise, the brain employs distinct cognitive mechanisms. Exploring these task-driven auditory processing variations sheds light on the intricate workings of the auditory system and holds promise for enhancing auditory-related technologies.
Methods: In two parallel tasks, human participants were presented with the same stimuli composed of speech, noise, and other sound categories with two different goals. In one task, participants were instructed to focus solely on the speech, while in the other task, they were required to scan the auditory scene to identify all present sound categories. EEG recordings were obtained throughout the tasks. An attentional decoding method was employed to reconstruct the original stimuli from the neural data. This method allowed for the examination of how task objectives affect neural tracking and auditory attention allocation across different sound categories and tasks.
Results: The results reveal that despite similar behavioral outcomes in both tasks, neural tracking exhibited distinct patterns, with stronger tracking of speech in the comprehension task compared to the detection task.
Conclusions: The findings indicate that different cognitive tasks manifest as differences in the neural tracking.

Neural Correlates of Learning the Social Meaning of Sounds in an Innate Behavior

Kai Lu[1], Kelvin Wong[1], Lin Zhou[1], Yike Shi[1], Chengcheng Yang[1], Robert Liu*[1]

[1]*Emory University*

Category: Auditory Cortex and Thalamus: Structure and Function

Background: Animals can learn new social meanings of sounds to modulate innate behaviors so that they can better adapt to the environment. For example, female mice can be naturally motivated to search and retrieve pups, based on a largely hard-wired motor program that benefits offspring survival. With experience, naïve mice can also learn to respond to infant ultrasonic vocalizations (USVs) and use USVs to search for pups. While plasticity within auditory cortex has been found to reflect this stimulus recognition, how the auditory cortex and brain areas involved in searching change during experience to incorporate new information is unclear.
Methods: Here we investigate this with naïve virgin mice 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.
Results: Initially, mice instinctively searched for pups where they last found them --a spatial-memory strategy reminiscent of a "win-stay/lose-switch" strategy. However, all animals learned to use the sound to locate pups within 3 to 7 days (N = 12, p less than 0.05, greater than 70% correct). Chemogenetic silencing AC (N = 15) significantly impaired the learning performance (p less than 0.01), compared to saline (N = 15) and CNO (N = 8) controls. We recorded single-unit/multi-unit spiking in auditory cortices (AC, N = 1279) and medial prefrontal cortices (mPFC, N = 966) during learning. ACx firing at the nest increased significantly with learning and correlated with subsequent search speed but not outcome (p less than 0.001). Surprisingly, ACx suppression rather than facilitation during search was more prognostic of correct sound-cued outcomes – even on the first day of training, before adopting a sound-cued strategy (p less than 0.001). Meanwhile medial prefrontal cortex, which is required for pup retrieval, encoded the last pup location, but this decayed as the spatial-memory strategy declined (p less than 0.001).
Conclusions: Our results suggest a neural competition between a weakening spatial-memory and strengthening sound-cued neural representation to mediate a strategy switch towards using sounds to guide the search for pups.

Different Modes of Attention in Dynamic Multi-Speaker Auditory Scenes

Stephanie Graceffo*[1], David Little[1], Emine Merve Kaya[1], Mounya Elhilali[1]

[1]*Johns Hopkins University*

Category: Auditory Cortex and Thalamus: Human Studies
Background: Auditory attention is a cognitive process that enables us to attend to sounds of interest in dynamic complex auditory environments. An illustration of this ability is the cocktail party effect, where our auditory system can hone in on one single speaker while filtering out the chatter from other speakers. Recent research has shown that it is possible to decode the attended speaker in a multi-speaker environment from brain activity recorded via electroencephalography (EEG) sensors. These paradigms typically explore neural correlates of attending to a specific speaker in the presence of a second competing voice. In the current study, we explore processes underlying different forms of attention in complex auditory settings. We specifically manipulate the attention of the subjects to the global scene (free-listening), a particular feature (acoustic space), or an object (one speaker), to contrast the effect of directed attention on the perception of the same scene.
Methods: Using stimuli consisting of three speakers with continuously varying spatial locations, we explore the listeners' ability to detect targets embedded in the stimuli while performing a variety of listening tasks. Listeners were presented with the same set of stimuli three times, each time varying their attentional focus: the first time they were asked to listen globally, the next two times they were asked to listen selectively, once to a specific speaker and once to a specific spatial location.
Results: Both behavioral and neural measures reveal strong evidence that perception of the same acoustic scene differs significantly according to the type of selective attention being deployed. Target detection accuracy is greater in both selective listening tasks than in the global listening task.
Interestingly, the results reveal that the salience of targets had a greater impact on performance when listeners were required to attend to the entire auditory scene, rather than more selectively. These results reveal an advantage of directed attention that is most evident for low-salience targets.

Conclusions: Overall, the study shows that the perception of the same acoustic scene differs according to the listening task being performed and that top-down and bottom-up processes interact to drive perception. These

results are consistent with the hypothesis that selective attention is partly comprised of a rapid learning process that adjusts the components of perception to the task and the dynamics of the current environment.

Modeling Speech-To-Language Transformations in the Human Brain

Edmund Lalor*[1], Jin Dou[1], Ole Bialas[1], Andrew Anderson[2]

[1]*University of Rochester,* [2]*Medical College of Wisconsin*

Category: Auditory Cortex and Thalamus: Human Studies

Background: How the human brain converts the complex acoustic patterns of natural speech into meaning remains unclear. Progress on this issue has been made in recent years by modeling electrophysiological responses to continuous, narrative speech in terms of various acoustic and linguistic features of that speech. In this presentation, we will describe three projects aimed at deepening the insights we can derive from this type of modeling. The first aims to address a debate over whether or not incorporating categorical phonetic feature representations into one's model allows one to explain additional variance in EEG responses to speech. The second aims to explore how prior context influences the speech-to-language transformations indexed by EEG. And the third aims to improve both model accuracy and interpretability by relaxing the typical linear assumptions used when implementing these kinds of speech-EEG models.

Methods: We used temporal response functions (TRFs) to model EEG responses from 19 participants who listened to a single-talker audiobook and from 33 additional participants who attended to one of two concurrently presented audiobooks. The first project sought to index how each phoneme gets weighted in the TRF model as a function of how acoustically variable that phoneme is across utterances – with the rationale that phoneme labels should add more explanatory value for phonemes that are more acoustically variable. The second project sought to model EEG responses to speech based on the activations in different layers of a state-of-the-art speech-to-language deep neural network known as Whisper – with the goal of assessing whether or not EEG reflects context-based speech-to-language transformations. And the third project introduced a non-linear dynamically warped temporal response function aimed at modeling EEG responses to words – with the goal of capturing nonlinear variations in those responses in terms of their amplitude, latency, and temporal duration.

Results: The first project revealed that phonemes that are spoken more variably are assigned larger weights in a TRF model of EEG responses to speech, suggesting that they index invariant categorical recognition of those phonemes. The second project found that the Whisper deep neural network provides an accurate, self-contained EEG model of speech-to-language transformation, and that the model is improved when including prior speech context. And the third project found that expected words are processed faster, that these effects depend on both the current word and the previous word, and that words that are quickly disambiguated from their phonetic neighbors also elicit earlier responses.

Conclusions: We conclude that: 1) we have found evidence for the invariant processing of phonemes in EEG responses to naturalistic speech; 2) context and attention shape EEG correlates of speech-to-language transformation; and 3) non-linear modeling of EEG responses to natural speech reveals faster processing of predictable and phonetically distinct words.

Intracranial Electrophysiology of Spectrally Degraded Speech in the Human Cortex

Kirill Nourski*[1], Mitchell Steinschneider[2], Ariane Rhone[1], Joel Berger[1], Emily Dappen[1], Hiroto Kawasaki[1], Matthew Howard[1]

[1]*The University of Iowa,* [2]*Albert Einstein College of Medicine, The University of Iowa*

Category: Auditory Cortex and Thalamus: Human Studies

Background: Stimulation of the auditory nerve with a cochlear implant (CI) is the method of choice for treatment of severe to profound sensorineural hearing loss. There remains considerable variability in speech perception outcomes despite advances in CI technology. Cortical function is a major contributing factor to this variability (Glennon et al., Curr Opin Neurobiol. 2020;60:108-14). Spectrally degraded stimuli presented to normal-hearing individuals approximate sensory input to the central auditory system in CI users (Shannon et al., Science. 1995;270:303-4). This study leveraged the high spatiotemporal resolution afforded by

intracranial electroencephalography (iEEG) to investigate the processing of spectrally degraded speech throughout the auditory cortical hierarchy and determine the relationship of cortical activity to task performance with CI-simulated speech.
Methods: Participants were adult neurosurgical epilepsy patients. Stimuli were utterances /aba/ and /ada/, spectrally degraded using a noise vocoder (1-4 bands) or presented without vocoding (clear). Clear and vocoded stimuli were presented in a one-interval discrimination task. Cortical activity was recorded using depth and subdural iEEG electrodes (greater than 2000 contacts). Comprehensive electrode coverage included auditory core in posteromedial Heschl's gyrus (HGPM), superior temporal gyrus (STG), ventral and dorsal auditory-related, prefrontal, and sensorimotor cortex. Cortical activity was examined in broadband gamma (30-150 Hz) and alpha (8-14 Hz) bands.
Results: Chance task performance occurred with stimuli degraded to 1-2 spectral bands and was near-ceiling in the clear condition. Performance was variable in the 3-4 band conditions, permitting segregation of participants into good, average, and poor performers. There was no significant relationship between task performance and participants' demographic, audiometric, neuropsychological, or clinical profiles. Several patterns of gamma augmentation and alpha suppression were identified based on response strength and differences between stimulus conditions. HGPM was characterized by strong bihemispheric responses to all stimuli. A preference for clear speech emerged within non-core auditory cortex. Good performers were characterized by strong gamma activation to all stimuli, whereas in poor performers, strong responses were generally limited to clear speech. This difference extended from posterior STG into dorsal auditory-related areas (e.g., supramarginal gyrus) and the precentral gyrus. A preference for vocoded stimuli was present at a minority of sites that clustered in the STG and supramarginal gyrus, particularly in good performers. Alpha suppression was generally more uniform across stimulus conditions than gamma augmentation and was more pronounced in good performers.
Conclusions: Examination of responses to noise-vocoded speech provides insights into the neural bases of speech perception outcome variability in CI users. The data emphasize the importance of the dorsal auditory cortical pathway in perception of spectrally degraded speech. Findings identify specific cortical regions that may have diagnostic and prognostic utility and suggest potential targets for neuromodulation-based CI rehabilitation strategies.

EEG as an Indicator for Perceptual Difficulties in Noise?

Jiayue Liu*[1], Josh Stohl[2], Tobias Overath[1]

[1]*Duke University,* [2]*MED-EL*

Category: Auditory Cortex and Thalamus: Human Studies
Background: A recent study in mice suggested that a loss of about 90% of the synapses connecting inner hair cells and auditory nerve fibers causes spontaneous hyper-synchronized neuronal activity ('internal noise') in the auditory cortex. Importantly, this 'internal noise' was only present for subsequently missed tone detection trials in noise (not in silence, or for hit trials), suggesting that it could explain degraded behavioral performance in noisy listening conditions (Resnik and Polley, 2021). The present study investigated whether EEG can capture the 'internal noise' in humans, and whether 'internal noise' correlates are associated with hearing difficulties.
Methods: 30 participants with near-normal hearing performed a monaural tone detection task in either quiet or noise while EEG was recorded. Participants also completed tasks that have been suggested to reveal cochlear synaptopathy (e.g., FM detection, SIN). The analysis aimed to determine whether single-trial EEG could predict behavior (hit vs. miss) and whether such EEG prediction correlated with other indicators of cochlear synaptopathy.
Results: Ongoing EEG analyses suggest that post-stimulus, but not pre-stimulus EEG activity predicts behavioral performance (e.g., hit vs. miss). Above-chance prediction performance of post-stimulus EEG likely reflects the presence of the P300 component for hit trials, but not earlier auditory processing stages. Higher prediction performance was also correlated with lower the Speech, Spatial and Quality of Hearing Scale (Gatehouse and Noble, 2004), but not with any of the other measures, such as the predicted consequences of cochlear synaptopathy (speech perception thresholds in noise, FM detection threshold), risk factors of cochlear synaptopathy (age or lifetime noise exposure) or physiological indicator of cochlear synaptopathy (ABR wave I amplitude).

Conclusions: Our results suggest perceived hearing difficulties in noise might be more related to higher-level processing, and less to measures that are thought to be related to auditory peripheral loss.

In Memoriam Symposium - Emotional Connections to Speech and Music Throughout Life: In Memory of Dr. Sandra Trehub

4:30 p.m. - 6:30 p.m.

Grand Ballroom Salon E

Emotional Connections to Speech and Music Throughout Life: In Memory of Dr. Sandra Trehub

Chair: Karen Gordon*, The Hospital for Sick Children*

Infants' Emotional Responses to Music and Song

Laura Cirelli

University of Toronto Scarborough

Individual Abstract: Infants' everyday soundscapes often include music. Live, recorded, sung or instrumental, music makes up a significant portion of early auditory input. Often, this input is social – parents, siblings, and other family members often sing to infants to soothe them or play with them. Infants respond to these vocalizations with interest and attention. Here, I will describe a series of studies exploring how infant responses to song varies across functional and social contexts. In the first study, we explored how song can be used to effectively mitigate infant distress. We asked parents to use either speech, a familiar song, or an unfamiliar song to cheer up their distressed infant. While song (familiar or unfamiliar) was more effective than speech at capturing infant attention, and reducing negative emotional expression, familiar songs were the most effective and the only condition that generated infant smiling. In the second study, parents sang *Twinkle, Twinkle, Little Star* to their infants, alternating between singing it in a playful or soothing style. Playful renditions were consistently faster, louder, and in a higher musical key than lullabies. These playsongs were better at capturing parent-directed infant attention while lullabies effectively down-regulated both parent and infant arousal. Study 3 replicates and extends this laboratory work by demonstrating that, even in a large audience context, playsongs captured infant attention and elicited more smiles than lullabies. Notably, infant attention in this audience context was more coordinated with their own parent's attention than a random parent in the same audience. Together, this research highlights the diversity of infant responses to song, depending on song features and social context. This may set the stage for the multifaceted functions of music throughout the lifespan, including emotion regulation, social cuing, and group bonding.

The Experience of Musical Chills Emerges Gradually During Development and Depends on Musicality and Auditory Perceptual Skills

Erin Hannon

University of Nevada Las Vegas

Individual Abstract: The phenomenon of musical chills is a hallmark of pleasurable emotional experience. While various musical features have been associated with chills, such as changes in dynamics or instrumentation, many elicitors of chills depend on the expectations of the listener. Even though musical expectations presumably depend on enculturation to a particular musical system during childhood, relatively little is known about whether or not children experience musical chills. This study investigated the experience of musical chills in a large sample of adults (N=163) and children ages 6-15 years (N=164). Participants (and their parents) completed self-report questionnaires assessing their experience of musical chills and musical

reward, personality, musical training, and they completed a child-friendly version of the Gold Musical Sophistication Index (Gold MSI). We also measured real time reactions by presenting music videos and asking participants to press a button for every momentary reaction such as tingles, shivers, or tears, and they provided arousal and valence ratings after each video. Listeners completed several tests of musical and auditory perceptual abilities, such as emotion recognition, sensitivity to speech prosody, musical tonality, major/minor mode, and beat and meter. Overall, the experience of musical chills was relatively common, with 80% of adults and 35% of children reporting experiencing chills at least sometimes, and 90% of adults and 40% of children indicating at least one emotional reaction to music videos during the study. Nevertheless, the experience of musical chills did not approach adult-like levels even in the oldest age group (13-15 years). Self-reported chills, reactions, and valence ratings were correlated with each other. We also observed correlations between the experience of chills and music training, the Gold MSI, musical reward sensitivity, as well as with auditory perceptual skills. Our findings provide preliminary evidence that the experience of musical chills begins to emerge relatively late in development and may be predicted by musicality and by developing music perception skills.

Music Listening Skills of Deaf Children With Cochlear Implants: Triumphs and Challenges

Tara Vongpaisal

MacEwan University

Individual Abstract: For cochlear implant (CI) users, much has been documented about the challenges of listening to sounds with reduced spectral cues that are particularly important for perceiving musical sounds and talker identity. However, in our program of research we have documented that when stimuli are engaging and listening tasks are optimized, CI children's listening performance resemble that of their hearing peers despite the disparities in their hearing input. For instance, they demonstrate high recognition performance in identifying popular songs, television theme songs, and their mother's voice in everyday task conditions. We discuss the perceptual, motivational, and rehabilitative influences that have enabled CI children to derive meaningful hearing experiences and enjoyment of musical sounds and identify future directions on how we can expand these gains.

Somewhere Between Music and Speech: Communicating Emotions With Cochlear Implants

Monita Chatterjee

Boys Town National Research Hospital

Individual Abstract: Emotions are expressed both by the words we speak (lexical semantic cues to emotion) and by the musical tone and cadence of our voice (emotional prosody), but emotional prosody dominates listeners' perception of the talker's intended emotion. For successful emotional communication, therefore, the talker must use prosody accurately in their speech and the listener must receive the prosodic signal and match it to the correct emotion. For individuals who are deaf or hard of hearing and use cochlear implants, prosodic cues are not as perceptually salient as for normally hearing listeners: therefore, children and adults with cochlear implants show significant deficits in their perception of emotional prosody. Currently, no clinical tools exist to assess or treat emotional prosody perception or production in children with cochlear implants. Compared to normally hearing counterparts, pediatric cochlear implant recipients with no acoustic experience have deficits in both emotion perception and production. In both areas, large intersubject variability is observed. Our results indicate that duration of device experience is a key predictor of emotion perception outcomes, as is nonverbal cognition. These predictors also interact with one another: we find a stronger association of emotion perception with cognitive function in children with less experience with the device. If this is indicative of greater availability of cognitive resources for the task in this population, then it seems reasonable to speculate that intervention programs will be more effective in children who are younger/have fewer years of experience with the device. Results of a cue-weighting study of emotion identification indicate that prelingually deaf children with cochlear implants are less able to utilize prosodic cues to emotion than postlingually deaf adults and normally hearing peers. This suggests that there is scope for training the pediatric cochlear implant population in better utilization of acoustic cues to prosody. Finally, we hypothesize that early experience with auditory input (acoustic or electric) benefits emotional prosody development in individuals

with cochlear implants. In preliminary support of this hypothesis, we find significant links between the prosodic features in emotional productions by children with cochlear implants and their accuracy in perceptually identifying emotions in speech. Taken together, these results underscore the need for clinical tools to address emotional prosody communication in children with cochlear implants, and also shed light on specific ways in which such interventions might be most effective.

Work supported by NIH grant nos. R01 DC014233, R01 DC019943, P20

Considering the Valence and Arousal of Sounds Including Music in Cochlear Implant Users
Erin Picou

Vanderbilt University Medical Center

Individual Abstract: Daily life is full of sounds that can inspire emotional responses; sometimes these sounds are speech sounds (e.g., an angry friend) and sometimes they are non-speech sounds (e.g., music). In response to speech and non-speech sounds, a wide range of emotional responses is important and expected. Moreover, pleasant and unpleasant emotions serve distinct purposes. Emotional responses to unpleasant sounds (e.g., to a crying baby) prepare a body for action. Conversely, emotional responses to pleasant sounds (e.g., peaceful music) broaden attention, enable creative thinking, and facilitate stress recovery. Adults with hearing loss exhibit a reduced range of emotional responses to non-speech sounds; their ratings of valence to pleasant and unpleasant sounds are less extreme than those of their peers with normal hearing. This suggests that adults with hearing loss are not experiencing the benefits of a full range of emotional responses to sounds, which has implications for mental health and well-being. Much less is known about the development of emotional responses to sounds. The purpose of this project was to evaluate emotional responses to sounds in children with normal hearing. School-aged children listened to non-speech sounds (e.g., music, animal noises, body sounds) and rated their emotional responses in terms of valence and arousal. The sounds and rating scales were the same ones used previously in studies with adult participants. The results of this study will be discussed in the context of the development of emotion perception for children with normal hearing. In addition, the implications of cochlear implant use will be discussed. Previous data with adult participants demonstrates that, even with appropriately fit assistive hearing devices (hearing aids, cochlear implants), adults with hearing loss demonstrate the aforementioned reduced range of valence responses. The implications of these findings will be discussed for children with hearing loss and who use cochlear implants. Finally, responses to music and other non-speech sounds will be compared to each other.

Tuesday, February 6, 2024

Symposium 8 - Insights into Regulation of Hair Cell Mechanotransduction Complex Composition and Environment

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

Grand Ballroom Salon E

Insights Into Regulation of Hair Cell Mechanotransduction Complex Composition and Environment

Chair: Shefin George*, Stanford University*

Co-Chair: Christopher L. Cunningham*, Pittsburgh Hearing Research Center, University of Pittsburgh School of Medicine*

Architecture of the C. Elegans TMC-1 Complex
Sarah Clark

Oregon State University

Individual Abstract: Mechanotransduction machinery located at the tips of hair bundles in the inner ear are responsible for our sensations of movement and sound. Deflection of the hair bundle by sound and fluid movement leads to opening of the mechanotransduction (MT) channel complex, resulting in an electrical signal. The MT complex is composed of five known subunits, including transmembrane channel-like proteins 1 and 2 (TMC-1/2), calcium and integrin binding protein CALM-1, and transmembrane inner ear protein TMIE. Studies of the MT complex have been hindered by its extreme scarcity in native tissue; it is estimated there are only ~3 attomoles of MT complex per mouse cochlea, approximately 1000x less than is necessary for detection by western blot. To overcome this limitation, we have developed methods to isolate the native TMC-1 complex from transgenic C. elegans to study its structure and composition. The cryo-electron microscopy structure of the C. elegans TMC-1 complex reveals that the complex is 2-fold symmetric and includes the auxiliary subunits CALM-1 and TMIE, as well as a previously undescribed subunit, arrestin (ARRD-6). Single particle reconstructions and molecular dynamics simulations highlight protein-lipid interactions that provide clues as to how the complex transduces mechanical force.

Investigating Contributions of the Deafness-Associated Protein TOMT to Localization of the Mechanotransduction Channel Subunit TMC1

Christopher L. Cunningham

Pittsburgh Hearing Research Center, University of Pittsburgh School of Medicine

Individual Abstract: Mechanoelectrical transduction (MET) in cochlear hair cells converts mechanical sound waves into electrical signals. These signals are then routed to the brain for auditory processing. MET function is mediated by a heteromeric, mechanically-sensitive ion channel complex. The MET complex comprises distinct combinations of protein subunits which are precisely assembled and localized to the tips of stereocilia. Recent studies have unveiled the molecular identities of many of the individual components of the MET channel complex. Individual mutations in these components disrupt MET channel localization and lead to loss of MET function. However, the processes by which MET complex proteins are assembled and trafficked to the tips of stereocilia are not entirely understood. Transmembrane channel protein 1 (TMC1) forms the major pore-forming subunit of the hair cell MET complex. We recently demonstrated that mutations in Transmembrane-O-methyltransferase (Tomt, also known as LRTOMT/COMT2), cause disruption of TMC1 stereocilia localization, loss of mechanotransduction, and profound deafness. To further investigate the contributions of TOMT in the cochlea, we used a newly generated TOMT-HA knock-in mouse to investigate expression patterns. We show that TOMT is specifically expressed in hair cells, but not in any other cell types in the cochlea. TOMT is distributed in the endoplasmic reticulum of hair cells but is absent from stereocilia. This suggests that TOMT has a role in the protein handling of TMC1, rather than in MET channel function per se. To better understand how TOMT regulates TMC1, we carried out a structure/function analysis of TOMT. Using a series of deletions, we examined biochemical interactions of TOMT with TMC1. We discovered a small region in TOMT that is critical for TMC1 binding. We also investigated the impacts of known TOMT deafness-linked mutations on biochemical interactions with TMC1. For functional analysis, we tested the impacts of Tomt deletions and mutations on AAV-mediated gene delivery rescue of TMC1 localization in Tomt mutant mice. Our results have identified specific domains in TOMT that are essential for biochemical interaction and TMC1 localization to stereocilia. Our work suggests that direct interaction of TOMT with TMC1 in the hair cell endoplasmic reticulum is critical for proper TMC1 localization to stereocilia, mechanotransduction function, and hearing. Critical domains in TOMT mediate these functions. We continue to elucidate the mechanisms by which TOMT and associated proteins contribute to the proper assembly and trafficking of MET channel complex proteins within hair cells.

LOXHD1 is Required for TMC1 Localization at the Lower Tip-Link Insertion Area

Pei Wang

Stanford Medicine

Individual Abstract: LOXHD1 is a large gene (160 kb) associated with age-related and congenital hearing loss in humans, dogs, and mice. It consists of 41 exons encoding 15 repeats of PLATs (Polycystin/Lipoxygenase/Alpha-Toxin), which is known in other proteins to bind proteins and lipids. Our lab previously discovered that the hearing loss caused by mutations in the 10th PLAT repeat results from a hair cell mechanical-electrical transduction (MET) defect onset between postnatal day (P) 7 and P11 (Trouillet et al., 2021). However, the exact mechanism of how LOXHD1 affects MET currents remains unclear, and alternative splicing isoforms may mask additional functions of LOXHD1. In addition, the localization of LOXHD1 in the hair bundle is uncertain. To investigate these questions, we generated Loxhd1Delta, a complete loss-of-function allele, eliminating all Loxhd1 PLAT domain coding sequences. Different from PLAT10 mutants, Loxhd1Delta showed ABR/DPOAE thresholds elevated not only in homozygous but also in heterozygous animals. Additionally, earlier MET defect was observed. These findings suggest that Loxhd1 isoforms can partially compensate for mutations affecting PLAT10. Through immunofluorescence (IF) and SUB-immunogold SEM, a novel technique to detect sub-membranous proteins at nanoscale (Miller et al., in preparation), HA-tagged LOXHD1 (Loxhd1HA) was found to be localized at the lower tip-link insertion area. To investigate if the lower tip-link localization of the known MET channel complex components were affected by the absence of LOXHD1, we tracked TMC1, TMC2, TMIE, LHFPL5, and CIB2 using tagged knock-in alleles (Cunningham et al., 2020) or specific antibodies. By IF, we detected a reduction of TMC1 signal at the tip of second-row stereocilia. SUB-immunogold SEM further demonstrated that TMC1 is mislocalized from lower tip-link area of IHCs in Loxhd1Delta/Delta mouse. Our results indicate the essential role of LOXHD1 in connecting TMC1 to the lower tip-link area, highlighting LOXHD1 as a crucial component of the mature MET machinery.

Myosin XVA Isoforms are Required for the Mechanotransduction-Dependent Remodeling of the Stereocilia Actin Cytoskeleton

A. Catalina Velez-Ortega

University of Kentucky

Individual Abstract: Inner ear hair cells have cellular projections known as stereocilia that are organized in rows of increasing height and harbor mechano-electrical transduction (MET) channels at the tips of shorter row stereocilia. The core of each stereocilium consists of a highly crosslinked paracrystalline array of actin filaments, which we previously reported exhibits activity-dependent remodeling (Velez-Ortega, et al., eLife, 2017). We showed that the blockage of MET channels or the breakage of the tip links that gate these channels lead to the selective shortening of transducing stereocilia (i.e. the stereocilia that harbor MET channels), while the non-transducing tallest row stereocilia remain unaffected. Once the MET blockage is removed or the tip links regenerate, the stereocilia regrow. In addition, we found that this MET-dependent stereocilia remodeling can affect the resting tension within the MET machinery in seconds (Dragich et al., in review). Thus, this process may dynamically regulate the sensitivity of hair cells to sound-induced vibrations and, hence, the sensitivity of our hearing. However, the exact molecular mechanisms of MET-dependent stereocilia remodeling are still unknown. Given that myosin XVA delivers molecular components of the actin core elongation machinery to the tips of stereocilia we explored the role of myosin XVA isoforms in the MET-dependent remodeling of the stereocilia cytoskeleton. Shaker2 mice (Myo15ash2/sh2) have hair cells with abnormally short stereocilia due to a mutation in the myosin XVA motor domain that affects all isoforms, but hair cells still exhibit MET currents. In the presence of MET channel blockers or an increase of intracellular Ca2+ buffering, no changes to the stereocilia morphology are observed in shaker2 auditory inner or outer hair cells. In mice lacking the long isoform of myosin XVA only (Myo15aΔN/ΔN), auditory hair cells exhibit normal bundle morphology and nearly normal MET but exhibit selective degeneration of transducing stereocilia (Fang et al., eLife, 2015). In these mice, MET channel blockage leads to exaggerated remodeling of the stereocilia cytoskeleton, including remodeling in stereocilia from the tallest row, in both inner and outer hair cells. In conclusion, our results suggest that myosin XVA isoforms deliver the molecular machinery that not only enables but also fine-tunes the MET-dependent remodeling of the actin cytoskeleton in mammalian auditory stereocilia.

Supported by R21DC017247 and R01DC021325 to A.C.V.

Stepwise Activation Mechanism of a Calcium-Activated Lipid Scramblase nhTMEM16

Valeria Kalienkova

University of Bergen

Individual Abstract: Lipid composition of the plasma membrane is asymmetric, with some lipid species localizing predominantly to the outer leaflet, and others – to the inner leaflet, respectively. Breakdown of the lipid asymmetry on the plasma membrane leads to extracellular exposure of lipids normally confined to the cytoplasm. This serves as a signal for numerous cellular processes, such as blood coagulation and apoptosis, to name a few. There are several classes of membrane proteins that facilitate rapid translocation of lipids from one leaflet to the other. On one hand, flippases and floppases transport lipids in an ATP-dependent manner against their concentration gradient and maintain the asymmetry. On the other hand, scramblases, randomize the lipid distribution between the two membrane leaflets and disrupt the asymmetry. To date, several unrelated protein families were characterized as lipid scramblases, with calcium-activated TMEM16 scramblases being the most studied. The first structural insight was brought forward by an X-ray structure of a fungal homolog nhTMEM16. The structure revealed a novel fold and interesting features potentially relevant for lipid scrambling. nhTMEM16 possesses a hydrophilic membrane-exposed groove, and it was hypothesized that this facilitates the passage for hydrophilic lipid headgroups across the hydrophobic membrane core. The structure was obtained in presence of calcium, revealing the location of ligand-binding site. In order to understand how nhTMEM16 is activated, we have used cryo-EM and have solved a number of structures of a fungal homolog nhTMEM16 in detergent and lipid environments, with and without its ligand calcium. We have observed that nhTMEM16 conformation is extremely sensitive to the surrounding environment. In detergent the protein assumes the conformation observed in the x-ray structure, irrespective of the presence of calcium. In contrast, when reconstituted into lipid nanodiscs, nhTMEM16 conformation is quite dynamic. We could identify a range of states, starting with the catalytic hydrophilic groove shielded from the membrane in the absence of calcium, and gradually opening to the membrane once calcium is added. We have identified residues that might be important for these conformational transitions and verified their role using mutagenesis and a liposome-based scrambling assay. Our results allowed us to propose a stepwise activation mechanism for nhTMEM16, which could possibly be extended to other less-characterized TMEM16 members. We could also observe the interaction of nhTMEM16 with the surrounding lipids in our nanodisc structures, providing relevant insight into transport mechanism.

Regulation of Membrane Homeostasis by TMC1 Mechanoelectrical Transduction Channels is Essential for Hearing

Angela Ballesteros

NIDCD-NIH

Individual Abstract: The mechanoelectrical transduction (MET) channel complex of auditory hair cells converts sound into electrical signals, allowing us to hear. After decades of research, the transmembrane-like channel 1 and 2 (TMC1 and TMC2) have been recently identified as pore-forming subunits of the MET channels, but the molecular peculiarity that differentiates these two proteins and makes TMC1 essential for hearing remains elusive. Interestingly, while TMC1 or TMC2 are sufficient for MET, expression of TMC2 is insufficient for maintaining normal hearing in mice lacking TMC1, suggesting that TMC1 must have additional functions required for hearing. Moreover, although some TMC1 deafness-causing mutations have been studied extensively, the deafness phenotype of these mutations does not appear to relate to the functional properties of the MET channel. Thus, the molecular mechanisms of TMC1-related deafness remain enigmatic. Inspired by the evolutionary and structural relation between the TMC proteins and the TMEM16 lipid scramblases, we investigated the potential role of TMC proteins in the regulation of hair cell membrane homeostasis using Airyscan super-resolution confocal microscopy and organ of Corti explants from 13 different transgenic mouse lines. mT/mG reporter mice expressing a membrane-targeted tomato fluorescent protein in a wild type or TMC1/TMC2 knockout background were used to visualize and study the hair cell

membrane. Here we show that TMC1, but not TMC2, is essential for a regulatory mechanism activated by a decrease in intracellular calcium that triggers membrane remodeling and lipid and protein mislocalization at the hair cell mechanosensory organelle. We demonstrate that pharmacological inhibition of MET channels, breakage of the tip links, or buffering of intracellular calcium lead to pronounced phosphatidylserine externalization, membrane blebbing and ectosome release at the hair cell sensory organelle, culminating in the loss of TMC1 protein. Importantly, our findings show that TMC1 dominant (M412K and D569N) and recessive (D528N) deafness-causing mutations (DFNA36 and DFNB7/B11) alter phosphatidylserine externalization by different mechanisms, and that the constitutive externalization of phosphatidylserine correlates with the deafness phenotype in TMC1 mice carrying the mutation in heterozygosity or homozygosity. Our work reveals a novel role for TMC1 in regulating hair cell membrane homeostasis that is essential for hearing and has consequences in hair cell development, repair, and death.

TMCs Regulate Membrane Viscosity in Mammalian Cochlear Hair Cells

Shefin George

Stanford University

Individual Abstract: Auditory mechanotransduction occurs in the hair bundle, an organelle composed of rows of linked stereocilia that increase in height in a staircase-like manner. Hair bundle deflection exerts force onto the tip link that is translated to mechanically gated (MET) ion channels located at the tops of the shorter stereocilia. There is a limited but growing body of data suggesting that stereocilia membrane properties modulate MET channels. Stereocilia membrane are more fluidic than the cell body membrane and are selectively sensitive to calcium and voltage. Transmembrane Channel Like proteins (TMCs) are considered part of the MET channel machinery, but also have membrane scramblase activity that regulates membrane homeostasis in hair cells. To further investigate the role of the membrane in modulating MET channel behavior, we used a novel viscosity sensor BODIPY 1c whose changes in fluorescence lifetime allowed precise spatial and dynamic monitoring of membrane properties within live hair cells. BODIPY1c can also enter hair cells through MET channels and fluorescently label the cytoplasmic membranes allowing us to identify hair cells with functional MET channels. We show that the membrane viscosity of stereocilia and soma of mammalian cochlear hair cells vary during development. Membrane viscosity decreases and strongly correlates with the onset of MET. TMIE and TMC1 mutant mice, both of which lack TMC1 in stereocilia have significantly higher membrane viscosity compared to litter mate controls at P10. In TMC1 mutants, the stereocilia membrane viscosity strongly correlates to the level at which the bundles are transducing. Inhibition of MET channel current in P10 rats for 30 mins with 1 mM curare, however, did not alter the membrane viscosity. Together this data suggests that the membrane viscosity of the stereocilia and hair cell soma undergoes developmental changes that correlated strongly with the onset of MET even though MET current is not driving the decrease in membrane viscosity with development. Lack of TMC1 inhibits these developmental changes to the membrane. Further studies are ongoing to determine the functional relevance between MET channel gating and membrane mechanics.

Unveiling the Role of Glycine Residues in Prestin: Insights Into Voltage and Mechanosensitivity in Mammalian Cochlear Amplification

Navid Bavi

University of California, Los Angeles, UCLA School of Medicine

Individual Abstract: Outer hair cells (OHCs) alter their length in response to changes in membrane potential (electromotility). This process is responsible for sound amplification and distortion-product otoacoustic emissions in mammals. OHCs' electromotility is mediated by millions of "motor" proteins called Prestin (SLC26A5) located at their basolateral membrane. Disruption or loss of Prestin function results in disabling hearing loss. Recent 3D structures of Prestin have begun to unravel how this protein detects voltage and converts it into in-plane areal expansion. However, the specific structural elements conferring voltage and mechanosensitivity to Prestin remain elusive. To address this, we introduced mutations in dolphin Prestin that

shift its voltage sensitivity across various membrane potentials (in increments of +100 mV). By employing single-particle cryo-electron microscopy, we determined the high-resolution 3D structures of these mutant variants. Our investigation revealed the significance of conserved glycine residues located on one of the peripheral transmembrane helices, TM6. These glycine residues serve as hinges at the interface between the protein and the lipid bilayer, influencing Prestin's voltage sensitivity and, consequently, its mechanosensitivity. Patch-clamp electrophysiology and molecular dynamics simulations further corroborate these structural findings. The detailed structures of Prestin, coupled with electrophysiology experiments and mutagenesis studies, provide valuable insights into the modulation of lipids and the functional mechanisms of this motor protein in the mammalian cochlea.

Podium Session 15 - Speech Perception: Hearing Loss, Noise, and Other Challenges

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

Platinum Salon 6

Audiometric Markers of Cochlear Synaptopathy and Speech in Noise Deficits in Humans

Lukas Rüttiger*[1], Jakob Schirmer[1], Stephan Wolpert[1], Konrad Dapper[1], Moritz Rühle[1], Jakob Wertz[1], Marjoleen Wouters[2], Jerome Bourien[3], Etienne Gaudrain[4], Deniz Başkent[5], Sarah Verhulst[2], Matthias H. J. Munk[6], Ernst Dalhoff[7], Christoph Braun[8], Marlies Knipper[1]

[1]*Hearing Research Centre Tübingen, Molecular Physiology of Hearing, University of Tübingen,* [2]*Ghent University,* [3]*Institute for Neurosciences Montpellier, University of Montpellier, Institut National de la Santé et de la Recherche Médicale,* [4]*Lyon Neuroscience Research Center, CNRS UMR 5292, INSERM UMRS 1028, Université Claude Bernard Lyon 1, Université de Lyon,* [5]*University of Groningen, University Medical Center Groningen,* [6]*University Hospital Tübingen; Technical University Darmstadt,* [7]*University of Tübingen,* [8]*MEG-Center, University of Tübingen; Hertie Institute for Clinical Brain Research*

Category: Speech Perception

Background: Aging individuals may experience difficulties in perceiving speech in a noisy environment, even when their clinically determined audiometric hearing thresholds are normal and therefore give no indication for the presence of a sensorineural hearing loss. The development of poor speech processing in older humans has recently been associated with the progression of cochlear synaptopathy. How cochlear synaptopathy alters the discrimination of spectrally and temporally distinct acoustic signals, and in turn, speech processing, is still unclear. We therefore studied the impact of age-related speech perception deficit with the help of clinically applicable measurements of the cochlear output and the auditory pathway responsiveness and analyzed the connection between hearing sensitivity, discrimination ability, and speech understanding.

Methods: We examined a cohort of young, middle-aged, and older individuals with mild impairments of auditory sensitivity appropriate for their age for sensory-neural hearing loss, cochlear synaptopathy, temporal processing, and speech comprehension. We applied pure-tone extended high-frequency audiometry (0.25 - 16 kHz), DPOAE (IO functions, DP-grams, level maps), ABR, ASSR, psychoacoustic discrimination of 4 pairs of syllables (/du/-/bu/, /di/-/bi/, /i/-/y/, /o/-/u/) and the Oldenburger Matrix Test (OLSA). The general good condition of the 90 participants was confirmed by tests for depression (Yesaga, Becks), the Mini Mental State Examination, and custom questionnaires.

Results: The audiometric results were used to score the speech performance of individual participants in quiet, with ipsilateral, or with contralateral noise masking. This score allowed to analyze the individual psychometric performances in respect to good, poor, or indifferent (normal) speech comprehension. The syllable discrimination ability went along with the specific noise masking condition and syllable spectral and temporal envelope contrast (low-contrast difficult and high-contrast easy tasks).

Conclusions: We discuss the results derived from the objective functional audiometric biomarkers for their suitability as predictors for speech discrimination disorders. As it is crucial to understand the impact of cochlear synaptopathy on speech coding to develop effective therapeutic interventions in the future, we take a special look at the age-related decline of hearing sensitivity over age. This age-related decline probably

describes a ‘healthy’ form of synaptopathy, which therefore needs to be considered in the clinical diagnostic of speech coding deficits.

Changes in the Fundamental Frequency of Speech in Response to Background Noise and Hearing Loss

Paolo Mesiano*[1], Johannes Zaar[1], Helia Relaño-Iborra[2], Lars Bramsløw[1], Torsten Dau[2]

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Category: Speech Perception

Background: The fundamental frequency (F0) is one of the many features of the voice that talkers can alter in adverse communicative situations with the intent to produce "clear speech" and increase the intelligibility of the transmitted message, e.g., when speaking in presence of background noise or with hearing-impaired (HI) interlocutors. Compared to "conversational speech", clear speech has been shown to be characterized by a higher average F0 and a larger F0 dynamic range. However, these changes in F0 were often measured in laboratory simulations with normal-hearing (NH) talkers, sometimes speaking in the absence of an interlocutor. The present study explored changes in F0 occurring in clear speech by analyzing the F0 statistics of naturalistic dialogues between NH and HI talkers, conducted in quiet and in background noise.

Methods: Speech recordings of Danish dialogues, recorded in two previous studies, were analyzed. The dialogues were conducted by 19 pairs of NH interlocutors and by 13 pairs each consisting of a NH and a HI interlocutor. The dialogues between NH interlocutors were conducted in quiet and in the presence of speech-modulated noise at 70 dBA sound pressure level. The dialogues between NH and HI interlocutors were conducted in quiet and in 20-talker babble noise at 60, 65 and 70 dBA sound pressure level. The recordings were divided into speech segments of minimum two seconds duration and the F0 information of each segment was extracted. For each segment, the average F0 (calculated as F0 median) and F0 dynamic range (calculated as F0 median absolute deviation) were calculated. The two statistical measures were analyzed at the individual and group level for each group of interlocutors and each background-noise condition.

Results: On the group level, it was found that in the presence of background noise both NH and HI talkers increased their average F0 and F0 dynamic range compared to the quiet condition. However, not all individual interlocutors adopted the same strategy when modifying their F0 production during conversations in noise, compared to when they were speaking in quiet: some interlocutors relied more on increasing their average F0, some others on increasing the F0 dynamic range and some on enhancing both aspects of F0 production. Furthermore, on average, NH talkers speaking with a HI interlocutor exhibited a higher average F0 and a larger F0 dynamic range than NH talkers speaking with a NH interlocutor.

Conclusions: These results provide further evidence of how talkers modify their voice and speaking style in response to noise and hearing loss. The obtained findings extend the available knowledge to more realistic dialogue recordings and to communicative situations involving HI interlocutors. It remains an open question whether the observed modifications in F0 adopted by the talkers do result in improved speech intelligibility for the listeners.

Predicting the Effect of Hearing Loss on Speech Intelligibility Using a Physiologically Inspired Auditory Model

Johannes Zaar*[1], Laurel H. Carney[2]

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Category: Speech Perception

Background: Several speech-intelligibility (SI) prediction models have been developed that mostly focus on predicting SI across a large range of acoustical conditions, such as different types of noise, noise-reduction processing, and reverberation. Most of the available models were designed and validated based on reference data for normal hearing (NH), for which individual differences in SI are typically small. In many cases, simplistic linear simulations of auditory processing proved sufficient for predicting such NH group data. However, although many of these models have later been extended to incorporate aspects of hearing loss, it has remained challenging to accurately predict SI differences between listener groups with NH and hearing

impairment (HI) and even more challenging to predict within-group individual differences in the HI population.
Methods: We recently introduced an SI prediction model [Zaar and Carney, (2022), Hear Res., 426:108553] based on the recently proposed hypothesis that across-frequency fluctuation profiles in auditory-nerve (AN) responses are relevant for (speech) sound discrimination. A phenomenological model provided simulated AN responses for NH and individual HI listeners. The model is here evaluated using several data sets consisting of speech reception thresholds (SRTs), measured in a range of noise conditions, and auditory profiling data, all collected in both NH and HI listeners. The model was calibrated using NH data obtained in a single noise condition; predictions were then obtained as a result of differences in the stimuli (different noise conditions) and by incorporating pure-tone thresholds and estimates of OHC and IHC loss into the auditory model (different HI listeners). Special attention was paid to the interpretation of pure-tone thresholds in terms of the underlying OHC and IHC contributions.
Results: The model accounted very well for SI across noise conditions in the NH group and accurately predicted the elevation of SRTs and the reduced masking release due to hearing loss. The measured and predicted SRTs for the HI listeners were strongly correlated for one of the data sets and moderately correlated for another, smaller, data set. The model predictions for the HI listeners were strongly dependent on the interpretation of the pure-tone thresholds with respect to the underlying OHC and IHC contributions, where stronger IHC contributions led to an elevation and stronger OHC contributions to a reduction in predicted SRTs. However, when individualizing the OHC/IHC impairments based on loudness-scaling data, the predictive power did not increase.
Conclusions: The results indicate that the proposed model accounts well for effects of additive noise and hearing impairment on SI, simply based on pure-tone thresholds. The differential effects of OHC and IHC impairment in the model warrant further investigation – while individualization based on loudness-scaling estimates was not successful in the present study, other diagnostic measures may yield better results.

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Predicting Supra-Threshold Speech Reception Deficits Using the Audible Contrast Threshold Test

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Category: Speech Perception
Background: The pure-tone audiogram is the main clinical diagnostic used for assessing hearing loss and provides the basis for the hearing-loss compensation applied in hearing aids. However, the audiogram does not necessarily reflect the hearing deficits that remain when audibility has been restored, for instance the crucial ability of individuals to understand speech in adverse conditions. These supra-threshold speech reception deficits can be measured using speech tests, but it has proven challenging to test speech reception directly in clinical settings due to limitations with respect to equipment, testing time, and standardized speech materials. A clinically viable test that is connected to supra-threshold speech reception deficits would thus represent a highly useful addition to the clinical assessment of an individual's hearing abilities.
Methods: The present study assessed to what extent the Audible Contrast Threshold (ACT) test, a novel quick-and-simple clinical spectro-temporal modulation detection test with built-in audibility compensation, can predict supra-threshold speech reception in noise. Ninety-eight hearing-impaired participants, consisting of 79 native speakers of German and 19 native speakers of Japanese, participated in the study. The audiogram and ACT were obtained along with speech-reception thresholds (SRTs). SRTs were measured with the participants using hearing aids in a challenging setting with spatially distributed speech interferers. Four different hearing-aid settings were tested: amplification only, mild directionality and noise reduction (DIR+NR), medium DIR+NR, and strong DIR+NR.

Results: On the group level, SRTs were highest for the amplification-only setting and decreased with increasing levels of DIR+NR processing. The individual SRTs collected with amplification only were strongly correlated with ACT and – to a lesser extent – with the 4-frequency pure-tone average (PTA4). The predictive power of ACT and PTA4 was found to be complementary, as they both contributed significantly to predicting the amplification-only SRT in a two-predictor linear regression model. Furthermore, the two measures were also associated with the individual SRT benefit induced by the DIR+NR processing.
Conclusions: The results indicate that the ACT test yields a measure of spectro-temporal modulation (or audible contrast) sensitivity that is predictive of supra-threshold speech reception in a realistic environment. The ACT yielded better SRT predictions than the audiogram while also adding significantly to the predictive power of the audiogram. This suggests that the ACT test indeed represents a clinical test that predicts supra-threshold speech reception deficits, which may be used to complement the information obtained from the audiogram in the clinic. More research is needed to identify meaningful interventions for individuals with substantial supra-threshold speech reception deficits and to assess said deficits in different populations of listeners, including those with audiometrically normal hearing.

The Effect of Fundamental-Frequency Dynamics on Speech Intelligibility in Competing-Talker Scenarios for Normal-Hearing and Hearing-Impaired Listeners

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Category: Speech Perception
Background: Differences in the fundamental-frequency (F0) dynamics between competing voices (i.e., an F0-dynamic-range contrast) have been shown to facilitate the perceptual separability of target speech from interfering speech for normal-hearing (NH) listeners. Similar research directed to older hearing-impaired (HI) listeners has shown that this benefit is limited or absent in presence of sensory-neural hearing loss. In these previous studies, the available evidence of this phenomenon was obtained by pairing natural voices with different levels of intonation and using a fixed combination of target and masking talkers. The employed speech manipulation methods may have modified other features of speech beside the F0 dynamics, that may have confounded the effects on the results. The present study aimed at extending the previous findings by using a larger variety of talkers, F0-dynamic-range levels and F0-dynamic-range contrasts, together with a speech-processing method that solely modifies the F0 information without affecting other speech features.
Methods: Target-speech intelligibility was measured in 19 young NH listeners and 13 older HI listeners as a function of the F0-dynamic-range contrast between two competing sentences that had either the same or different average F0 (0 and 6 semitones, respectively). The desired F0-dynamic-range contrast and average F0 difference were obtained by digitally manipulating the F0 trajectories in the competing speech signals whilst keeping the F0 dynamics within the limits of realistic conversational speech. NH listeners were tested at a target-to-masker ratio (TMR) of -4 dB, while HI listeners, provided with individualized linear-gain amplification, were tested at TMRs of 0 and 4 dB. In each experimental trial, the competing speech signals were spoken by the same talker and were diotically presented over headphones.
Results: For both NH and HI listeners, speech intelligibility was unaffected by the F0-dynamic-range contrast and moderately facilitated when a difference in average F0 was present between the competing speech signals. In NH listeners only, speech intelligibility was lowest when both speech signals had the smallest F0 dynamics and increased substantially when a moderate level of F0 dynamics was introduced in at least one of the speech signals, regardless of the F0-dynamic-range contrast between them.
Conclusions: These findings suggest that moderate levels of F0 dynamics in one or both competing speech signals, rather than their mutual F0-dynamic-range contrast, provide a perceptual dissimilarity between the F0 trajectories that aids speech intelligibility in NH listeners. However, HI listeners do not seem to benefit from the F0 dynamics levels used in this experiment. Further research should be directed to investigating whether HI listeners can benefit from F0 dynamics that are enhanced beyond the conversational values used in this study.

The Role of Periodicity in Speech-on-Speech Understanding in Normal-Hearing and Hearing-Impaired Listeners

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Category: Speech Perception

Background: Understanding speech in the presence of one or multiple competing talkers is a challenging auditory task that occurs often in daily life. While 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. The periodicity information of the competing speech signals, which is connected to the characteristics of their fundamental frequency, can provide useful auditory cues for segregating the target speech from the interfering speech, but it is unclear how hearing deficits interfere with the access to such cues. This study investigated how the periodicity information in target and interfering speech contributes to speech intelligibility in young NH and older HI listeners.

Methods: 10 NH and 30 HI listeners participated in a two-competing-voices experiment. The HI group was divided into two subgroups: 15 listeners affected by high-frequency hearing loss (HI1) and 15 listeners affected by both low- and high-frequency hearing loss (HI2). In the experimental stimuli, the periodicity information of target and/or masker signals was either fully available (natural speech) or removed using noise vocoding (vocoded speech). Additionally, speech intelligibility in quiet was measured as a reference condition. The stimuli were played through two frontal loudspeakers (one for each competing signal). HI listeners were provided with linear-gain amplification.

Results: In quiet, NH and HI1 listeners showed high speech intelligibility scores for both natural and vocoded speech, while HI2 listeners showed lower scores, particularly for the vocoded speech. In the masked conditions, NH listeners performed best when natural speech was masked by natural speech. Vocoding the target or the masking speech separately did not affect intelligibility substantially, but vocoding both target and masker signals reduced the performance, producing the lowest speech reception thresholds (SRTs) overall. Compared to NH listeners, HI listeners showed overall worse performances (with HI2 being worse than HI1 in all experimental conditions) and larger variability at the individual level. For both HI1 and HI2 listeners, with natural target speech, vocoding the masker did not affect speech intelligibility substantially. In contrast, vocoding the target worsened speech intelligibility, with the highest SRTs measured when target and masker signals were both vocoded.

Conclusions: The obtained findings suggest that (i) the severity of (low-frequency) hearing loss is a predictor of speech-on-speech understanding, (ii) NH listeners are challenged only when the periodicity information is removed from both target and masker signals and (iii) HI listeners rely mostly on the periodicity information in the target speech, while the presence of periodicity information in the masker is useful to them only when no target periodicity information is available. Further research may be directed at exploring potential strategies for enhancing the relevant periodicity information to improve speech intelligibility for HI listeners.

The Effect of Age in Normal Hearing and the Use of Cochlear Implants on Voice Discrimination in the Background of Noise

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Category: Speech Perception

Background: Understanding speech in noisy environments is crucial for successful communication in various settings. One strategy for enhancing listening in noise involves tracking the speaker's vocal cues, including the fundamental frequency (F0) and formant frequencies (reflecting vocal tract length). Studies found that in quiet, individuals with normal hearing (NH) tend to rely more on formant cues and less on F0 cues for voice discrimination (VD). Although background noise adds a new layer of difficulty because it can obscure both spectral and temporal information critical for VD, its effects on VD have not been investigated to date in either normal hearing or in special populations. The aims of the present study were: (1) to assess the influence of noise on VD in different age groups of normal hearing (NH), and (2) to explore the effect of noise on VD in cochlear-implant (CI) users compared to NH peers.

Methods: The study involved four groups of participants:16 NH children (7-10 years old), 24 NH young adults (18-35 years old), 12 NH older adults (over 65 years old), and 10 prelingually-deaf young adults with

CIs. First, speech reception thresholds in noise (SRTn) were assessed using an adaptive sentence-in-noise test. Next, VD was assessed for 3-word sentences based on F0 only, Formants only, or F0+formant cues, using a three-interval three-alternative, two-down one-up adaptive procedure. Thresholds were assessed in quiet and in the presence of speech-shaped noise at SNRs that were 3-5dB higher (i.e., easier) than the individual SRTn.
Results: (1) In quiet (and as expected), NH listeners exhibited better VD with the formants compared to the F0 cues; (2) Background noise significantly disrupted the utilization of formant cues across all NH groups resulting in similar or worse thresholds for formants compared to F0 thresholds for VD. This adverse effect of background noise was particularly challenging for older adults and children, as both groups exhibited poorer F0 perception than young adults; (3) CI users demonstrated poorer VD compared to the NH listeners across voice cues in quiet, with no advantage to formant cues over F0 cues for VD; and (4) The addition of background noise had no influence on the VD of the CI group compared to the quiet condition.
Conclusions: The finding that robust cues such as formants are compromised for VD in noisy conditions, even when the noise levels permit good SRT, has significant clinical implications. Specifically, it underscores the difficulties that individuals with limited F0 perception, such as children, the elderly, and those with CI, encounter in noisy environments. This emphasizes the importance of conserving low-frequency hearing during cochlear implantation when relevant and enhancing the accessibility to F0 cues in developing speech-processing strategies designed for implantable hearing devices.

Decoding Selective Attention in Cochlear Implant and Normal Hearing Individuals' Single-Trial EEG

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[1]*Seoul National University,* [2]*University of Iowa*

Category: Speech Perception
Background: Cochlear Implant (CI) users are known to have challenges that extend beyond peripheral limitations, exhibiting high variability at the cerebral level, notably in selective attention. While it remains difficult to recover peripheral functions, improving cortical functions that operate selective attention may serve as a more feasible option to enhance auditory processing abilities in CIs. Neurofeedback training (NFT) emerges as a novel approach for improving selective attention, exhibiting its efficacy for improving speech-in-noise performances and underlying cortical activations in normal hearing listeners.
One of the essential elements of NFT is the real-time decoding of selective attention from single-trial EEG signals. However, decoding attention from CI users' EEG poses immense challenges due to significant EEG channel dropouts and large device artifacts. Most of the existing attention decoding algorithms developed for normal hearing remain unvalidated for CI users. Thus, this study aims to develop an attention-decoding pipeline suitable for EEG signals from both normal hearing and CI users.

Methods: Twenty-eight normal hearing participants and fifty-one CI users underwent identical selective attention tasks. Participants were instructed to concentrate on either of two speech streams that repeat the word "Up" five times or "Down" four times, which are presented simultaneously but at disparate rates, and to identify the place of a pitch deviant. After a visual cue, participants concentrated on one of the speech streams while EEGs were recorded using a 64-channel Biosemi system.
The acquired EEG signals underwent preprocessing, where severely contaminated trials and channels are manually rejected based on their respective maximum amplitudes. Subsequently, ICA was implemented for artifact removal related to eye movements and CI device interferences. Signals were re-referenced to near-ear electrodes, baseline-corrected, and bandpass filtered (2-8Hz) to isolate auditory evoked activities. They were then downsampled to 20Hz. Three classifiers: logistic regression, random forest, and SVM were trained to categorize attention to either the Up or Down stream using the amplitude sequence extracted from EEG waveform.

Results: Despite a slight drop in accuracy, the classifiers are still valid in CI users, with particular emphasis on higher accuracies in the central area for both normal hearing and CI users. Moreover, the performance varied across subjects, hinting that some had more pronounced attention modulation. For CI users only, there was a significant correlation between the speech-decoding accuracy and their performance in a speech-in-noise attention task.

Conclusions: Our study demonstrates that EEG-based attention decoding is also available for CI users. Furthermore, decoding accuracy has the potential to serve as an indicator of the strength of attentional modulation, especially for CI users. This study lays the groundwork for exploring different decoders in NFT to enhance training effects for CI users.

Podium Session 16 - Inner Ear Development

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

Platinum Salon 5

POU4F3 Deletion Causes Developmental Arrest of Utricular Hair Cells

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Category: Development: Cellular/Systems

Background: The transcription factor and deafness gene Pou4f3 is expressed in hair cells soon after they begin to differentiate. Recently, POU4F3 was shown to act as a pioneer factor that assists ATOH1 in accessing its transcriptional targets, thereby facilitating the expression of genes critical for hair cell (HC) development. While the cochlear HCs of Pou4f3DTR/DTR and Pou4f3-/- mutants degenerate postnatally, vestibular HCs appear to survive in an abnormal, and possibly non-functional, state. Previous reports characterizing these Pou4f3 mutant hair cells have noted the expression of exclusively immature or Type II-associated markers, and the number of HCs present does not change between P0 and P30, suggesting these cells may actually be Type I precursors. We sought to more fully characterize Pou4f3-/- utricular HCs, determine whether they appear developmentally arrested at the transcriptional level, and shed light on their developmental origin.

Methods: To cover the period of Type I and Type II HC development and differentiation, we collected cells at P1, P5, P11-12 and P28-30, with the final time point representing mature, functional HCs. At each time point, we dissected and pooled utricles from 4-11 animals of each genotype (Pou4f3+/- and Pou4f3-/-) and used the 10X Chromium system to capture individual cells for transcriptional profiling.

Results: Using canonical cell type and regional markers, we identified both striolar and extra-striolar Type I and Type II HCs from mature (P28-P30) Pou4f3+/- animals and report novel markers for these cell types. Additionally, we detected a group of HCs which appeared to be immature, newly differentiated Type IIs arising from supporting cells or their progenitors. Consistent with this hypothesis, these cells were relatively more abundant at earlier time points, and clustered adjacent to Type II HCs. By contrast, HCs from Pou4f3-/- animals regardless of collection time point appeared transcriptionally similar regardless of time point, forming just two distinct clusters, one of which appeared to be relatively more abundant at earlier time points. Interestingly, several of the top 10 differentially expressed markers of Pou4f3-/- HCs were shared with the cluster of immature Type II hair cells we identified in normal utricles.

Conclusions: Our results suggest that Pou4f3-/- HCs are, indeed, arrested at an early stage of their development. In future work, we will collect utricles from embryonic time points and P0/P1 to profile immature Type I HCs. Using transcriptional data from both immature Type I and immature Type II HCs, as well as inducible mouse lines for lineage tracing, we will then seek to elucidate the developmental origin of Pou4f3-/- HCs. Additionally, because currently available public single-cell datasets for the utricle contain only tens or hundreds of HCs, these data may significantly improve our understanding of the dynamic transcriptional processes giving rise to utricular HC subtypes.

GJB2-Mediated Transcriptional Genomic Changes in the Postnatal Development of the Cochlea Analyzed by RNA-Seq

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Category: Development: Cellular/Systems

Background: GJB2 (Cx26) mutations can induce a high incidence of hereditary deafness, responsible for 70-80% of nonsyndromic hearing loss in the clinic. Previous mouse models showed that Cx26 deficiency can produce cochlear developmental disorders, hair cell and spiral ganglion neuron degenerations, endocochlear potential (EP) reduction, and active cochlear mechanics reduction. These data suggest that Cx26 is required and plays a critical role in the cochlear development. In this study, we used the advanced RNA Sequencing (RNA-Seq) technique and investigated GJB2-mediated transcriptional changes during the cochlear postnatal development.
Methods: Cx26 conditional knockout (cKO) mice were used and created by LoxP-Cre technique. Cx26 FloxP/FloxP mice were crossed with Pax2-Cre mice. The mouse cochlea at postnatal day 1 (P1), P3, P5, and P10 were collected. Bulk Poly(A) RNA Sequencing was performed.
Results: In comparison with WT mice, Cx26 cKO mice at P3 had the most significant changes. It has been found that the major changes in the pathways of the primary Biological Process (BP) were related to positive regulation of cell projection and cell migration. In Cell Component (CC) analysis, it has been found that the actin cytoskeleton and synapse formation and specialization had the most primary changes. In primary Molecular Function (MF) analysis, the tubulin and microtubule binding had significant changes. In addition, in comparison with WT mice, the downregulations of some genes in the Cx26 cKO mice were identified by significant q-value and logFC changes. These notable genes included Zfhx4, Hey1, and so on. Previous studies demonstrated that deficiency of these genes could also cause hearing loss.
Conclusions: Cx26 deficiency can cause significant changes of gene expression in the transcriptional level during the cochlear development. Cx26 deficiency can also cause other genes downregulations, which can cause hearing loss as well. These data provide valuable information for developing efficient gene therapy for GJB2 mutation induced hearing loss.
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Revisiting the Potency of Tbx2 Expression in Transforming Outer Hair Cells Into Inner Hair Cells at Multiple Ages in Vivo

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Category: Development: Cellular/Systems
Background: The cochlea, the auditory organ, contains two types of sound receptors: inner hair cells (IHCs) and outer hair cells (OHCs). Tbx2 is expressed in IHCs but repressed in OHCs. The neonatal OHCs with Tbx2 misexpression transdifferentiate into IHC-like cells. However, the extent of the switch from OHCs to IHC-like cells and the underlying molecular mechanism remain poorly understood. Furthermore, it is unknown whether Tbx2 can transform fully mature adult OHCs into IHC-like cells.
Methods: In this study, we contrast two new mouse genetic models. The first is Rosa26-LSL-Tbx2-P2A-Tdtomato/+ by which we are able to specifically and conditionally overexpress Tbx2 in cochlear OHCs at different ages. The second is Rosa26-LSL-Tbx2-P2A-Ikzf2-T2A-EGFP/+ strain that allows us to conditionally overexpress Tbx2 and Ikzf2 in OHCs at the same time. The two models are thoroughly analyzed by single-cell transcriptomic analysis, immunostaning and SEM analyses as well as ABR test.
Results: We find 85.6% of IHC genes, including Slc17a8, are upregulated, whereas only 38.6% of OHC genes, including Ikzf2 and Slc26a5, are downregulated in neonatal OHCs with Tbx2 misexpression. Thus, our findings suggest that Tbx2 cannot fully reprogram neonatal OHCs into IHCs, contrary to previous assumptions. Consistently, Tbx2 also fails to fully reprogram cochlear progenitors into IHCs. Finally, Ikzf2 restoration alleviates the abnormalities present in the Tbx2+ OHCs, supporting the notion that Ikzf2 repression by Tbx2 contributes to the transdifferentiation of OHCs into IHC-like cells.
Conclusions: Overall, our study reevaluates the effects of ectopic Tbx2 expression on OHC lineage development at different stages and provides molecular insights into how Tbx2 disrupts the gene expression profiles of OHCs. This research also lays the groundwork for future studies on OHC regeneration.

HMGA2 Integrates Tonotopic Morphogen Signaling and is Required for Low-Frequency Hearing in Mice

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Category: Development: Cellular/Systems

Background: High-mobility group AT-hook 2 (HMGA2) is a protein known to regulate transcription by influencing chromatin modification and the assembly of protein complexes that regulate gene expression. In the mouse organ of Corti, Hmga2 is expressed in an apex-to-base decreasing gradient that is established as early as E12.5 and maintained throughout postnatal life. Experiments using organotypic cultures of the organ of Corti suggest that the tonotopic expression pattern of Hmga2 is shaped by morphogens such as retinoic acid. In humans, aberrant expression of HMGA2 is commonly associated with cancer, but its role in organ of Corti development remains unknown.

Methods: To fill this gap in knowledge, we studied the influence of retinoic acid levels on the formation of the HMGA2 mRNA and protein gradients in vivo using a loss-of-function (Aldh1a3-KO) mouse model. Furthermore, conditional knockout of Hmga2 from the otocyst stage was established to study the relevance of HMGA2 using histological staining, and hearing function via ABR and DPOAE measurements.

Results: Retinoic acid signaling activity decreases from the base towards the apex. Hence, retinoic acid and Hmga2 form inversely oriented gradients in the embryonic mouse cochlea. Analysis of Aldh1a3-KO mice revealed that reduced levels of retinoic acid result in an extension of the Hmga2 gradient towards the cochlear base. This finding indicates that positional information mediated by the retinoic acid morphogen gradient shapes the formation of the Hmga2 gradient in vivo. Conditional knockout of Hmga2 did not affect the cellular composition of the organ of Corti at birth, but the mice have significant auditory function deficits as adults, which are more profound in the low and mid frequencies.

Conclusions: In this work, we used different transgenic mouse lines to establish that embryonic patterning via retinoic acid modulates the Hmga2 gradient along the apex-to-base axis. Our results also show that this chromatin modifier is necessary for the normal adult hearing, particularly of low-frequency sounds. Together, the results indicate that HMGA2 is an integral part in mediating tonotopic patterning in the mouse cochlea.

Notch1 is Required for Sensory Maturation in the Mammalian Vestibular System

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[1]*University of Rochester Medical Center,* [2]*University of Rochester*

Category: Development: Cellular/Systems

Background: Notch signaling is an evolutionarily conserved pathway that has roles in cell proliferation, cell differentiation and cell death. During inner ear development, early expression of Notch and its ligand Jagged1 are required for proper formation of the vestibular sensory region. Later, Notch is important in the cell fate decision to become either a hair cell or supporting cell by mediating lateral inhibition. However, once sensory cells have adopted a cell fate and begun differentiating, the role Notch has in the maturation of the vestibular sensory organs is unknown.

Methods: To delete Notch1 in the five sensory organs of the vestibular region, tamoxifen (37.5mg/kg) was administered by IP injection into Sox2CreER Notch1fl/fl pups at postnatal day 1 and 2 (P0/P1) and utricles were collected and processed for experiments at several time points after birth. Vestibular function was measured at 2 months by vestibular sensory evoked potentials (VsEPs) and analyzed through standard behavior assays, including open field, balance beam and swim tests. Whole mount immunofluorescence and confocal imaging were performed to examine the effects of Notch1 deletion on supporting cells and Type I and Type II hair cells, as well as afferent innervation, during vestibular maturation.

Results: We found that loss of Notch1 after birth resulted in significant vestibular dysfunction in mutants compared to controls, as assessed by VsEP recordings. Additionally, behavioral tests revealed behaviors associated with balance problems in the Notch1 mutants. Whole mount immunohistochemical analyses showed significant loss of the specialized Type I hair cells in the striola, indicated by a loss of Oncomodulin (Ocm+) cells, as well as defects in hair cell innervation. Additionally, Notch1 mutants have significant

supporting cell loss in both the striolar and extrastriolar regions and supernumerary Type II hair cells. At P2, the early hair cell marker ATOH1 was detected in some supporting cells, suggesting Notch1 has a continued role in lateral inhibition during postnatal vestibular maturation.
Conclusions: Together, these results indicate that Notch1 continues to mediate lateral inhibition in the vestibular region during postnatal maturation of the sensory regions.

Deciphering the Contribution of Wnt Ligands From Cochlear Epithelium and Periotic Mesenchyme During Cochlear Development

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Category: Development: Cellular/Systems
Background: The planar cell polarity (PCP) pathway organizes cells in tissue planes, utilizing a set of core PCP proteins. Cochlear hair cells (HCs) are precisely oriented with hair bundles aligned radially. We previously showed that cochlear PCP, including cochlear extension, HC orientation and core PCP protein polarization, is regulated by Wnt secretion from the embryonic cochlear epithelium. Here, we hypothesize that the formation of cochlear PCP requires specific Wnt ligands secreted from the cochlear epithelium and periotic mesenchyme. To test this hypothesis, we analyzed single-cell RNA-sequencing (scRNA-seq) data from developing cochlear epithelium and mesenchyme to examine combinatorial Wnt-Frizzled interactions, and then evaluated the role of candidate Wnts as well as Wntless (Wls) using conditional KO (cKO) mice.
Methods: The CellChat algorithm (Jin et al., 2021) was used to analyze scRNA-seq data from embryonic (E)16.5 cochlear epithelium (Kolla et al. 2020) and E15.5 cochlear mesenchyme to rank Wnt -Wnt receptors interactions. To evaluate effects from Wnt ligands, conditional knockout mice for Wls or individual Wnts crossed with Emx2-Cre, Dermo1-Cre, or UBC-Cre-ERT2 were examined. To assess for abnormalities of cochlear PCP, we analyzed the following at E18.5: 1) cochlear length, 2) HC orientation, and 3) polarized expression of Dvl2 and Fzd6 in HCs.
Results: The CellChat algorithm inferred that Wnt5a, Wnt7a, and Wnt7b are the three most active ligands from the cochlear epithelium acting on Wnt receptors on cochlear HCs, while Wnt5a is the most active from the cochlear mesenchyme. Deletion of Wntless from the cochlear duct led to severely shortened cochlear duct, malrotated HCs, and loss of polarization of Dvl2 and Fzd6. On the other hand, deletion of the following combinations from the cochlear epithelium: 1) Wnt5a; Wnt7b, 2) Wnt7a; Wnt7b, and 3) Wnt4, 5a, and 7b led to shorter cochlea and mildly misoriented HCs, but not loss of polarization of Dvl2 or Fzd6, suggesting additional Wnts or Wnts from the periotic mesenchyme also contribute to PCP. Deletion of Wnt5a from the cochlear duct or periotic mesenchyme using Emx2-Cre or Dermo1-Cre mice caused no cochlear PCP abnormality. Ongoing experiments will evaluate the effects of ablating Wnt secretion from both the cochlear duct and periotic mesenchyme.
Conclusions: The PCP in the developing cochlea is regulated by the combined effects of multiple Wnts secreted from the cochlear epithelium, including Wnt4, Wnt5a, Wnt7a, and Wnt7b, and possibly also Wnts secreted from the periotic mesenchyme.

The Transcription Factor TOX Potentiates ATOH1 Activity and Regulates Hair Cell Maturation in the Hearing Organ

Yoko Nakano[1], Susan Wiechert[1], Botond Banfi*[1]

[1]*University of Iowa*

Category: Development: Cellular/Systems
Background: The transcription factor ATOH1 is a master regulator of hair cell (HC) development. Genetic deletion of Atoh1 prevents differentiation of pro-sensory cells to HCs, and ectopic expression of ATOH1 results in trans-differentiation of greater epithelial ridge (GER) cells to HC-like cells in the immature cochlea.

However, the efficiency of ATOH1-induced reprogramming of GER cells declines with the progression of cochlear development. Thus, some potentiators of ATOH1 activity are probably expressed in both immature HCs and GER cells during early phases of cochlear development. Given that potentiators of ATOH1 activity could improve the efficacy of current HC-regenerating approaches, we set out to identify novel transcription factors that enhance the effects of endogenous and ectopically expressed ATOH1 in the developing cochlea.
Methods: Based on previous RNA-sequencing analyses of mouse cochlear cells, we identified transcription factors that are upregulated at the same time as Atoh1 in immature HCs and expressed in the GER during early phases of cochlear development. The effects of these proteins on ATOH1 activity were tested in transfected HEK293 cells using promoter assays. This screen revealed that one of the tested transcription factors (i.e., thymocyte selection-associated HMG box, TOX) enhanced ATOH1 activity. To evaluate the function of TOX in the cochlea, we used knock-out (Tox-/-) mice, gene-trapped (Toxgt/gt) mice, histological techniques, hearing tests, RNA sequencing, genetic interaction tests, and adeno-associated virus (AAV)-mediated gene delivery to the GER.
Results: The Tox-/- and Toxgt/gt genotypes were associated with stereocilium bundle defects, postnatal loss of cochlear HCs, and deafness. In Toxgt/gt mice, deletion of the gene trap specifically in immature HCs rescued stereocilium bundle morphology, HCs, and hearing. RNA sequencing analysis of the organ of Corti of newborn Tox-/- mice revealed that the expression levels of hundreds of genes were abnormal, and that many target genes of ATOH1 were expressed at abnormally low levels. Consistent with the notion of functional association between ATOH1 and TOX, deletion of one copy of the Atoh1 gene (Atoh1+/-) in Tox-/- mice accelerated dramatically the loss of inner HCs and increased the severity of morphological defects of outer HCs. Lastly, in GER cultures from 1-week old wild-type mice, trans-differentiation of GER cells to HC-like cells was more efficiently induced by a mix of ATOH1- and TOX-encoding AAVs than the ATOH1-encoding AAV alone.
Conclusions: Our data demonstrate that TOX is a crucial regulator of HC maturation and a candidate for use in HC regeneration-inducing gene cocktails.

Development of the Fetal Human Utricle Revealed by Single-Nucleus Multiomics

Weisheng Liang*[1], Ryosuke Yamamoto[2], Emilia Luca[2], Alain Dabdoub[3]

[1]*University of Toronto,* [2]*Sunnybrook Research Institute,* [3]*Sunnybrook Research Institute; University of Toronto*

Category: Development: Cellular/Systems
Background: The utricle is a vestibular organ responsible for detecting horizontal linear acceleration. Its sensory epithelium is composed of mechanosensory hair cells interdigitated by supporting cells, and the periphery is surrounded by nonsensory transitional epithelial cells. Single-cell studies with mouse utricles have begun to uncover cellular diversity and molecular networks involved in development. However, there is currently no data available on the developing human utricle at single-cell resolution. Therefore, we used single-nucleus multiomic sequencing to unveil the transcriptomic and epigenomic profiles of the fetal human utricle at two gestational ages, week 15 and 19.
Methods: We dissected the utricle from the inner ear at gestational week 15 and 19 (n=2 each), and separated the sensory epithelium and surrounding transitional epithelium from the underlying mesenchyme using thermolysin. After tissue lysis, the single nuclei suspension was used to perform the 10x Chromium Single Cell Multiome ATAC + Gene Expression protocol followed by sequencing. For each age, the multiomic dataset underwent quality control and batch correction between the two samples. We performed dimension reduction and clustering using various bioinformatic tools, followed by snRNA-seq and snATAC-seq data integration.
Results: Integration of transcriptomic and epigenomic data revealed distinct populations of hair cells, supporting cells, and transitional epithelial cells present in the utricle at both gestational ages. Cell-type specific or enriched genes were identified through differential gene expression analysis. By performing motif enrichment analysis in accessible peaks and calculating their correlation with gene expression, we inferred gene regulatory networks which depicted complex gene interactions that govern cell identity, and detected active transcription factors in each cell type.

Conclusions: Our work generated the first single-nucleus multiomic datasets of the fetal human utricle. We uncovered cellular heterogeneity and gene regulatory networks, thereby identifying novel genes and active transcription factor interactions in the sensory epithelium at gestational weeks 15 and 19. We also revealed the developmental state of the human utricle's sensory and non-sensory cell types. By establishing a better understanding of the human utricle, we anticipate that our data will contribute to preclinical studies to design therapies to treat vestibular dysfunction.

Symposium 9 - Plasticity of the Central Auditory System During the Life-Time

10:15 a.m. - 12:15 p.m.

Grand Ballroom Salon E

Plasticity of the Central Auditory System During the Life-Time

Chair: Josef Syka*, Institute of Experimental Medicine, ASCR*
Co-Chair: Nina Kraus*, Northwestern University*

Session Description: We propose a symposium that is concentrated on plastic changes in the auditory system appearing during the lifetime. It is evident that the auditory system is vulnerable during the early developmental period and on the other side it is possible to influence its development during the early developmental period in a positive way. Similarly, specific changes occur during aging and proper understanding of these changes can ameliorate their negative effects especially as concerns understanding of the human speech in a noisy environment. Our proposal of the symposium topics covers both these periods of life and in addition, it combines results of experimental animal approaches with results of the human studies. Josef Syka reports about series of experiments in rats when their auditory development was influenced in a negative or positive way. Other reports are based on human data. Oliver Profant describes reorganization of the central auditory system after unilateral hearing loss, Martin Meyer reviews age-related changes in the brain and their effects on hearing and speech, Samira Anderson concentrates on plasticity of the auditory system in aged human subjects and Jennifer Krizman reports about sexual dimorphism, their findings point to fundamental differences in how males and females engage with their auditory world. Finally, Nina Kraus summarizes auditory learning with the BEAMS hypothesis, which is based on the critical role of the efferent shaping the afferent auditory system.

The Plasticity of the Auditory System in the Early Developmental Period

Josef Syka

Institute of Experimental Medicine, ASCR

Individual Abstract: The structure and function of the auditory system undergoes modifications during a lifetime, with the most sensitive period for plastic changes being the early postnatal developmental period. In altricial animals, such as rats and mice, the period of higher plasticity of the auditory system starts with the onset of hearing at postnatal day 12, and then lasts for approximately a further two weeks. In the human auditory system, it occurs in the third trimester of pregnancy and lasts up to the age of 4-5 years. Interestingly, the end of the period of higher plasticity is associated with the appearance of perineuronal nets that fill in the extracellular spaces between the neurons in the brain, including the central auditory system. Experimentally, we were able to influence the development of the central auditory system (CAS) in the rat by exposure to specific sound stimuli and, by doing so, to change the structure and function of the CAS in adulthood. A brief (8 min) noise exposure with a high intensity, administered on postnatal day 14, resulted in almost normal hearing thresholds in adult animals, however, several parameters of the function of CAS neurons, such as sharpness of frequency tuning, were permanently deteriorated. The deterioration of functional characteristics was also accompanied by morphological changes in adult animals, e.g. the mean total length of apical

dendrites of pyramidal cells in the auditory cortex was smaller than in the control animals and the numerical density of spines was significantly lower. A different type of acoustical exposure applied during the critical developmental period, can influence the development of the CAS in a positive way. For example, rat pups exposed during the whole postnatal developmental period to a spectrally and temporally modulated complex sound with a moderate intensity displayed when adult: lowered excitatory thresholds, increased frequency selectivity, steeper rate —intensity functions and increased spontaneous activity. Rearing pups in the acoustically enriched environment led to a behavioral improvement in gap detection ability under more difficult testing conditions, i.e., with a worsened stimulus clarity. In addition, it resulted in a decreased stochasticity and a higher reproducibility of neuronal spiking patterns. Morphological changes in the auditory cortex involved in this case increases in the total length and volume of the basal dendrites of pyramidal cells and, in addition, increases in the number and density of spines on these dendrites.

Plastic Changes in the Auditory Cortex Caused by Unilateral Hearing Loss
Oliver Profant

Charles University

Individual Abstract: Unilateral hearing loss (UHL) is an irritating condition that leads to a decreased ability to process complex auditory stimuli such as speech, especially in a noisy environment, and also the inability to navigate in space without determination of the location of a sound source. It is most frequently of a peripheral idiopathic origin. In cases with an identified cause, UHL is most commonly caused by a viral infection. Retrocochlear UHL is often induced by vestibular schwannoma (VS), which is the most common tumor of the temporal bone and besides hearing loss it also leads to tinnitus and vertigo. Treatment of the VS (surgery or radiotherapy) often results in single sided deafness, the most extreme form of UHL. The animal models of UHL have revealed atrophy of neural structures, specifically spiral ganglion and neurons of the cochlear nuclei, and a decrease in the activity of the cochlear nerve on the ipsilateral side towards the lesion. At higher levels of the auditory system the potential lack of inhibition due to missing stimulation caused by the UHL, leads to increased activity in the ipsilateral inferior colliculus and auditory cortex (AC).
Human studies, besides the aforementioned, showed decreased ability to process complex auditory stimuli in a noisy environment and, in a navigation task, showed disturbed temporal processing. The UHL only results in minimal changes within the white matter of the auditory pathway, whereas the gray matter morphometry is disturbed not only in the AC but also in the regions specific for the working memory and cognitive processing such as parahipocampus, temporal lobe and precuneus. Chemical shifts of N-acetyl-aspartate and creatine within the contralateral AC (towards the UHL) were reported, assuming structural neuronal pathology and decreased metabolism. The function of the AC is also influenced by UHL. The main findings are a more balanced bilateral activation and increased activity within the ipsilateral AC (towards the normal hearing ear). There are also different effects of the left vs. right UHLs. UHL affects regional homogeneity (connectivity towards neighboring regions) within cognitive structures (insula, parahipocampus, sulcus calcarinus) and increased activity in the operculum as a key structure of the hearing and working memory. The aim of this presentation is to summarize the known effects of UHL on the processing of auditory stimuli, and its effects on the plasticity of the auditory system and associated regions.

Hormones Impact Sexual Dimorphism of Subcortical Auditory Processing in Humans and Rodents
Jennifer Krizman

Northwestern University

Individual Abstract: Numerous studies in humans have found that subcortical auditory processing differs between males and females. In general, these differences correspond to faster and larger evoked responses in females, relative to males. While anatomical differences, including differences in cochlea length or head size, may contribute to these sex differences, we have found they are neither the sole, nor the major, source of sexual dimorphism of subcortical auditory processing. We have collected the frequency-following response (FFR) to complex sound in thousands of humans across the lifespan. Using this measure, we discovered that

some differences in auditory processing, such as differences in peak latency, are present at 3 years of age, while other sex differences, such as differences in harmonic encoding, do not emerge until adolescence or early adulthood, and yet other measures, such as non-stimulus activity, never differ between males and females. Differences that emerge over development arise because changes in male auditory processing lead to smaller and later responses with increasing age. Females, in contrast, maintain the larger and earlier responses typical of early childhood. Given that auditory processing and circulating sex hormones begin to sexually diverge around the same age, and that estrogen regulates auditory plasticity in a sex-specific manner in seasonally breeding animals, we hypothesize that estrogen signaling supports auditory sex differences. To test this, we recorded FFRs in male and female rats to the same sound used in humans. Female FFRs were collected during both low and high levels of circulating estrogen during the estrous cycle. Regardless of where females were in their cycle, female rodents had larger harmonic encoding than male rodents. However, the magnitude of this sex difference was greatest when the females were at the highest point of estrogen production. These results argue that hormonal differences, specifically estrogen, underlie sex differences in harmonic encoding in rodents and suggest a similar mechanism in humans. These findings have important implications. First, the multiple time courses of sexual dimorphism indicate the multifaceted nature of the FFR. Second, we have seen that the enhanced encoding seen in females results in unique auditory advantages for both healthy and concussed female athletes, with estrogen potentially offering a neuroprotective effect in the case of a head injury. In short, these findings point to fundamental differences in how males and females engage with their auditory world.

Neural Plasticity Over the Lifetime: The Beams Hypothesis

Nina Kraus

Northwestern University

Individual Abstract: The brain is an organ of prediction. Our behavior—how we react in any circumstance—is based on who we have become based on what we have learned throughout our lives. We operate from this bedrock using a combination of intuition and logic built from experience. Based on probabilities and past outcomes our brain is equipped to do what it must do at any given moment. How so we form this bedrock of who we are? In part from our memories. A chief role of the efferent pathway is to gather the right information under the circumstances. Over time, an important sound—perhaps your mother's voice—earns preference in processing due to careful sculpting of the afferent pathway by efferent signaling. The memory of the sound of your mother's voice now resides in your automatic neural circuitry. Primed to respond optimally to your mother's voice, your brain gives precedence to that sound. You are likely to hear it even if you're asleep. A prediction is made, based on memory, that it is a voice of consequence. I have summarized auditory learning in the BEAMS hypothesis [1]. The hearing brain interacts with the other senses, the motor, emotion, and cognitive systems of the Brain. This experience with sound effects changes through the Efferent (brain-to-ear) circuitry—which is massive. Over time, as importance is established, the Afferent (ear-to-brain) pathways change, representing Memories of the Sounds of our lives. Our neural pathways are sculpted by our lives in sound. It doesn't happen quickly. But over time it adds up. Memories for sounds are made, neural pathways are altered, and automaticity replaces labor. The signal overtakes the noise. Eventually, with music practice, with athletic competition, as we get older, the sounds of our past bring about a foundation of sound processing in the present. The hearing brain continues to accrue wisdom, building on the foundation of memories that makes us, us. The sounds of our lives shape our brains.

1. Kraus N (2021) Memory for Sound: The BEAMS Hypothesis. Hearing Research. 407: 108291.

On the Interaction Between Speech, Age-Related Hearing Loss, and Brain Atrophy

Martin Meyer

University of Zurich

Individual Abstract: Nowadays, there is a consensus regarding the dynamic nature of the central nervous system (CNS) in humans, which continuously undergoes transformations as a result of learning and

experiential factors. This characteristic, known as "neuronal plasticity," encompasses a multitude of triggers that stimulate plastic processes within the brain. Noteworthy, not all of these processes adhere to a controlled "top-down" modality, as a considerable portion of plastic changes occurs inadvertently, often yielding unforeseen ramifications. Moreover, the scope of neuroplasticity also encompasses language-related functions and neuroanatomical structures as well. Research has revealed that spoken language is widely distributed across both left and right perisylvian regions. However, distinguishing between the diverse aspects and outcomes of functional and structural plasticity frequently proves challenging. This discourse aims to direct attention toward the plasticity observed within the auditory and speech systems of the human population, with additional focus on various dimensions of speech and hearing throughout an individual's lifespan. With the understanding that age-related hearing loss leads to sensory deafferentation within the peripheral system, causing a disruption or attenuation in stimulus transmission from the inner ear to the auditory cortex, it becomes evident that this phenomenon contributes to a reduction in gray matter within areas of the brain related to auditory and speech processing. Consequently, this reduction exacerbates the difficulties encountered in speech comprehension. Furthermore, beyond peripheral hearing loss, there exists a form of suprathreshold hearing loss that progressively intensifies with the advancement of the underlying condition. In parallel, the process of normal aging introduces independent neurodegenerative mechanisms within the brains of older individuals, impacting the integrity of frontal brain regions. Consequently, the ability to compensate for impaired central speech and hearing systems through heightened attention and concentration becomes arduous. The greatest challenge presently confronting plasticity research in the realm of hearing and aging revolves around comprehending the interaction and interdependence among threshold-dependent, suprathreshold, and cognitive hearing loss. Our research group has undertaken several studies that illuminate the reciprocal relationship shared among different forms of hearing loss, language comprehension, cognition, brain aging, and neural plasticity. Of particular interest are recent findings, which offer glimpses of structural plasticity reliably attributed to at least one of the three forms of hearing loss. Notably, the traditionally less linguistically dominant right hemisphere of the brain has exhibited both structural and functional modifications, relinquishing its language-related features in favor of sustaining robust language comprehension, primarily localized within the left superior temporal lobe.

Evidence of Neuroplasticity in the Aging Brainstem and Cortex

Samira Anderson

University of Maryland

Individual Abstract: The brain’s ability to preserve the precise timing characteristics of the speech signal is an important factor in challenging listening situations, such as speech in noise, accented speech, or fast speech. Older listeners demonstrate declines in temporal processing in perceptual and neural measures, suggesting that it is a possible factor in their speech understanding difficulties. Hearing aids focus on restoration of signal audibility but may not compensate for auditory temporal processing deficits, particularly when hearing thresholds are near normal. This presentation will describe the results of a series of studies that used electrophysiology, magnetoencephalography, pupillometry, and perceptual testing to demonstrate training-related neuroplastic changes in older listeners. These studies have used both speech and non-speech raining materials that have varied in cognitive demand.

This research was supported by the National Institute on Aging of the National Institutes of Health under Award Number P01AG055365 (Gordon-Salant)

Podium Session 17 - New Frontiers in Auditory Research: Exploring Middle Ear Dynamics and Tinnitus Pathophysiology

10:15 a.m. - 12:15 p.m.

Platinum Salon 6

Acoustic Trauma Measured via Intracochlear Pressure Changes During Mastoidectomy

Emily Bacalao[1], Nam Lee[1], Juanantonio Ruiz[1], Brian Herrmann[1], Nathaniel Greene[1], Emily Bacalao*[2]

[1]*University of Colorado School of Medicine,* [2]*University of Colorado*

Category: Middle and External Ear

Background: Mastoidectomy is one of the most common procedures done in otologic surgery as it provides access to the middle ear through the mastoid bone. Mastoidectomy involves drilling the mastoid bone with cutting and diamond burr tips to the level of the middle ear space. While otologic surgery is usually done with the goal of improving hearing outcomes and or preserving hearing, it is unknown how much acoustic trauma drilling on the mastoid exposes the patient to. Many studies in literature discuss the risk and rates of sensorineural hearing loss after mastoidectomy, and while it is generally considered rare and attributed to surgical mistakes or inflammatory process from the surgery itself, substantial noise and vibration is generated during high-speed drill use, which could lead to a noise induced injury. This study will examine the acoustic trauma from mastoidectomy, particularly the vibration and noise transmitted to the inner ear via bone conduction, by directly measuring intracochlear pressure changes during a mastoidectomy.

Methods: Sound and vibration transmission to the inner ear was measured using fiber optic pressure sensors inserted into the cochlea of cadaveric heads. Cochleostomies were made into the scala vestibuli and tympani, through which the sensors were placed, then sealed and secured to the cochlear promontory with dental impression material and cyanoacrylate adhesive using a trans-canal approach. Briefly, a tympanomeatal flap was elevated, leaving the tympanic membrane intact, pressure sensors were inserted, then the flap was secured back in place with cyanoacrylate. Intracochlear pressures were measured with different drill bits, at different speeds (80,000- 20,000 RPM), during removal of outer cortex, mastoid air cells, opening of the facial recess, and cochleostomy. The equivalent sound pressure level in the ear canal was calculated from intracochlear pressure measurements using the middle ear transfer function. Microphones were also placed in the surgeon's ear and within three feet of drilling to capture noise exposure in the room.

Results: Significant intracochlear pressure changes were detected throughout drilling during mastoidectomy, including both continuous and impulsive noise events. Noise measurements exceeding 110 dB SPL were observed in scala vestibular and tympani measurements, with the highest values recorded during cochleostomy portion of the procedure. Substantial noise exposure was observed for the surgeon as well.

Conclusions: Results of our experiment have shown significant changes in intracochlear pressure during mastoidectomy, with levels high enough to potentially induce acoustic trauma. This data will be used to characterize the noise exposure mastoidectomy patients and surgeons experience during mastoidectomy procedures, and will be discussed in relation to occupational exposure to provide guidelines for safe maximum noise exposure and duration for patient and surgeon.

Defining Exclusion Criteria for GAP Inhibition of Acoustic Startle Reflex: Critical Factors for Assessment

Rod Braun[1], Selin Yalcinoglu*[1], Antonela Muca[1], James Warila[1], Aaron Apawu[1], Anthony Cacace[2], Avril Genene Holt[1]

[1]*Wayne State University School of Medicine,* [2]*Wayne State University*

Category: Tinnitus

Background: Gap inhibition of acoustic startle reflex (ASRgi) can serve as a promising technique to test the function of central auditory-related nuclei. Studies have linked inferior colliculus (IC) to be a critical part of the ASRgi pathway. Lesioning the IC diminishes ASRgi. However, the application of ASRgi measures is not as ubiquitous as expected. This may be due to the sheer volume of data obtained, the variability within groups, and the lack of standardized analyses. These factors complicate the distinction of differences amongst experimental groups. In this current study, we have compared two different exclusion criteria for the same ASRgi data set and have analyzed variability within the data set and differences observed across treatment groups.

Methods: Assessment of gap detection was performed in 10 noise-exposed (16 kHz 106 dB SPL 1 hr) Sprague-Dawley rats and nine age-matched controls. Following data collection two methods were applied to the same ASRgi data set. For Method 1 the exclusionary criteria were based on Fmax/Fmax,SOS ratios, where

the maximal response (Fmax) had to be greater than 10% and less than 200% of Fmax,SOS. For Method 2, each animal set their own exclusion criteria before Fmax ratio generation. Exclusion criteria were applied to raw data based on average parameters of startle responses recorded during each measurement. Criteria were set for each animal based on the average time to startle + 1.5 SD (Tmax) and force applied + 2.0 SD (Fmax) for each session.
Results: After the application of the exclusion criteria for method 1 2070 trials (21.3%) were excluded and for method 2 1818 trials (19.4%) were excluded. Method 2 exclusion criteria resulted in less variability when the ratios of "No Gap" to "SOS" startle response (Fmax No Gap /Fmax,SOS) were compared. Startle force patterns acquired with and without a silent gap were compared across parameters and fit a linear mixed-effects model. Significant differences between treatment groups across specific frequencies and intensities were demonstrated with predicted marginal means under gap and no gap conditions. For method 1, there was a ~32% average startle reduction, and for method 2 there was a ~50% average startle reduction.
Conclusions: Allowing animals to set their own exclusion criteria for each session (as done with Method 2) allows the complexity of ASRgi to be accounted for while maintaining the rigor desired for testing and determining significant differences. Reducing uncertainty in testing parameters and applying powerful statistical analyses are critical for utilizing ASRgi as a method to test differences among groups in studies involving damage and or dysfunctions like tinnitus that involve hyperactivity in central auditory nuclei.

Acoustic Estimation of Middle-Ear Input Impedance

Joshua Hajicek[1], James Lewis[2], Stephen Neely*[1]

[1]*Boys Town National Research Hospital,* [2]*University of Tennessee Health Science Center*

Category: Middle and External Ear
Background: Wideband acoustic immittance (WAI) measurements include contributions from sources unnecessary for audition and may compromise the tool's clinical utility. These sources arise from the ear canal and middle ear cavity resonances or portions of the tympanic membrane that do not contribute to stapes displacement. While these sources confound estimates of middle ear impedance, some sources may prove useful for diagnosing specific pathologies. Decomposing measurements with a numerical model of the middle ear is likely to improve the clinical utility of WAI measurements as sources pertinent to a pathology can be separated from those extraneous to the pathology. Additionally, the capability to isolate individual, source-specific contributions to WAI has the potential to provide advanced understanding regarding mechanics in dysfunctional ears. Moreover, if the model's components have anatomical correspondence to middle ear anatomy, it can be used to inform targeted intervention and promote positive outcomes.
Methods: An analog circuit model of ear-canal acoustics and middle-ear mechanics has been developed to improve predictions of middle-ear input impedance and identify contributions from various middle-ear sources. Model components coincide with physiological structures of the middle ear, such as the malleus, incus, icudostapedial joint, stapes, and the cochlea's resistive load. The model also predicts acoustic contributions from the ear canal, middle-ear cavity, and tympanic membrane vibrations independent of ossicular chain motion. To evaluate model performance, its parameters were fit to 28 wideband acoustic absorbance measurements from 14 normal hearing ears. In each ear, measurements were made at two probe insertion depths, a deep but comfortable fit and 2 mm shallower (data were collected as part of a previous study: Lewis and Neely, 2015, Non-invasive estimation of middle-ear input impedance and efficiency J. Acoust. Soc. Am. 138(2)).
Results: The model's absorbance estimates closely matched absorbance measurements and identified the most salient features within and across ears. A shift at low frequencies in middle-ear admittance was observed when probe insertion depth was reduced. This shift was likely due to an induced reduction in ear-canal static pressure that caused corresponding decreases in stiffness of the tympanic membrane and the annular ligament, which the model predicted. The model also predicts a commonly observed notch in ear-canal absorbance near 8 kHz due to middle-ear cavity resonance.
Conclusions: The middle ear network in the present study supports the association of components in the network with anatomical structures in the middle ear. The similarity between the estimated middle-ear input impedance in the present study and published temporal bone measurements supports the validity of this modeling approach. It may be clinically feasible to estimate air-bone gaps in individual ears based on acoustic measurement of ear-canal impedance.

Metabolic Dysregulation and Risk of Developing Persistent Tinnitus in Two US Cohorts

Oana Zeleznik*[1], Raji Balasubramanian[2], D. Bradley Welling[3], Konstantina Stankovic[4], Gary Curhan[5], Sharon Curhan[5]

[1]*Brigham and Women's Hospital, Harvard Medical School,* [2]*University of Massachusetts,* [3]*Massachusetts Eye and Ear, Harvard Medical School,* [4]*Stanford School of Medicine,* [5]*Brigham and Women's Hospital, Harvard Medical School*

Category: Tinnitus

Background: The pathways leading to persistent tinnitus are unclear, but accumulating evidence suggests metabolic dysregulation may play a role in tinnitus pathoetiology. Metabolite levels are 'downstream' of transcriptional and translational processes and reflect genetic, dietary, and environmental characteristics, thus metabolomics studies can offer valuable insights into the influence of metabolic factors on this complex condition.

Methods: We investigated the association of plasma metabolites and risk of developing incident persistent tinnitus among 1789 participants in two large cohorts of women, Nurses' Health Study (NHS) and NHSII. We used logistic regression and nested models adjusted for potential confounders to identify individual metabolites significantly and independently associated with risk of developing persistent tinnitus (defined as daily tinnitus lasting ≥5 minutes) during the 10-year period after the blood collection among 500 cases and 500 1:1 matched controls (mean age 57y, NHSII) and 40 cases and 749 controls (mean age 42y, NHS). Information on tinnitus and potential confounders was collected on biennial questionnaires. Liquid-chromatography mass spectrometry was used to measure 1364 metabolites (Metabolon). We used random-effects meta-analysis to summarize findings from the two cohorts and Metabolite Set Enrichment Analysis (MSEA) to identify metabolite classes associated with risk of developing persistent tinnitus. We used the number of effective tests (NEF-p, regression) and the false discovery rate (FDR, MSEA) to account for testing multiple correlated hypotheses.

Results: In fully adjusted models, 32 individual metabolites (14 lipids, 11 amino acids, 3 cofactors and vitamins, 2 xenobiotics, 1 nucleotide and 1 peptide) were significantly associated (p less than 0.05) with risk of developing persistent tinnitus and showed the same direction of association in both cohorts. Only one amino acid, methionine sulfoxide, remained statistically significant at NEF-p less than 0.05. Perturbations of several metabolic pathways were significantly associated (FDR less than 0.2) with risk of persistent tinnitus. Upregulation of tocopherol metabolism, vitamin A metabolism, acyl choline metabolism, benzoate metabolism and sphingomyelins was associated with higher risk of persistent tinnitus, whereas downregulated metabolism of branched-chain amino acids, nicotinate and nicotinamide, tyrosine, acyl carnitine, the tricarboxylic acid cycle and dihydrosphingomyelins was associated with lower risk. Importantly, several of these findings (sphingomyelins, acyl choline and acyl carnitine metabolism) are consistent with our previous metabolomics findings in two different subsets of participants, one with prevalent tinnitus and one with incident tinnitus at the time of the blood draw in which plasma metabolomics data were assayed using a different platform (MIT/Harvard Broad Institute).

Conclusions: This metabolomics study of incident persistent tinnitus in two US cohorts of women identified metabolites and metabolic pathways significantly associated with risk of developing persistent tinnitus. Together with our previous studies, these findings suggest metabolic dysregulation plays a role in tinnitus etiology. Results from this study could inform discovery of novel disease biomarkers and therapeutic targets for effective tinnitus treatment.

An Integrated High-Speed 3D-Digital Image Correlation and Schlieren Methodology for Studying Tympanic Membrane Exposed to Blast

Anahita Alipanahi*[1], Jonathan Oliveira Luiz[1], Cosme Furlong[2], Jeffrey Cheng[1]

[1]*Mass. Eye and Ear, Harvard Medical School,* [2]*Worcester Polytechnic Institute*

Category: Middle and External Ear

Background: Understanding the dynamics of the tympanic membrane (TM) under high-pressure blast waves is critical for advancing research on damage mechanics of hearing and developing effective protective methods to prevent damage. Limited studies have been conducted to quantify and define how the TM responds to blast waves, particularly on its nonlinear mechanical behaviors and fracture mechanics during such events, due to the lack of effective methodologies and instrumentation. To address this issue, we introduce an integrated methodology that combines high-speed 3D-Digital Image Correlation (3D-DIC) and Schlieren imaging techniques with a customized shock tube. This methodology allows for a comprehensive examination of the TM's rapid response to blast waves, encompassing both its complex deformation and its interaction with blast waves.
Methods: We develop 3D-printed artificial membrane samples to assess the feasibility and reliability of our integrated methodology and apply analytical tools for describing various stages of the membrane's dynamic response to blasts. The core techniques utilized in our methodology include high-speed 3D-DIC, which enables the measurement of the membrane's transient deformations induced by blast waves, and Schlieren imaging that visualizes blast wave flow structures and their interactions with the membrane. Additionally, high-frequency pressure sensors are used to record overpressure values at different locations along the shock tube and near the sample. Pressure measurements are synchronized with imaging acquisitions to provide unique and complete datasets for comprehensive analyses. The accuracy of 3D-DIC is validated by well-established Laser Doppler Vibrometry (LDV), while the solid-fluid interaction visualization is done via high-speed Schlieren imaging.
Results: Results demonstrate the feasibility of our developed methodology, and they also lay the groundwork for future investigations on TM's dynamic response to blasts, including its nonlinear mechanical behavior. We show a good agreement between displacements of the membrane obtained through 3D-DIC and LDV. Schlieren imaging visualizes shock waves propagating in the air and interacting with the membrane, which allows us to investigate the dynamics of the membrane induced by blast including its damage and fracture mechanics.
Conclusions: This study provides a comprehensive approach to recording and quantifying rapid responses of the membrane exposed to blasts. Future studies will be using cadaveric human ears to gain critical insights into blast-induced TM responses and potential injuries in the human ear. The outcomes of this research are expected to have significant implications for the development of more effective protective gear to enhance the safety and well-being of individuals exposed to damaging noises.

Dynamics and Fluid-Solid Interaction of Human Tympanic Membrane Exposed to Blast

Jonathan Oliveira Luiz*[1], Anahita Alipanahi[1], Cosme Furlong[2], Jeffrey Cheng[1]

[1]*Mass. Eye and Ear, Harvard Medical School,* [2]*Worcester Polytechnic Institute*

Category: Middle and External Ear

Background: Comprehending the intricate interplay between blast waves and fluid-solid interactions with the human middle ear is crucial for understanding blast-induced auditory injuries, particularly the rupture of the tympanic membrane (TM), which has been suggested as a potential biomarker for assessing ear damage by blasts. However, there is still a lack of methodologies and instrumentations that are capable of effectively evaluating the rapid dynamic responses of TMs exposed to high acoustical events. In this work, we report a recently developed methodology that integrates high-speed quantitative imaging techniques to a customized instrumented shock tube to evaluate and elucidate the response of the TM to shock waves, shedding light on the biomechanical and physiological aspects of the TM’s dynamics and fracture mechanics.
Methods: Cadaveric human temporal bones with intact middle ear are used in this study. The external ear canal is removed to expose the TM for 3D-Digital Image Correlation (3D-DIC) measurements. The specimens are subjected to blast waves generated by a customized shock tube, with peak pressures ranging from 175 to 200 dB SPL. High-speed Schlieren imaging is applied to record the propagation and fluid-solid interaction of shock waves with the TM at a frame rate up to 150k frames per second (fps). Simultaneously, the rapid deformation of the TM in response to the shock wave is measured using 3D-DIC at the same frame rate. In addition, high-speed pressure sensors record the overpressures along the shock tube and near the TM. These experimental results are utilized to compute strain distributions over the TM surface induced by blasts, which may be associated with its damage. Additionally, a simplified finite element TM model is created to simulate its response to blasts and assist in data interpretation.

Results: The combined experimental and computational results help us gain a holistic understanding of the TM's dynamic response and its fluid-solid interaction with blast waves. The results allow us to estimate the overpressure values that can lead to TM damage. Also, the results provide insight into the vulnerable areas in the TM that may be more prone to rupture while being exposed to blast.
Conclusions: By utilizing advanced experimental techniques and computational modeling, the study enhances our understanding of the complex biomechanics and physiological aspects of TM fracture mechanics. Furthermore, the knowledge gained from this study has immediate applications in improving blast injury prevention and treatment. In addition, it enriches our broader understanding of fluid-solid interactions in biological tissues, with potential applications across various fields.

Middle-Ear Sound Transmission in Cadaveric Temporal Bones: Fresh Versus Fresh-Frozen Human Temporal Bones

Bastian Baselt*[1], Merlin Schaer[2], Alexander Huber[2], Jae Hoon Sim[2]

[1]*University of Zürich,* [2]*University Hospital Zurich*

Category: Middle and External Ear
Background: Human cadaveric temporal bones are widely used in otologic research to reveal sound transmission mechanism via the middle and inner ear or to assess clinical rehabilitation such as middle ear implants. Though Fresh cadaveric temporal bones obtained immediately after death are preferable in such experiments, their availability is limited because immediate access to cadavers after death is difficult and strict regulations are applied for their usage. As an alternative, fresh-frozen cadaveric temporal bones, which are acquired from donors and are kept frozen, are frequently used for experiments in otologic research.
To verify a normal middle-ear functionality of the cadaveric samples before the experimental investigation, the middle ear transfer function (METF), defined as the ratio of velocity of the stapes footplate relative to pressure at the tympanic membrane, is measured and checked in comparison with the corresponding standards.

Freezing the cadaveric samples may cause changes in mechanical properties of the middle-and inner-ear structures and thus sound transmission via the middle- and inner ear. However, such changes have not been investigated systemically.

This study aims to investigate on effects of freezing temporal bones by comparing the METFs between the two types of the cadaveric samples.

Methods: A posterior tympanotomy was performed on 43 fresh-frozen and 57 fresh human temporal bones, keeping all middle and inner ear structures intact. Stimulating the ear drum acoustically, the corresponding vibrations at multiple points on the stapes footplate were measured using a Laser Doppler vibrometer. The measurements were checked for outliers and the magnitude and phase of the METF were calculated. Then, the obtained METFs were compared frequency wise between the two types of cadaveric samples. Further, each METF was fitted with a rational polynomial, allowing for shape-preserving comparison of average frequency response curves between the two groups.
Results: Preliminary results show that most of the cadaveric temporal bones of the two types satisfies the ASTM standards. However, the magnitudes of the METF in fresh-frozen temporal bones were considerably lower than the corresponding magnitudes in fresh ones, especially at lower frequencies.
Conclusions: The results indicate that middle-ear sound transmission is attenuated after freezing, suggesting possible drawbacks when fresh-frozen temporal bones are used in experiments for otologic research.

Rapid Evaluation of Middle Ear Muscle Reflex Level Growth Functions Using a Swept Elicitor

Shawn Goodman*[1], Ehsan Khalili[1], Julia Roemen[1], Rachel White[1], Megan Wright[1], Daniel Tay[1], Jeffery Lichtenhan[2]

[1] *University of Iowa,* [2]*University of South Florida*

Category: Middle and External Ear

Background: Moderate to intense sounds activate stapedius muscle contraction, which alters sound transmission throughout the auditory periphery. This Middle Ear Muscle Reflex (MEMR) may reduce the spread of cochlear excitation and minimize the masking effects of low-frequency background noise. MEMR has also been demonstrated to be a non-invasive metric for assessing cochlear afferent synaptopathy. Clinically, MEMR measurements are made using a 226 Hz probe tone and a series of sound elicitors that are discretely varied in level and frequency, emphasizing MEMR threshold estimation. Here, we investigate additional aspects of MEMR measurements that may advance clinical diagnostics and understanding of how the ear works: onset delay, growth rate, phase, maximum change, and hysteresis. These measures are not commonly assessed, partly because of the time involved using discretely varying clinical test paradigms. We present a novel technique to rapidly collect all the aforementioned measurements in 2 minutes.
Methods: We tested 30 young, normal-hearing participants (16 female) using a 2-channel probe system (ER10X, Etymotic Research). MEMRs were measured with an acoustic probe consisting of an 8-second train of clicks (80 dB peSPL, spaced 50 ms apart). The MEMR elicitor was a broadband noise swept in level from 45 to 115 dB SPL and back to 45 dB SPL in 8 seconds (17.5 dB/s rate). Probe clicks were presented to the left ear, and noise elicitors were presented to the right. Thevenin-source calibration was used to flatten the magnitude of the probe and elicitor (0.1-20 kHz) at the eardrum of each individual ear tested. The 8-second sweep was repeated 15 times per test. The test was repeated four times per subject, with the probe system removed, replaced, and recalibrated for each measurement. Speech-in-noise performance was assessed using the QuickSiN.
Results: We assessed the MEMR using changes in the ear canal SPL of the probe clicks. Each click was time-windowed, transformed by FFT, and expressed relative to the initial clicks in the sweep. Changes were examined in 100-Hz bands from 0.125-8 kHz. When changes in magnitude only were considered, some growth functions were non-monotonic. Considering combined changes in magnitude and phase resulted in monotonic growth functions, which are consistent with expected MEMR-induced changes in middle ear stiffness. Results will be presented for MEMR threshold, growth with stimulus level, average growth rate, maximum change, reflex delay, hysteresis, and test-retest reliability. MEMR measurements will be regressed on QuickSiN measurements.
Conclusions: Multiple parameters of the MEMR can be quantified quickly and repeatably using a swept elicitor.

Podium Session 18 - Cochlear Mechanics: Modelling

10:15 a.m. - 12:15 p.m.

Platinum Salon 5

Using a Cochlear Model to Evaluate the Correlation Between Intracochlear Tuning Sharpness and Stimulus Frequency Otoacoustic Emission Group Delays

George Samaras*[1], Yiwei Xia[1], Julien Meaud[1]

[1]*Georgia Institute of Technology*

Category: Otoacoustic Emissions
Background: Stimulus frequency otoacoustic emissions (SFOAEs) are sounds emitted by the cochlea in response to an external stimulus at its given frequency. According to coherent reflection theory, SFOAEs arise due to small mechanical heterogeneities (termed roughness) in the cochlea. The resulting local reflections of traveling waves receive outer hair cell (OHC) active feedback and form standing waves inside the cochlea that are measurable as SFOAEs in the ear canal. SFOAEs are characterized by rapidly rotating phase and quasiperiodic fine structure. The phase-gradient delay of SFOAEs (NSFOAE), which characterizes the latency of emission has been linked to basilar membrane (BM) group delay (NBM) and tuning sharpness (QERB) in response to pure tones, but these links are not fully understood. This work aims to expand understanding of these relationships.
Methods: The links between NSFOAE, NBM and QERB are evaluated using a computational model of the gerbil cochlea. This model consists of a 3D fluid domain of the cochlear ducts, a mechanical domain to represent vibration of organ of Corti structures, and an electrical domain which drives OHC force feedback

via somatic electromotility. We simulate SFOAEs by introducing roughness to the electromechanical coefficient (couples OHC transmembrane potential to OHC force). A variety of roughness profiles (generated using a random number seed), yield SFOAEs with varied fine structure and phase. This mimics experimentally observed SFOAEs in multiple cochleae. We apply time-frequency filtering to separate SFOAEs into successive reflections. NSFOAE is calculated using the first reflection, facilitating comparison to NBM. To validate the baseline model, we compare model characteristics of pure tone responses (NBM, QERB), SFOAEs (NSFOAE), and group delay ratio (NSFOAE to NBM) to available experiments in gerbils, and the effect of frequency on NSFOAE and QERB. From there, we individually vary three parameters: activity level of mechanoelectrical transduction current (to mimic loss of cochlear amplification), strength of tectorial membrane (TM) longitudinal coupling (to mimic effects of genetic mutations on TM proteins), and impedance of organ of Corti structures.
Results: We establish a clear correlation between cochlear tuning and SFOAE characteristics at the population level when the parameters are varied. While for each individual model (i.e., for one roughness profile), there is no apparent correlation between QERB, and NSFOAE, averaging NSFOAE over many roughness profiles reveals direct correlation between QERB and NSFOAE for varied activity level and TM longitudinal coupling. For varied organ of Corti impedance, there is weaker correlation at the population level.
Conclusions: The results show that measurements of SFOAE group delay can reflect changes in intracochlear tuning due to cochlear activity and TM longitudinal coupling but may not reflect changes due to altered cochlear micromechanics. Overall, this work provides an important step forward in improving assessments of cochlear function using noninvasive SFOAE measurements.

3D Finite Element Modeling of Blast Wave Transmission to the Organ of Corti in Human Ear

John Bradshaw*[1], Marcus Brown[1], Alexander Bien[2], Rong Gan[1]

[1]*University of Oklahoma,* [2]*University of Oklahoma Health Sciences Center*

Category: Inner Ear: Cochlear Mechanics
Background: Military Service members are frequently exposed to blast overpressures (BOPs), and BOP-induced damage to tissues in the ear may contribute to sensorineural hearing disabilities. There is an urgent need to understand blast-induced hearing damage and improve hearing protection devices for Service members. Recently, a 3D finite element (FE) model of the entire human ear with 3-chambered spiral cochlea was developed by our lab. The model is able to predict the cochlear basilar membrane's response to BOP applied at the ear canal entrance. However, the organ of Corti (OC) and hair cells behavior is not included in the model. The present study aims to develop an OC model that incorporates the membranes, sensory cells, and supporting cells of the OC as well as the endolymph fluid in the cochlea to predict their response to blast wave transmission. The preliminary results over 2 ms of blast exposure by using macroscale model-derived basilar membrane (BM) displacements as input are reported in this paper.
Methods: The fluid-structure coupled microscale model of the OC is divided into structural and fluid components. The structural model consists of the BM, Deiters' cells, outer hair cells (OHCs), reticular lamina (RL), hair bundles (HBs), tectorial membrane (TcM), Hensen's cells, inner pillar cells, outer pillar cells, inner hair cells, and osseous spiral lamina. The fluid components represent the endolymph and scala media. Both models are connected using the System Coupling service in ANSYS Workbench, with ANSYS Mechanical and Fluent simulating the structures and fluids, respectively. The BM displacement 16.75 mm from the BM base end calculated by the macroscale human ear model during the transmission of a 31 kPa BOP wave was applied as input to the center of the BM in the OC model, and the simulation was run for 2 ms.
Results: The maximum displacement of the OHCs, RL, and HBs was derived from the OC model to illustrate the movement of the sensory cells response to the BOP wave. In addition, the displacement distribution on the RL and TcM was assessed as a potential indicator of where damage to those membranes is most likely to occur. Similarly, the maximum principal strain on the OHCs, RL, and HBs was used as an indicator of blast-induced tissue injury.
Conclusions: This microscale OC model is the first 3D FE model of the OC including coupled structural and fluid components in organ of Corti. It is important progress for the simulation of blast wave transmission through the peripheral auditory system and for a better understanding of blast-induced hearing loss. Future

work on this model will focus on properly simulating the nonlinearity of the membranes and supporting cells and validating the modeling results.

Acknowledgement: This work was supported by DOD award HT9425-23-1-1013.

Steep Cochlear Filters, Noise Squelching and Spatial Filtering: Everywhere, All at Once in Your Ears!

Alessandro Altoe*[1], Christopher Shera[1]

[1]*University of Southern California*

Category: Inner Ear: Cochlear Mechanics

Background: Cochlear transfer functions measured at one location—a.k.a. "cochlear filters"—are characterized by a steep roll-off as the frequency increases above the local best (responding) frequency (BF). The functional role of the sharp "cut-off" is not well understood, although it was previously hypothesized that the cut-off plays a pivotal role in encoding sound frequency—whereas BF dramatically changes with level, the cut-off remains fairly invariant. Less hypothetical in nature, our work elucidates the role of the steep cut-off to improve signal detection in cochlear-like amplification strategies.

Methods: We first study the effects of signal amplification in one-dimensional active gain media, including internal noise sources that generate waves propagating in two directions. We demonstrate that the optimal strategy for boosting the signal-to-noise ratio (SNR) at a given location "x" requires high gain basal to "x", followed by rapid attenuation (i.e., sharp cut-off) beyond it.

Results: This strategy of amplification followed by a sharp cut-off is precisely how the cochlea processes traveling waves of a given frequencies. That is, the cochlea implement a near-optimal strategy to maximize SNR near the characteristic frequency (CF) location. Simulations in cochlear models show that this strategy greatly boosts SNRs, both to narrow- and broadband signals.

Conclusions: Regardless of the many unknowns in cochlear mechanics, our work demonstrates that cochlear wave amplification greatly enhances cochlear SNR. The strategy is simple and biologically robust: It boils down to a peculiar form of spatial filtering where waves coming from the "signal side" are amplified, while waves coming from the direction where there is noise but no signal are squelched. This noise "squelching" action manifests in measured transfer functions as the steep high-frequency roll-off, and we demonstrate here that it is crucial for cochlear performance.

Did Constraints Imposed by Outer Hair Cell Electromechanics Drive Cochlear Evolution?

Richard Rabbitt*[1], Nastaran Gholami[1]

[1]*University of Utah*

Category: Inner Ear: Cochlear Mechanics

Background: Outer hair cells (OHCs) first appeared approximately 125 million years ago, and over time endowed mammals with the ability to hear frequencies far exceeding other vertebrate species. We hypothesize that electromotility appeared in hair cells early, and that the cochlea subsequently evolved to take advantage of the somatic motor. We previously demonstrated, based on analysis of piezoelectric OHCs, that electro-mechanical power conversion is tuned to a specific characteristic frequency (CF) set by cell length, level of prestin expression, resting basolateral conductance and properties of the mechano-electrical transduction (MET) current (J. R. Soc. Interface 2023, 2020220762). Here, we examine in silico if evolutionary pressure to maximize the frequency bandwidth of hearing might have constrained cochlear design to meet requirements imposed by OHCs.

Methods: We built a relatively simple 3-chamber mathematical model of the cochlea by applying conservation of momentum and conservation of mass to the fluid-filled scala vestibuli, the scala tympani and deformable organ of Corti (cochlear partition, CP) in one spatial dimension. Fluids were modeled using high-frequency Womersley flow, which accounts for hydraulic resistance and hydraulic mass resisting longitudinal

fluid velocity in each chamber. The portion of the reticular lamina (RL) overlying OHCs (denoted as the hotspot, HS) was allowed to move relative to the basilar membrane (BM) driven by changes in OHC length, while the remaining regions of the RL were constrained to move with the BM. OHC biophysical properties were fixed at the optimum values for electro-mechanical power conversion efficiency. Spatially-dependent scalae areas and mechanical properties of the BM and RL were then selected to match the impedance of OHCs at each location, thereby designing the cochlea around OHC constraints. A two-state Boltzmann driven by deflection of the reticular lamina was used for mechano-electrical transduction, and gain set near the critical point of dynamic instability. Equations were solved numerically and compared to a long-wave WKB approximation.
Results: Similar to OCT data in the cochlea (Nat Commun 2018, 9(1):3054), numerical simulations reveal distinctly different compressive nonlinearities for the traveling wave on the BM compared to the traveling wave on the RL HS. Amplification of the BM traveling wave was restricted to the region near CF, while amplification of the RL traveling wave at the HS occurred primarily near CF but also at every spatial location basal to CF. The 3-chamber WKB solution reveals that traveling waves on the BM and the RL arise from the superposition of two distinct traveling wave modes, each with a unique location-dependent wavenumber and mode-shape.
Conclusions: Results suggest the cochlea might have evolved to maximize electro-mechanical power conversion under rigid constraints imposed by OHC biophysics.

A Radial-Slice Model of the Gerbil Cochlea With Reticular Lamina Motion Amplification

Julius Kraut*[1], Sunil Puria[2]

[1]*Massachusetts Eye and Ear,* [2]*Harvard Medical School, Mass. Eye and Ear Infirmary*

Category: Inner Ear: Cochlear Mechanics
Background: A pressure difference across the organ of Corti sets it into motion. What happens after that in the active cochlea is the subject of this work. Shearing between the tectorial membrane and the reticular lamina (RL) produces hair bundle (HB) deflections resulting in a mechano-electric transduction current that produces an intercellular receptor potential (VRP). The outer hair cells (OHC) generate forces due to electromotility, which is essential for increasing the motion of the organ of Corti. However, amplification critically depends on the phases along the feedback path. Some cochlear models have assumed that OHC force is directly proportional to the basilar membrane (BM) pressure (e.g., Motallebzadeh et al., 2018), which ignored the various transfer functions between the BM pressure and OHC force generation. The goal of this study is to determine the magnitudes and phases introduced at different steps along the feedback path leading to amplification.
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 OHCs containing one cell each. Floquet boundary conditions simulated longitudinal wave motion (similar to Tubelli et al., 2022 bioRxiv). A physiologic model representing prestin activated OHC electromotility was used (Santos-Sacchi et al., 2019). For each OHC, HB displacement determined its VRP, which was used as the input for the OHC model that generated a frequency dependent force. A frequency dependent phase was introduced to represent the phase between the HB displacement and VRP. Another parameter that we explored was the RL Young's modulus which determined its stiffness and subsequent HB displacement. The model was solved in response to a prescribed fluid velocity at the scala tympani wall as a proxy for the oval window.
Results: Only a small range of phases introduced between HB displacement and OHC VRP produced OHC forces capable of amplifying RL displacement. The phase needed to achieve amplification increased with stimulus frequency, which corresponds to a delay. Stiffness of the RL had a significant impact on the generated OHC force. Some low values produced high forces but motions were asynchronous across the three rows. Higher values resulted in the three OHCs moving in a synchronized fashion, which amplified RL displacements. Experimental results that RL3 (OHC3) moves more than RL1 (OHC1) (Cho and Puria, 2022) were evident in the model.
Conclusions: Despite incorporating a physiological model for OHC electromotility, results indicate that phase needs to be introduced between HB displacement and OHC VRP to enable amplification at RL. RL stiffness

is a crucial parameter as it coordinates the movement of the three OHCs to be in phase. [Work supported by R0 DC07910 from the NIDCD of NIH.]

Analyzing Cochlear Amplification Through a Two-Dimensional Organ of Corti Micro-Electro-Mechanical Model

Wen Cai*[1], Karl Grosh[1]

[1]*University of Michigan*

Category: Inner Ear: Cochlear Mechanics

Background: The active, nonlinear electromechanical function of mammalian outer hair cells (OHCs) is necessary for sensitive, frequency specific hearing. A piezoelectric-like OHC constitutive behavior is hypothesized to realize a high frequency, cycle-by-cycle conversion of electrical to mechanical energy to boost cochlear responses at low-level sound. This hypothesis has been challenged for decades because of the low-pass OHC transmembrane impedance which attenuates the active energy transfer process by reducing the mechanoelectric transducer (MET) current-induced voltage at high frequencies. In this study, we analyze a coupled electromechanical model of the organ of Corti (OoC) specifically including the OHC transmembrane impedance to explore mechanisms capable of producing high-frequency amplification through system-level coupling between different components of the OoC.

Methods: We use a thermodynamically consistent constitutive theory to describe the rate-dependent response of an isolated OHC. Both this model of somatic electromotility and of a model of MET function are linearized around the equilibrium position to simplify the analysis. Kinematic analysis is used to relate the motional constraints among the different components of the OoC components and then Lagrange's method is employed to derive the governing equations of the OoC vibration. In the electrical domain, Kirchhoff's laws are used to obtain the governing equations. Because of the OHC piezoelectric-like behavior and the hair bundle deflection-related MET current, the OoC model represents the coupled electromechanical system which then predicts responses to either electrical or mechanical stimulation.

Results: We find that this OoC model is able to fit the in vitro experimental data of the hair bundle geometric gain and the BM-RL relative motion under mechanical and electrical stimulation. To investigate the high-frequency amplification, the mechanical responses at a location where the characteristic frequency is greatly above the RC cut-off frequency are examined. We find that the transmembrane impedance together with other parameters, such as structural damping, TM resonance, and prestin rate dependence, introduce a phase delay between the mechanical and electrical responses, and how this phase difference counteracts damping.

Conclusions: The OHC amplification effect is a combined influence of the global system parameters. As present in many models and mentioned in recent literature, the RC filtering of the OHC must be included in the modeling, and is a feature rather of the response rather than some inherent "problem".

The Role of the Osseus Spiral Lamina on Human Cochlear Mechanics Examined With a Finite-Element Model

Andrew Tubelli*[1], Paul Secchia[2], Hideko Heidi Nakajima[2], Sunil Puria[2]

[1]*Mass. Eye and Ear Infirmary,* [2]*Harvard Medical School, Mass. Eye and Ear Infirmary*

Category: Inner Ear: Cochlear Mechanics

Background: The human cochlear partition differs from that of typical lab mammals. In the base, the relative radial width of the osseous spiral lamina (OSL) is approximately ten times that of the basilar membrane (BM) whereas the ratio is less than one at the apex (Raufer et al., 2020 JARO). The osseous spiral lamina is formed by calcified porous plates. The structure of the OSL in human, measured with laser Doppler vibrometry and optical coherence tomography (OCT) (Stenfelt et al., 2003 Hearing Research; Raufer et al., 2019 PNAS), indicates a more compliant structure in comparison to the essentially immobile OSL commonly found in laboratory animals. Additionally, there is a soft tissue bridge region between the OSL and the BM proper that is present in humans but not in animals. As such, the foot of the inner pillar cell, which acts as a pivot point for the motion of the organ of Corti (OoC) that ultimately translates to hair bundle transduction, is separated from the tip of OSL, whereas it is not in animals. This structure of the human cochlear partition is hypothesized

to have a profound effect on hair bundle transduction. The present study examines this OSL structure-function relationship with computational modeling.
Methods: We created a finite-element model of the human cochlear partition in COMSOL Multiphysics as an uncoiled cochlear box model. The partition consists of a BM, bridge, and an OSL sectioned into three radially spaced regions to account for regions of differing porosity (Braga et al., 2023 JARO). At the apical end, a helicotrema region consisted of a tapering of the partition with fluid coupling between the scalae. The model was stimulated with a velocity input at the oval window to simulate the stapes and terminated with a round window membrane. To calibrate the model, the output was compared with the expected Greenwood (1990 JASA) frequency-place map for the passive human cochlea and several experimental motion measurements, including radial profile and frequency response measurements in fresh human cochleae using OCT.
Results: The most sensitive parameters of the model used to tune results to experimental data were the Young's moduli and thicknesses of the OSL and BM, i.e., the parameters that most impact stiffness of the partition. Further altering the Young's modulus of the OSL to account for porosity has an important impact on the motion of the BM. In addition, preliminary results show that an OoC structure on top of the partition is instrumental in capturing the radial profile and phase response.
Conclusions: Future work on the model will include additional parameter studies including a detailed OoC structure on top of the partition. [Work supported by R01 DC013303 from the NIDCD of NIH.]

Modeling the Effect of Intracellular Calcium on Hair Bundle Adaptation

Varun Goyal*[1], Karl Grosh[1]

[1]*University of Michigan*

Category: Inner Ear: Cochlear Mechanics
Background: The intracellular calcium concentration in cochlear outer hair cells (OHCs) has been observed to regulate the adaptation of the mechano-electric transducer (MET) current in the OHC hair bundle (HB), manifest shifts in the activation curves, and alter the resting state of the displacement and MET current (Caprara et al., Science Advances 6, no. 33 (2020)). We developed a three-row model of an isolated HB to predict the effects of changes in intracellular calcium.
Methods: Our three-row model includes the nonlinear kinematics, viscoelastic HB mechanics, and the nonlinear response of the MET channels coupled to adaptation complexes in each of the upper tip link densities (UTLDs). A two-state Boltzmann function models the opening probability of the gating mechanism in each of the lower tip link densities. We replicated the experimental three-force-pulse protocols of Caprara et al., Science Advances 6, no. 33 (2020), where the first pulse was applied from rest, and the second and third pulses were preceded by an adaptation step. The experiments used two intracellular calcium concentrations, one near-normal and the other low (modulated through BAPTA). We altered our model's resting properties of UTLD putative adaptation complexes to simulate the different calcium concentrations.
Results: We denote the near-normal calcium concentration case as the NCC model and the low calcium concentration case as the LCC model. For both cases, the displacement and current profiles of the HB response are quantitatively matched. Rightward shifts due to adaptation steps in the activation curves follow the trends of the experiments for both calcium concentrations. The reduction of calcium in our model reduces adaptation, as seen in experiments. Furthermore, a leftward shift seen in experiments upon calcium chelation is observed from the model, with a shift prediction error of 0.1%, 8%, and 30% for the third, second, and first pulses, respectively. Our linearized model predicts three time constants- 0.75 ms, 5.5 ms, and 9 ms from the NCC model and 0.66 ms, 6.4 ms, and 13 ms from the LCC model, where the lowest value represents the activation, and two larger values represent the slow adaptation time constants. As noted in experiments, the slow adaptation time constants are faster in NCC than in LCC.
Conclusions: We modeled different intracellular calcium concentrations to study their effects on adaptation. We found that varying properties of the adaptation complex in the model ensure lower feedback, reducing the MET channels' reclosure magnitude and imitating the consequences of decreased intracellular calcium seen in experiments. The presence of the three time constants is a hallmark of our three-row model (where a two-row model would give only two). As a next step, we will investigate the correlation between creep and slow adaptation and use our model to provide mechanistic explanations for various observed phenomena.

Poster Session 4

1:15 p.m. - 3:15 p.m.

Marquis Ballroom

T1. A Multi-Lab EEG Replication and Extension of "Tagging the Neural Entrainment to Beat and Meter"

Karli Nave[1], Erin Hannon[2], Joel Snyder*[2]

[1]*University of Western Ontario,* [2]*University of Nevada, Las Vegas*

Category: Auditory Cortex and Thalamus: Human Studies

Background: There is a great need to replicate influential studies in auditory cognitive neuroscience, but it is expensive and time-consuming to do so, resulting in a paucity of such studies. Nozaradan et al. (2011) found enhanced frequency-tagged EEG activity at beat-related frequencies when listeners imagined a pattern as being in a duple or triple meter while presented an ambiguous isochronous auditory stimulus. However, it is unclear whether this represents repeatable evidence for conscious perception of the beat being reflected in brain activity–especially given the small participant sample (N=8)–and whether this effect is moderated by musical or dance training.

Methods: The original study was replicated in 13 different laboratories with a total of 154 participants, all using the same pre-registered and provisionally-accepted protocol, with an added behavioral task that measured beat perception on each trial. We estimated the meta-analytic effect sizes for differences between imagery conditions (duple vs. passive, triple vs. passive, duple vs. triple), as well as moderating effects of music and dance training. Non-registered analyses of variance (ANOVAs) were also performed to detect significant effects of imagery on beat-related brain activity in a large sample of participants.

Results: Meta-analytic effect sizes across labs expressed as voltage difference between different imagery conditions (mean=0.04 uV) were consistently smaller than in the original study (mean=0.16 uV), and all confidence intervals encompassed 0 uV. No moderating effects of musical or dance experience occurred. Exploratory ANOVAs showed a significant effect of imagery condition for beat-related frequencies, but effect sizes were considerably smaller (mean partial eta-squared=0.11) than in the original study (mean partial eta-squared=0.67).

Conclusions: There may be a small effect of imagery on beat-related brain activity. Moderating effects of musical or dance training may require much larger samples to detect. Our finding of smaller effect sizes is consistent with recent studies across many fields, and underscores the need to widely embrace practices such as pre-registration, a priori power analysis, and replications.

T2. Interactions Between Fundamental Frequency and Spectral Centroid in the Cortical Encoding of Pitch and Timbre

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[1]*University of Minnesota*

Category: Auditory Cortex and Thalamus: Human Studies

Background: Fundamental frequency (F0) and spectral centroid (Fc) usually define the pitch and timbral brightness of a harmonic complex tone, respectively. Perceptual studies have suggested that the perception of these two features is interdependent. In terms of neural encoding, tuning to both dimensions has been observed in human auditory cortex, but it remains unclear whether the F0 mapping within auditory cortex is dependent on Fc, or vice versa.

Methods: This study used functional magnetic resonance imaging (fMRI) to measure BOLD responses of human participants when they listened to harmonic complex tones varying in both F0 and Fc. Different encoding models were built and tested on the BOLD responses to reflect independence and interaction of the cortical tuning of these two features.

Results: We found that the model incorporating interaction between the two dimensions outperformed the independent model in auditory cortical regions, especially for those near the boundary between Heschl's Gyrus (HG) and Heschl's Sulcus (HS).
Conclusions: Our results suggest that the cortical mapping of F0 and Fc are interdependent, providing evidence for the interaction between pitch and timbre within auditory cortex. [Supported by NIH grant R01 DC005216.]

T3. Chimeric Music: Exploring Pitch-Time Interaction on the Neural Encoding of Music Expectation

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Category: Auditory Cortex and Thalamus: Human Studies
Background: Cognitive processes involving prediction and anticipation are activated when listening to music, shaped by its inherent structure. Studies investigating the brain response to music sequence have shown the violation of expectation elicits specific neural responses (e.g., MMN). An EEG study (Di Liberto et al, 2020) using continuous music with a time-resolved regression analysis (temporal response function; TRF) showed the combination of acoustic and expectation features better explain the variance of corresponding brain activity than with acoustic features alone. These musical expectation features are derived from a predictive Markov chain model (IDyOM; Pearce, 2005), encapsulating surprise and uncertainty of note pitch and timing. However, the expectation feature of each dimension (i.e., pitch or time) is computed solely on its respective probabilistic structure. Perceptually, music expectation is shaped with an interaction between the two dimensions, and isolating them individually may not fully explain the underlying expectation process. In this study, we incorporated the same TRF paradigm and proposed a new design of stimulus – "Chimeric Music", to investigate how pitch and time interact on the neural encoding of music expectation.
Methods: Our basic stimuli were European folk songs selected from the Essen Folksong Collection. We designed chimeric music where we paired two distinct songs and exchanged their pitch contours and note onset-times to create two new melody lines. Therefore, within each set of stimuli, we had two intact music and two chimerae with the same acoustical and expectation statistics. Two ABR channels and 32-channel EEG were used to record the response to these two different categories at the subcortical and cortical level. The TRF was used to relate brain activity to music features including acoustical features (spectral flux and onset time) and expectation feature extracted from IDyOM model (pitch surprisal, onset time surprisal, pitch uncertainty and onset time uncertainty). Inverse modeling was also used to reconstruct the EEG from those features.
Results: Preliminary data show that, consistent with previous studies, using both acoustical and expectation features better correlate with neural activity for intact music. This also holds true for chimeric music, indicating there is an expectation process during music listening even if the music is less structured. However, we have seen a smaller expectation effect (the correlation coefficient difference between the reconstruction of expectation model and acoustic only model) in chimeric than intact music (Cohen's d greater than 0.5).
Conclusions: Chimeric music provides a new way to study music expectation by decoupling musical pitch and timing while keeping the marginal statistics of all stimulus features intact. The data collected in this experiment will allow us to test for joint encoding of musical expectation features that is not accounted for by musical models.

T4. Open Board

T5. Photobiomodulation Alters Cell Proportions in Auditory Cortex in Photo-Thrombosis Hearing Loss in Vivo Model

Ji On Park*[1], So-Young Chang[2], Jin-Chul Ahn[3], Ji-Eun Choi[1], Min Young Lee[1], Jae Yun Jung[1]

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Category: Auditory Cortex and Thalamus: Structure and Function

Background: The sudden hearing loss can be caused by ischemia to inner ear. The pathologies can result in secondary symptoms such as tinnitus and aural fullness. Moreover, persistence of these symptoms could lead to psychiatric disease such as depressions and anxiety. These cascades of symptoms could be due to abnormal connections and signaling pathway in brain. Photobiomodulation (PBM) is a therapeutic tool that application of red and near infra-red (NIR) light radiation. PBM elicits a beneficial biological response in cells and tissue. PBM is used as a treatment for various neuronal diseases and disorder. In this study, we analysis effect of transtympanic (transcanal) PBM treatment on cells in the cochlea and auditory cortex in a photo-thrombosis (PT) hearing loss model.
Methods: We used male Sprague-Dawley rats (7 weeks old) for PT models. Fore development of PT, the rats were injected with Rose Bengal (RB) in the femoral vein. After an intravenous injection of RB solution, the vessel was occluded with a photochemically induced thrombus with transcanal laser irradiation (532nm, 175mW, 15 min). In this study, we used transcanal laser of central wavelength 808nm for PBM which was administrated for rat for 5 times (1 time/day, 270 J/cm², 330 min). Hearing of animal were measured with ABR. Brains and cochleae were harvested 1 week after insult. Immunohistochemistry (IHC) was performed to analysis the effects of PBM on hair cell recovery and CNS neuronal cell recovery.
Results: In the PT model, mean value of the threshold measured by auditory brainstem response (ABR) were increased after PT. On day 3, 7 after surgery, the PT group's value of threshold was increased compared to the control group. But, the PT+PBM group's value were tend to be decreased compared to the PT group. After PT, deterioration of cochlear structure was observed. In the PT+PBM group, slight less cochlear structure damage was shown. As to auditory cortex, the PT+PBM group's anti-Neuronal Nuclear (NeuN) immunofluorescence intensity increased compared to the PT group. However, Anti-Glial Fibrillary Acidic Protein (GFAP) that recognized astrocyte immunofluorescence intensity in PT+PBM group tended to be decreased compared to the PT group. These results show PBM alter cell proportions in auditory cortex in PT model.
Conclusions: In this study, the PT models resulted the hearing threshold shift and the damage in the cochlear. Transcanal PBM immediately after PT caused slight increase of hearing threshold and hair cell survival. Transcanal PBM also affected auditory cortex. Possible neurons have increased, and possible astrocyte has been decreased with PBM after PT insult.

T6. Characterizing the Morphology of Auditory Evoked Loudness Dependent fNIRS Responses in Sleeping Infants

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Category: Auditory Cortex and Thalamus: Structure and Function
Background: Functional near-infrared spectroscopy (fNIRS) is a developing technology that uses near-infrared light to image brain activity in the surface layers of the cortex. It measures changes in oxyhaemoglobin (HbO) and deoxyhaemoglobin (HbR) in response to stimuli. The haemodynamic response can measure the brain's response to distinguishing between different temporal structures in the acoustic signal and can measure hearing ability in auditory neuropathy. The features of fNIRS make it suitable for objectively assessing hearing levels by examining the morphology of sound-evoked fNIRS response.
Methods: Twelve sleeping infants with no known hearing loss participated in the study. A natural recording of the /ba/ speech token was used as the stimulus, which was trimmed and concatenated into a 5.4 s stimulus block. The stimulus was presented monaurally between 35 to 90 dB SPL using an insert earphone. fNIRS responses were recorded from bilateral pre-frontal and temporal regions.
Results: We observed a positive peak at around 5 – 6 s from stimulus onset and followed by a negative trough at around 10 – 20 s from stimulus onset. The amplitudes and latencies of this response varied with different stimulus intensity levels. To better understand how the independent neural responses are associated with auditory processing in sleeping infants, we conducted Independent Component Analysis (ICA) on the data. The results showed that two ICA components with the highest signal power consist of a positive component and a negative component. Our ICA result confirmed our hypothesis that there are two independent response mechanisms that contributes to the auditory evoked fNIRS responses the sleeping infant.

Conclusions: Our findings suggest the importance of the multiple independent response mechanisms in the analysis of sleeping infant fNIRS responses to speech sounds.

T7. Neural Correlates of Periodicity Pitch Perception in Mouse Auditory Cortex

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Category: Auditory Cortex and Thalamus: Structure and Function

Background: Pitch perception is a fundamental component of hearing that is critical for everyday listening tasks, such as speech comprehension and music appreciation. Temporal periodicity is an acoustic cue for pitch perception, yet it is poorly understood how the auditory system transforms temporal periodicity into 'periodicity pitch'—a percept that is heard in-common across sounds with the same temporal periodicity but different spectral profiles. Here, we used mice as an animal model to study the neural basis of periodicity pitch in auditory cortex. Because mice have poor hearing below 2 kHz, and their auditory filters above 2 kHz blur spectral profiles, we hypothesized that pitch perception in mice may largely derive from temporal periodicity in sound and is represented in auditory cortex as periodotopy.

Methods: To investigate periodicity pitch perception, we trained a cohort of mice on a go/no-go auditory operant conditioning task in which they were rewarded for licking a waterspout after hearing sounds with a low fundamental frequency (F0), and punished with a time-out after licking in response to a high F0. The stimulus set included 4 kinds of periodic broadband sounds (2-45 kHz), each with different spectral profiles: harmonic stacks (HS), click trains (CT), iterated ripple noise (IRN), and amplitude modulated noise (AMN). Each type of sound was presented in randomized order, and with F0=55 Hz (go-signal) or F0=330 Hz (no-go-signal). We used in vivo widefield imaging of auditory cortex in awake transgenic thy1-GCaMP6s mice to study cortical periodotopy in response to HS, CT, AMN, and IRN stimuli.

Results: Our preliminary behavioral data show that mice learned to correctly discriminate F0 (d' greater than 1) for each type of sound, except IRN. However, we found that mice could learn to correctly discriminate IRNs when spectral cues were made available by increasing F0. Importantly, our results suggest that mice initially trained only with HS immediately generalized the task to HS, CT, and AMN stimuli. Our preliminary widefield imaging results show that HS, CT, and AMN each have similar periodotopy in cortical space, though the exact spatial pattern differed across individual mice. In contrast, IRN showed weak periodotopy that was often spatially inconsistent and more variable compared to the other stimuli.

Conclusions: Our results suggest that mice may perceive periodicity pitch. Furthermore, the robustness of periodotopy for a given sound may correlate with how well mice hear periodicity pitch.

T8. Myeloarchitecture of the Feline Auditory Cortex

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Category: Auditory Cortex and Thalamus: Structure and Function

Background: For more than a century, neuroscientists have been interested in how anatomical features vary across subregions of the cerebral cortex, and how this structural variability relates to regional differences in neurophysiology. Efforts to identify the neural correlates of complex behaviours have benefited from advances in functional neuroimaging; however these approaches have inherent limitations (e.g., functional localizers are of little use in identifying deafferented sensory cortex). Thus, there is renewed interest in developing approaches to parcelating cortex based on structural features. Accordingly, the current study sought to determine whether regional differences in the density of myelin (the fatty substance that ensheathes neurons to make communication between brain regions more efficient) might be useful for identifying subregions of the auditory cortex.

Methods: Brains were sectioned and myelinated fibers were visualized using a modified Gallyas protocol. The boundaries of the 13 subregions comprising the auditory cortex were defined using patterns of neurofilament expression, and stereological estimates of myelin density were computed for each region using a Spaceballs probe. These values were compared to in-vivo and ex-vivo quantifications derived from myelin-

sensitive MRI sequences to determine the extent to which these different approaches converge on common estimates.
Results: Stereological estimates of myelin density varied across auditory cortical regions in a way that conformed to previously described functional hierarchies. Namely, regions comprising the auditory core (A1 and AAF) were most heavily myelinated, with decreasing myelin density in higher order areas. Additionally, histologically-derived estimates were highly correlated with values obtained using diffusion-weighted and magnetization transfer saturation imaging.
Conclusions: Myelin density in the feline auditory cortex varies by region, and does so in a manner that conforms to proposed functional hierarchies. This is in accordance with previous neuroimaging work in humans and neuroanatomical studies in non-human primates that have shown increased myelin density in core auditory regions. Moreover, we have shown that myelin-sensitive imaging sequences reliably recapture this distribution, suggesting that auditory cortical subregions can be delineated noninvasively. Importantly, these studies suggest that regional differences in myelin density may allow for the parcelation of auditory cortex in cases where functional localizers are inappropriate (e.g., identifying the boundaries of auditory cortex in deaf individuals).

T9. A Novel Approach for the Automation of Spiral Ganglion Neuron Quantification Using an ADARB1 Antibody

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Category: Auditory Nerve
Background: Spiral ganglion neurons (SGNs) are the primary neurons that transmit sound sensation in the inner ear. These neurons degenerate under a number of conditions, including auditory neuropathies, concussions, and aging. Accurate quantification of SGNs is essential for studying auditory disorders and evaluating therapeutic interventions. However, counting SGNs is an arduous, time-consuming process, due largely to their high density crowding as well as their lack of immunoreactivity for nuclear enriched NeuN, a traditional marker for neuron quantification. As the RNA editing enzyme ADARB1 has been reported to be enriched in the nuclei of glutamate receptor expressing neurons, we investigated a novel approach utilizing ADARB1 immunofluorescence and Image-J software to automate the counting of SGNs.
Methods: Mid-modiolar cochlear sections from 3-week old CD1 mice were immunolabeled using antibodies targeting ADARB1 and neurofilament. Images were acquired by laser scanning confocal microscopy using a 40X objective. Manual thresholding, noise removal, and the analyze particles function were used in ImageJ (Fiji) to quantify ADARB1-positive SGN nuclei. The accuracy and precision of automated counts were compared to those acquired by an independent observer.
Results: ADARB1 immunolabeling provided specific and robust staining of SGNs in cochlear sections. The automated image-J counting method offered a high degree of accuracy compared to manual counts and significantly decreased the time and effort required to quantify SGNs.
Conclusions: This study provides a novel and reliable method for quantifying SGNs using anti-ADARB1 immunofluorescence and open-source Image-J software. The automated counting approach should enhance the efficiency and reproducibility of SGN quantification and reduce bias. Further validation with larger sample sets, other ages, species, and pathological conditions will enhance the level of evidence. Still, this method has great potential to improve our investigations of auditory disorders and enhance the evaluation of neuroprotective interventions. The automation of neuron quantification can also be applied to other areas where neurons have enriched expression of ADARB1.

T10. Auditory Phenotype in a Mouse Model of Neurofibromatosis Type 2 is Associated With Proliferation of Glial Cells and Peripheral Myelinopathy

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Category: Auditory Nerve

Background: Merlin, also known as neurofibromatosis type 2 (NF2), is a tumor suppressor gene. Its loss leads to development of Schwann cell tumors, predominantly of the vestibular portion of the 8th cranial nerve. Schwannomas of the vestibular nerve are typically associated with varying degrees of sensorineural hearing loss (SNHL), but the etiology of this impairment remains to be elucidated. Leading current theories implicate the secretion of pro-inflammatory and potentially neurotoxic factors. In this study, we examined the auditory and vestibular nerves in a NF2 mouse model (Periostin-Cre; Nf2flox/flox) to investigate the underlying cochlear NF2 phenotype with an emphasis on cochlear Schwann cells.

Methods: Periostin-Cre; Nf2flox/flox mice were compared to littermate controls with FVB/NJ background to characterize SNHL at 2 months and at later ages. For analysis of myelination, nodes of Ranvier and heminodes, cochlear whole mounts were stained for nodal (Gliomedin), paranodal (Caspr) and myelin (MBP) markers and imaged by confocal microscopy. Frozen cochlear sections were analyzed for glial and neuronal markers; Schwann cells and neurons were quantified per 100 µm area in Rosenthal's canal, Scarpa's ganglion, and the auditory nerve trunk on serial sections. Cochlear spiral ganglion mRNA was isolated and quantitative PCR was performed for Schwann cell markers.

Results: At 2 months of age, months prior to onset of hearing loss and development of proliferating Schwann cell tumorlets, Periostin-Cre; Nf2flox/flox mice have normal ABR and DPOAE thresholds, but the first peak of the ABR waveform has lower amplitudes and longer latencies than control mice. Analysis of heminodes and nodes of Ranvier was consistent with myelinopathy. At later ages, ABR demonstrated significant hearing loss in all NF2 mice compared to control mice.

Conclusions: Here we demonstrate that the Periostin-Cre; Nf2flox/flox mouse model displays a cochlear phenotype that may be associated with dysregulation of myelination and a subsequent neuronal dysfunction that initially resembles synaptopathy and progresses to loss of spiral ganglion neurons. These findings may, in part, explain the SNHL in patients with vestibular schwannomas.

T11. Lateral Olivocochlear Efferent Inputs Contribute to Setting Auditory Nerve Fiber Spontaneous Firing Rates in Vitro

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Category: Auditory Nerve

Background: The lateral olivocochlear (LOC) efferent fibers originate from the lateral superior olive in the brainstem. LOC fibers project towards the cochlea to make axo-dendritic synapses onto the unmyelinated endings of type I auditory nerve fibers (ANFs), close to where they contact the inner hair cells (IHCs) to form the first afferent synapse in the auditory pathway. LOC fibers are known to release multiple neurotransmitters, including acetylcholine, dopamine, GABA, and neuropeptides like Neuropeptide Y, Substance P and CGRP (Puel, 1995). In vivo intracochlear perfusion of acetylcholine (Felix and Ehrenberger, 1992) or dopamine (Ruel et al., 2001) during single unit recordings from ANFs in vivo have shown changes in ANF firing rates. The LOC efferent system is thought to operate as a feedback loop, dynamically changing ANF activity in response to sound exposure (Wu et al., 2020; Frank et al., 2023), and possibly protecting the IHC afferent synapse from noise damage (Maison et al., 2012). However, it is unclear how the LOC efferent system precisely regulates the IHC/ANF encoding properties, and which underlying mechanisms contribute to this regulation.

Methods: Channelrhodopsin was expressed in LOC fibers using the Cre-dependent choline acetyltransferase (ChAT) promoter to activate LOC fibers. ANF firing rates were recorded in acutely excised apical cochlear coils from 4-week-old C57BL/6J mice using extracellular loose patch recordings from their bouton endings (Wu et al. 2016), while stimulating LOC fibers optogenetically. At room temperature, ANFs with spontaneous rates (SRs) less than 10 spikes/s were defined as 'low SR' (typically located modiolar on the IHC), and with SRs greater than 10 spikes/s as 'high SR' (typically pillar).

Results: In response to light activation of LOC fibers, the firing rate of low SR fibers increased from a median value of 1.74 to 14.8 spikes/s, turning low SR into high SR fibers. This effect was blocked by the perfusion

of scopolamine, a non-selective metabotropic acetylcholine receptor (mAChR) blocker. Similar recordings performed from high SR fibers did not change their firing rate in response to LOC fiber activation. However, the perfusion of scopolamine during recordings from high SR fibers, in the absence of LOC stimulation, decreased high SR fiber firing rate from a median value of 29.64 to 5.82 spikes/s, suggesting that tonic release of ACh by the LOC fibers contacting high SR fibers is setting their firing rate in the in vitro experimental setting.
Conclusions: Here we demonstrate that cholinergic LOC efferent input can dramatically change ANF firing rates, turning low SR fibers into high SR fibers, when LOCs are activated, and turning high SR fibers into low SR fibers, when a mAChR blocker is used. These results make it likely that lateral olivocochlear cholinergic efferent inputs contribute to setting ANF SR rates in vivo.

T12. Molecular Subtypes of Bushy Cells in Mouse Cochlear Nucleus

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Category: Brainstem: Structure and Function
Background: The cochlear nuclear complex (CN) is the first relay station for central auditory signal processing. Bushy cells in the ventral cochlear nucleus (VCN) convey the timing and fine structure of sounds from auditory nerve fibers to the superior olivary complex and the auditory midbrain. Our single-nucleus RNA sequencing (snRNA-seq) analysis of CN identified two molecularly distinct bushy cell populations with specific expression of Atoh7 and Hhip, which may correspond to spherical and globular bushy cells respectively. In addition, snRNA-seq subclustering analysis suggested further molecular subtypes within each major bushy cell population.
Methods: The bushy cell population was computationally isolated from the snRNA-seq dataset of all CN cells. Subclustering analyses were then performed on the bushy cell population to identify the potential subtypes within each main population. Meanwhile, using Patch-seq, which is a multimodal analysis method for single cells, bushy cells were assigned to each molecular subtype, thus comparing spatial-morphological-electrophysiological properties with comprehensive transcriptomic profiles.
Results: Subclustering analysis identified five potential molecular subtypes of bushy cells, including three subtypes of Atoh7+ bushy cells (Dchs2+, Tox+, and Sorcs3+) and two subtypes of Hhip+ bushy cells (Calb1+ and Galnt18+). To support these subtypes, we examined the properties of each Patch-seq cell that was assigned to each subcluster, including morphological, electrophysiological properties and anatomical locations. In Atoh7+ bushy cells, Dchs2+ cells appeared to be restricted to the nerve root area of the VCN, while Sorcs3+ cells and Tox+ cells appeared to have less spatial preference. Electrophysiologically, Dchs2+ cells had distinct firing properties from Sorcs3+ and Tox+ cells, while Sorcs3+ and Tox+ cells appear to share similar firing properties. Morphologically, Dchs2+ cells in general had the longest dendritic trunks among the three subtypes. In Hhip+ bushy cells, Calb1+ cells appeared to be preferentially localized in the posteroventral cochlear nucleus, while Galnt18+ was preferentially localized in the dorsoanterior cochlear nucleus. Calb1+ cells had distinct firing properties from Galnt18+ cells. In addition, the Calb1+ cells, in general, had shorter dendritic projections, but more complex dendritic tufts than Galnt18+ cells.
Conclusions: These analyses support distinct, functionally relevant subtypes within each bushy cell population and indicate potential novel bushy cell types in the cochlear nucleus. Further work will be needed to identify the functions of these new bushy cells in auditory signal processing. These analyses support distinct, functionally relevant subtypes within each bushy cell population and indicate potential novel bushy cell types in the cochlear nucleus. Further work will be needed to identify the functions of these new bushy cells in auditory signal processing.

T13. Bilateral and Symmetric Glycinergic and Glutamatergic Projections From the LSO to the IC in the Mouse

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Category: Brainstem: Structure and Function

Background: Auditory space has been conceptualized as a matrix of systemically arranged combinations of binaural disparity cues that arise in the superior olivary complex (SOC). The computational code for interaural time and intensity differences utilizes excitatory and inhibitory projections that converge in the inferior colliculus (IC). The challenge is to determine the neural circuits of this convergence and to model how the binaural cues encode location. It has been shown that midbrain neurons are largely excited by sound from the contralateral ear and inhibited by sound leading at the ipsilateral ear. Ascending projections from the lateral superior olive (LSO) to the IC have been reported to be ipsilaterally glycinergic and contralaterally glutamatergic. Using different methods, however, we found distinctly different results in terms of LSO projections to the IC.

Methods: This study used CBA/CaH mice (3-6 months old) where unilateral or bilateral retrograde tracer injections were made in the IC to label projecting cells in the ipsilateral and contralateral LSO. Standard immunohistochemical techniques using glycine and glutamate transporters were applied to counterstain this tissue. Means, standard deviations, and p values are provided where appropriate.

Results: Glycinergic and glutamatergic neurons are spatially intermixed within the LSO. On average, 21% ± 5.3% of GlyT2 neurons projected ipsilaterally and 19% ± 6.2% of GlyT2 neurons projected contralaterally (n=7; p=0.55). In contrast, 15% ± 0.8% of vGLUT2 neurons projected ipsilaterally and 17% ± 0.9% projected contralaterally (n=5; p=0.26). 28% of the IC-projecting cells do not label with GlyT2 or vGLUT2 antibodies. The total average number of ipsilaterally and contralaterally projecting cells was similar ($\bar{x}$ ipsi = 732.3 ± 19.1; $\bar{x}$ contra = 673.3 ± 16.8; p=0.48). On average, 60% of GlyT2 and 53% of vGLUT2 neurons did not project to the IC. The average somatic size of the GlyT2 (100.4 ± 24.8 μm2) and vGLUT2 (101.1 ± 29.8 μm2) neurons that did or did not project to the IC was also similar [F(457,305) =1.45, p = 0.71]. A distinct population of small (less than 40 μm2) neurons that did not project to the IC were labeled by GlyT2.

Conclusions: Our findings indicate a symmetric and bilateral projection of glycine and glutamate cells from the LSO to the IC in the CBA/CaH mouse. The difference between our results and those from previous studies cannot be easily explained but emphasizes excitation and inhibition in binaural processing and highlights the importance of techniques and comparative neuroscience. These data will be important for assessing the number of the different LSO cell types and for modeling how excitatory and inhibitory systems converge to create auditory space in this mouse strain.

T14. FMRP Regulation of Axon Targeting and Synaptogenesis for Binaural Processing

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Category: Brainstem: Structure and Function

Background: The binaural circuit in the brainstem computes interaural time differences, a critical cue for determining the source of a sound. This skill relies on the precise organization of the circuit in which inputs from each ear are channeled into the same binaural coincidence detectors via their segregated dendritic domains. The binaural coincidence detectors are located in the medium superior olive (MSO) in mammals and the nucleus laminaris (NL) in birds. Dendrites of MSO and NL neurons are sensitive to changes in afferent inputs and various developmental insults. The goal of this project is to understand the molecular mechanisms governing the dynamic organization of the binaural circuit, focusing on the role of an RNA-binding protein, Fragile X messenger ribonucleoprotein (FMRP).

Methods: We utilize the chicken circuit because of its simple anatomy, which allows spatially and temporally controlled genetic manipulations during development while having a high similarity to that of humans.

Results: We have previously developed a strategy of selective knockdown of FMRP expression from the nucleus magnocellularis (NM, homogenous to the mammalian ventral cochlear nucleus) via in ovo electroporation. This strategy includes a short hairpin RNA (shRNA) and a tetracycline response element for inducible shRNA expression at specific developmental stages. Normally, the single axon of NM neurons bifurcates and terminates on dorsal dendrites of the ipsilateral NL and ventral dendrites of the contralateral NL. We found that FMRP reduction leads to misguided axons that don’t stop at their intended destinations,

which is expected to impair the accuracy of biannual processing. Multiple-photon live imaging further revealed that many distal protrusions along developing dendritic and axonal branches of transfected NM neurons exhibit a high level of dynamism with continuous direction-changing, growth, or retractions. Quantitative analyses are ongoing to determine the effects of FMRP reduction on structural dynamics of axons and dendrites as well as their physical contacts (synaptogenesis), by comparing to neighboring un-transfected NM neurons and neurons transfected with a scrambled shRNA. Additionally, we identify the involved molecular players by assessing changes in the level and distribution of a selected set of presynaptic proteins following shRNA-induced FMRP reduction. We have observed a reduction in the terminal level of the synaptotagmin 2 (Syt2), a primary calcium sensor on presynaptic vesicles. Given the critical role of Syt2 in triggering neurotransmission, our next investigation will assess the effect of FMRP reduction in presynaptic neurons on synaptic vesicle dynamics and neurotransmitter release.
Conclusions: Together, our results are expected to provide a better understanding of the molecular basis of auditory circuit maturation under the control of RNA-binding proteins.

T15. Calcium Signaling During Development of Mouse MNTB Neurons

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Category: Brainstem: Structure and Function
Background: In the central auditory system, Ca2+ signaling plays a pivotal role in the regulation of cellular processes including neuronal development and maturation. Disruption of Ca2+ homeostasis and signaling leads to physiological deficiencies, abnormal development of auditory circuits, and compromised auditory processing and behavior. Here, using Ca2+ imaging, we investigated the underlying mechanisms for Ca2+ regulation of neurons during a critical developmental period around hearing onset, in the medial nucleus of the trapezoid body (MNTB), a brainstem nucleus involved in sound localization.
Methods: Brainstem slices with visually identified MNTB were prepared from GlyT2-Cre/tdT-GCaMP6f mice of either sex, which is a transgenic line obtained by crossing the glycine transporter 2-Cre recombinase mouse to the reporter line ROSA26-tdT-GCaMP6f. Ca2+ imaging experiments were performed at several milestone ages of development including P0, P7, P14, P21 and P30, at 35 °C. Fluorescence images of cells were obtained under excitation wavelength of 480 nm. Ca2+ signals were recorded from MNTB neurons in response to activation of various transmitter receptors such as Ca2+ permeable ionotropic glutamate receptors (iGluRs) - NMDARS and AMPARs, and group I metabotropic glutamate receptors (mGluRs) through bath application of their respective agonists. Furthermore, a hearing loss model of the mice was employed to investigate the effects of hearing deprivation on the Ca2+ signaling in MNTB neurons.
Results: Ca2+ signals were produced in MNTB, before the onset of hearing when NMDARs, AMPARs and group I mGluRs were activated by bath application of their respective agonists (NMDA 500 μM, AMPA 500 μM, 3,5-DHPG 200 μM). After hearing onset, Ca2+ signals for NMDARs and group I mGluRs declined dramatically, whereas AMPAR-mediated signals continued to elevate until maturity. To further test the sensitivity of the Ca2+ imaging method, we electrically stimulated the afferent fibers innervating MNTB while performing Ca2+ imaging. A 100 Hz pulse train stimulation delivered at the rate of 1 train/s triggered release of glutamate which elicited Ca2+ signals via glutamate receptors and potentially voltage-gated Ca2+ channels.
Conclusions: These data demonstrated the feasibility of performing Ca2+ imaging in genetically identified glycinergic neurons in mouse MNTB. The preliminary results suggested that in MNTB neurons Ca2+ signaling mediated by various transmitter receptors was developmentally regulated.

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T16. Sex Differences in Young Adult Frequency Following Responses to Speech and Musical Sounds

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Category: Other, Music Processing

Background: Male and female young adults differ in the timing and magnitude of their frequency following response (FFR) to sound. Krizman et al. (2019) investigated sex differences in FFRs to speech sounds across development and, in young adults, demonstrated greater response latencies in males and weaker encoding of high-frequency harmonics in the male temporal fine structure response. Some of these sex differences arise in adolescence, suggesting an underlying hormonal mechanism. There is a breadth of research documenting sex differences in response timing and amplitude in click ABRs and FFRs to the syllable 'Da'; However, there is little evidence of sex differences in FFRs to other complex sounds, such as musical notes.
The goals of the current study are twofold: (1) to replicate sex differences in timing and response amplitude in young adult FFRs to speech sounds found in Krizman et al. (2019); and (2) to investigate whether similar sex differences extend to FFRs to musical notes.

Methods: FFRs from young adults (18-25 years of age) were collected in response to a 40 ms 'Da' stimulus and three 200-ms musical note stimuli sampled from a Rhodes Electric Piano (A2, A#2, B2) with an average frequency content similar to that of 'Da'. Participants had normal hearing thresholds as determined by a standard and extended high-frequency audiogram (125 - 14 kHz). 3000 responses were collected to the 'Da' stimulus, while 500 responses were collected to each musical note, all presented in alternating polarities.
Results: Early results show that sex differences in Da responses were largely similar to those previously reported. Especially striking were onset timing differences with males having longer onset latencies than female responses.

In their musical note responses, males have longer onset latencies than females. Males have weaker encoding of high-frequency harmonics in the temporal fine structure response than females. Analyses of the fundamental frequency and other harmonics are ongoing.

Conclusions: These results provide evidence for a timing delay in male auditory processing through early adulthood that is not stimulus specific.
A similar pattern of sex differences to both 'Da' and the musical notes suggests the validity of the musical notes for assessing sound encoding in the auditory midbrain. This will be especially relevant for future studies in which feature encoding of musical sounds or sound processing in musical populations is of interest. Future research could also benefit from the broad frequency content of this stimulus set and the more pleasant nature of a melodic stimulus presentation.

Supported by the Knowles Hearing Center

T17. Noise-Induced Hearing Loss Increases Ca2+ Channel Conductances Underlying Burst Firing Pattern of Lateral Olivocochlear Neurons

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Category: Brainstem: Structure and Function
Background: Lateral olivocochlear (LOC) efferent neurons may protect hearing sensitivity. We have examined their electrophysiological properties in brain slices from juvenile mice. Previously we showed that LOC neurons exhibit an infra-slow (~0.1 Hz) burst firing pattern, whose genesis is dependent on L-type Ca2+ channels. However, it has not been clear what triggers the termination of burst firing. Moreover, how burst firing and the underlying ion channels change with noise-induced hearing loss is unknown.
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.
Results: Noise-exposed LOC neurons showed significantly longer bursts than controls. Broad-spectrum Ca2+ channel blocker Cd2+ and Ni2+ abolished burst firing, highlighting the significance of Ca2+ channels in these neurons. The voltage sensitivity and pharmacology of LOC Ca2+ current indicated expression of multiple

Ca2+ channel subtypes, including T- and L-type channels, but mainly high-voltage activated (HVA) channels were active during long depolarizations that accompany bursts. HVA Ca2+ current was significantly elevated with noise exposure, likely contributing to longer bursts of LOC neurons. Moreover, we found that termination of Ca2+ channel activity by Ca2+-dependent Ca2+ channel inactivation (CDI) may contribute to the termination of bursts. With 0.1 mM EGTA in the intracellular solution and 1.2 mM Ca2+ in the bath, the inactivation phase of Ca2+ current could be fitted with a double exponential, with a tau-1 of ~50 ms and a tau-2 of ~600 ms. When 10 mM BAPTA was used to chelate intracellular Ca2+, tau-2 was significantly prolonged, thus reducing the degree of inactivation. A more drastic effect was observed by switching the external Ca2+ to 1.2 mM Ba2+. Ba2+ prolonged both time constants, and reduced the fractional contribution of the fast tau. The use of BAPTA and Ba2+ together reduced the degree of inactivation (measured at the end of a 2-s pulse) from 95% to 67%. Interestingly, the elongation of tau-2 with BAPTA or Ba2+ was absent in noise-exposed LOC neurons, suggesting CDI was partially compromised by noise exposure. Finally, we found that K+ leak conductances and in a subset of neurons, Kv7 current, work synergistically with CDI to contribute to the termination of bursts.
Conclusions: Noise-induced hearing loss caused increased Ca2+ channel conductances with altered inactivation properties in LOC neurons. 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.

T18. Impact of KCC2 Phosphorylation on Synaptic Development in the Lateral Superior Olive (LSO)

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Category: Brainstem: Structure and Function
Background: In prehearing animals, inhibitory inputs onto LSO neurons are depolarizing, thus bringing the cell membrane closer to action potential threshold. Action potential firing increases intracellular Ca2+ levels that are crucial for regulating a variety of signaling pathways during development. The depolarizing effect of GABA and glycine extends to postnatal day 8, after which inhibitory inputs are hyperpolarizing throughout the rodents lifespan (D/H shift). The nature of the inhibitory response (hyperpolarizing or depolarizing) is determined by cytoplasmic chloride concentration ([Cl-]i) as GABAA and glycine receptors are chloride-permeable. In most auditory brainstem nuclei, the Cl--extruding cotransporter (KCC2) is the key protein to regulate developmental Cl- homeostasis. Although KCC2 is present in the cell membrane of LSO neurons from birth, its transport activity starts only at the end of the first postnatal week. Following the gradual activation of KCC2, [Cl-]i is lowered and the inhibitory inputs become hyperpolarizing. While the developmental timeline of KCC2 activation is well-studied, the mechanism of activation remains elusive. Previous studies pointed to post-translational modification as regulator of KCC2 activation and deactivation.
Methods: Here, we tested the effects of phosphorylation on KCC2 transport activity by generating a KCC2 Thr934Ala/Ser937Asp transgenic mouse line (KCC2AD/AD), which mimics the phosphorylated state at Ser937, while Thr934 is inaccessible to phosphorylation to avoid compensatory side effects. We performed transfections in HEK293 cells, as well as gramicidin-perforated and whole-cell patch-clamp recordings.
Results: We demonstrate that HEK293 cells transfected with KCC2AD/AD show higher transport activity in comparisson to the KCC2aWT tranfected HEK293 cells. Using gramicidin-perforated patch-clamp recordings to measure [Cl-]i in acute brainslices of LSO neurons, we found that KCC2 in KCC2AD/AD is activated as early as P3.
Conclusions: This early D/H shift suppresses the excitability of LSO neurons during the first postnatal week, but the long-term consequences on synaptic development are unknown and remain to be determined.

T19. In Utero Exposure to Paracetamol Disrupts Auditory Brainstem Responses in Rats

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Category: Brainstem: Structure and Function

Background: Paracetamol (PAR; acetaminophen) is an analgesic and antipyretic drug typically regarded as the safest over-the-counter pain and fever relief option for use during pregnancy. PAR and its metabolites are known to reach the developing fetus and neonate through direct placental transfer and excretion in breast milk. However, recent epidemiologic evidence suggests that PAR exposure may increase the risk of neurodevelopmental conditions, including autism spectrum disorder (ASD) and attention deficit hyperactivity disorder in humans. In utero PAR exposure has also been shown to result in behavioral abnormalities in rat pups. Since hearing abnormalities are a common feature of ASD, we hypothesized that in utero PAR exposure would result in elevated hearing thresholds and delays in the auditory brainstem response (ABR).

Methods: We examined this hypothesis using Sprague-Dawley rats from dams exposed to either saline or PAR (350 mg/kg) from embryonic day 6 to partition. Animals were handled from postnatal day (P) 15-20 and weaned on P21. Auditory brainstem responses were recorded on P21 and P28 in response to broad-band clicks.

Results: Our results revealed that approximately one third of PAR-exposed animals had normal thresholds and wave latencies at P21. However, approximately two-thirds of PAR-exposed animals had elevated thresholds and increased wave latencies at P21. Accordingly, PAR-exposed animals were split into exposed and affected groups. At P28, both PAR-exposed and PAR-affected animals had significantly higher click thresholds compared to controls. PAR-affected animals had longer latency responses for waves III, IV and V. Further, PAR-affected animals had less improvement in ABR latencies from P21 to P28 compared to control and PAR-exposed animals.

Conclusions: Together, these results demonstrate impaired ABRs in the majority of rats exposed to PAR in utero. Further anatomical analyses will be needed to identify regions of the peripheral and/or central auditory pathway impacted by PAR.

T20. Sources and Termination Patterns of Cholinergic Input to the Ventral Nucleus of the Lateral Lemniscus

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Category: Brainstem: Structure and Function

Background: Acetylcholine (ACh) plays a substantial role in modulating neuronal activity in the auditory system but for many brainstem nuclei the source of cholinergic innervation is unknown. We characterized cholinergic inputs to the ventral nucleus of the lateral lemniscus (VNLL), one of the largest sources of inhibitory projections to the inferior colliculus.

Methods: We bred Chattm1(cre)Lowl mice (Jackson Labs #031661) with CBA/CaJ mice and selected offspring to obtain mice with two copies of the wildtype cdh23 gene, eliminating the early-onset hearing loss that characterizes the C57BL/6J line. To validate the cre expression, we crossed the mice with Ai14 reporter mice and stained the tissue with anti-VAChT to identify cholinergic neurons. Across four brainstem cholinergic nuclei, 89% of reporter-labeled cells were VAChT-positive, indicating a high percentage of cre-expressing neurons are cholinergic.

We placed red RetroBeads into the VNLL and subsequently stained the tissue with anti-VAChT to identify cholinergic cells. We observed VAChT-positive, tracer-labeled cells in multiple nuclei. The majority were located bilaterally in the pedunculopontine tegmental nucleus (PPT), laterodorsal tegmental nucleus (LDT) and superior olivary complex (SOC). In addition, a small number of cells were located bilaterally in the lateral paragigantocellular nucleus (LPGi).

Results: We then injected adeno-associated viral vectors into each cholinergic region in 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: PPT, LDT, SOC and LPGi. Each cholinergic source labeled axons bilaterally in the VNLL. Labeled axons from different sources exhibited many similarities. Most axons were very thin and travelled in or along the VNLL in parallel with lemniscal fibers. Nearly all axons exhibited boutons along their length and many exhibited branches within the VNLL. The one unexpected finding from the viral studies was the surprising amount of cholinergic innervation from the LPGi. Our retrograde studies indicated only a small proportion of the innervation arises from LPGi cells, but the axonal labeling following viral deposits in LPGi was

substantial, with the labeling in individual cases rivaling that seen after viral deposits in any of the other cholinergic sources. This suggests that individual LPGi cholinergic axons may have relatively much more extensive axonal arbors in the VNLL.
Conclusions: We conclude that the VNLL receives cholinergic input from the PPT, LDT, LPGi and SOC. Axons from each source terminate throughout the VNLL, suggesting that individual VNLL neurons could be modulated by multiple cholinergic sources. The different sources are likely to be active under different conditions, suggesting different roles in modulating VNLL neurons and their responses to sound.
Supported by NIH R01 DC004391.

T21. Diversity of Subthreshold and Action Potential Properties in Octopus Cells of the Posteroventral Cochlear Nucleus: Implications for Temporal Coding

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Category: Brainstem: Structure and Function
Background: Octopus cells (OCs) of the posteroventral cochlear nucleus integrate large numbers of auditory nerve fiber inputs and encode transient, broadband acoustic stimuli as well as frequency sweeps. With their dendrites oriented approximately perpendicular to the path of the auditory nerve fibers, OCs require near-simultaneous arrival of tens of inputs to reach action potential threshold. The action potentials are unusually small and narrow in OCs and appear specialized for precise temporal coding at rates of hundreds of hertz. However, the nature of action potential initiation is poorly understood in OCs.
Methods: We made whole-cell patch recordings in current or voltage-clamp from octopus cells under IR-gradient contrast optics in parasagittal brain slices of the cochlear nucleus of the mouse (CD1 strain; P21-P45; 34-35°C), and initiated action potentials either with direct current injection through the recording electrode or through electrical activation of auditory nerve fiber (ANF) inputs. Cells were filled with 1% biocytin and either Alexa-488 or 568 for post-hoc morphological reconstruction using a Neurolucida system.
Results: We found that both intrinsic membrane properties and action potential characteristics were more diverse than previously reported. While some cells exhibited low input resistances and fast membrane time constants similar to prior reports, both of these parameters were far more diverse than previously reported (steady-state Rin, 14 to 72 MΩ; tm, 0.2 to 1.7 ms). Just-suprathreshold action potentials were also diverse, ranging from 6 to 65 mV, with their halfwidths ranging from 0.35 to 1.75 ms. In addition, the amplitude and shape of OC action potentials were highly dependent on stimulus strength, with many OCs exhibiting slower, presumed calcium components that increased the duration and amplitude of the AP up to ~ 2 fold. During trains of synaptic potentials changes in action potential shape were typically associated with strong active repolarizing conductances that hyperpolarized OCs well below the resting potential. OCs with strong repolarizing conductances also exhibited spontaneous hyperpolarizing potentials with similar rise and decay times (~5 ms). These events were eliminated when calcium stores were depleted in the presence of 20 μM cyclopiazonic acid (CPA), and in preliminary experiments these events were blocked by 100 nM iberiotoxin, a blocker of calcium-activated potassium channels.
Conclusions: Our results reveal that OCs exhibit subthreshold membrane properties and action potential characteristics more diverse than previous reports. It has been assumed that Kv1 potassium channels were the dominant mechanism underlying the fast repolarization of EPSPs and spikes. Our current results suggest an important role for calcium channels and calcium-activated potassium channels in regulating the precision of OC spiking output.

T22. Mapping Vasculature in Human Midbrain With MRI

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Category: Brainstem: Structure and Function
Background: While midbrain vasculature was laboriously delineated by Duvernoy (1978), the vasculature has not been mapped and made available using three-dimensional data. T2*–weighted images—including

BOLD functional MRI—are sensitive to blood vessels and make the immediate vicinity of vessels distorted. As advances in functional MRI enable sub-millimeter resolution in deeper brain structures, particularly at 7 Tesla, these vascular effects are increasingly prominent (Viessmann et al., 2019).
Methods: Postmortem MRI was collected from a 65-year-old male without any known neurological conditions. The brainstem and thalamus were removed, flushed with saline, and immersed in formalin for 2 weeks before being re-hydrated for 1 week in 0.1 M solution of phosphate buffered saline doped with 1% (5 mM) gadoteridol. Imaging was conducted on a small-bore 7 Tesla MRI. T2*-weighted anatomical images were collected at 50 μm isotropic spatial resolution encompassing the entire brainstem and thalamus specimen. The MR images were inverted, masking out non-brain tissue. Vasculature was identified as high-signal voxels with high spatial frequency using the Segmentator software package. The orientation of vessels was identified using structure-tensor modeling. Regions of interest were flattened and laminated using LAYNII.
Results: We generated three-dimensional maps of vasculature in human dorsal midbrain including inferior and superior colliculus. In the rostral–caudal plane, vasculature was oriented orthogonal to the neuraxis that aligns with the cerebral aqueduct. In perpendicular slices through each colliculus, vasculature fanned out laterally from the cerebral aqueduct.
Conclusions: Using 50 μm isotropic T2*-weighted MRI in a postmortem sample, we identified a neuraxis-based vasculature organization scheme in human dorsal midbrain. This stands in contrast to an alternative surface-based vasculature organization scheme that is more typical in neocortex. As ultra-high field imaging moves to higher and higher resolution deeper in the brain, T2*-based functional MRI will be increasingly biased by vasculature effects. Next steps include quantitative mapping of T2 in vivo (Gulban et al., 2021) in midbrain and directly assessing the vascular impact on blood oxygenation level-dependent functional MRI.

T23. ABR+: Combining Click and Neurophonic Stimulus Types to Assess Multifaceted Neural Function in a Single Test

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Category: Brainstem: Structure and Function
Background: The auditory brainstem comprises nuclei with an astonishing variety of functions. While different evoked potential parameters have been developed to assess these functions, each test reflects a constrained set of system properties. For example, the click-evoked Auditory Brainstem Response provides information about the threshold and integrity of transient, synchronous neural firing in nuclei along the peripheral pathway, while the FFR reflects suprathreshold, phase-locked, functional properties of subcortical and early cortical parts of the auditory system. Our long-term goal is to expand application of brainstem-evoked potentials so that multiple facets of function can be assessed in the clinic. Here we present a novel method of combining two stimulus types, clicks and neurophonic vowels, to assess audibility and complex processing in a single test. In order to determine whether the combined response compromised the integrity of each individual test presented alone, we varied each stimulus type along parameters known to produce well-established response shifts (e.g., level and frequency).
Methods: 20 young adults (8 males) with no history of neurological disorders or hearing impairment participated after consent. A three-channel vertical evoked potential recording montage was applied to subjects while seated comfortably in a sound attenuating booth. Stimuli were presented (Intelligent Hearing Systems, Inc.) in three pseudo-randomized conditions : 1) ABR alone, consisting of a 100 us click at 70 or 40 dB nHL, 2) FFR alone where synthetic /a/ vowels of 40 ms duration were presented at 80 dB SPL with F0s of either 100 or 200 Hz, and 3) ABR+, where clicks at 40 and 70 dB nHL were followed by the vowel with an F0 of 100 or 200 Hz. Stimulus Onset Asynchrony between clicks and vowels was and the Interstimulus Interval for all three conditions was 900 ms, yielding a rate of 11.1/s. Data were analyzed in two stages. First, we compared the latency and amplitude of Waves I-V between ABR alone and ABR+ conditions across the two levels. Second, we compared the RMS amplitude of the FFR waveforms between FFR-alone and ABR+ conditions across the two F0s.
Results: Results suggest no differences in ABR peak latencies elicited by a click stimulus presented at the two levels either alone or as part of the combined stimulus in either frequency condition. No differences were observed between FFR amplitude in either frequency condition, recorded alone or as part of the combined stimulus.

Conclusions: Taken together, these results suggest that the ABR and FFR can be reliably recorded in a time-efficient manner using a combined "ABR+" stimulus. Such work provides an important step to increase the feasibility of clinical recording of FFR, allowing suprathreshold measures of auditory function to be more widely assessed in clinical populations.

T24. Central Auditory Deficits in Post-Acute COVID Syndrome

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Category: Brainstem: Structure and Function

Background: Post-acute COVID syndrome (PACS), also known as Long-COVID, presents a complex challenge due to its association with subtle neuroinflammation and subjective cognitive fatigue symptoms. Objective methods to diagnose or track PACS are needed. The auditory system may offer such measures. Self-reported auditory symptoms seen with PACS include peripheral hearing issues, speech perception difficulties, and hyperacusis. Central auditory tests can detect dysfunction in neurocognitive processing, making them potentially sensitive to PACS-related neuroinflammation. This study investigated peripheral, central, and electrophysiological auditory tests in individuals with and without PACS.

Methods: 76 subjects participated, including 40 with PACS (mean age: 48.3, Female: 80%) and 36 healthy controls (mean age: 44.8, Female: 80%). Control group eligibility criteria included no history or diagnosis of COVID-19, while the PACS group was defined by persistent cognitive fatigue, assessed using the Chalder Fatigue Score, at least 2 months after a confirmed COVID-19 diagnosis via PCR. Peripheral auditory function was assessed using Békésy Audiometry (0.5-8.0 kHz) and speech reception thresholds. Central auditory function was evaluated with the Hearing in Noise Test (HINT), Triple Digit Test (TDT), Gap Detection Threshold (Gap), and Staggered Spondaic Words (SSW). Electrophysiological evaluations included the Acoustic Brainstem Response (ABR) elicited by a slow (21.1 per second) and fast (61.1 per second) click train, as well as the Frequency Following Response (FFR) elicited by a /ba/ (~180ms). Descriptive statistics compared demographics, peripheral auditory, and electrophysiological measures, while logistic regression and ANOVA models explored the relationship between auditory function and PACS diagnosis, with Bonferroni corrections for multiple comparisons.

Results: Demographics and peripheral auditory function showed no significant differences between groups. Across all measures central auditory function trended toward worse performance in those with PACS. PACS individuals exhibited significantly worse scores on the SSW test (p less than .009). The SSW score independently predicted PACS (p=0.011) in logistic regression, independent of age and peripheral auditory function. FFR results trended toward larger F0s, smaller F1s, and reduced stimulus to response measures, though without statistical significance. ABR findings resembled those in tinnitus patients, with elevated V/I amplitude ratios. When PACS subjects were divided by age (□45 years) young adults with PACS had reduced wave I amplitudes in combination with heightened Wave V relative age-matched controls (p=.019).

Conclusions: Our study suggests a subtle, yet observable decline in central auditory function among individuals with PACS compared to controls. Despite similar peripheral auditory function, significant differences were observed in dichotic auditory processing, as seen in the SSW test, and ABR amplitude of peaks I and V in PACS subjects. Consequently, PACS may lead to premature auditory aging, with maladaptive compensatory increases in central auditory function. While preliminary, our data suggest central auditory differences in PACS and underscore the potential for using these tests for assessing PACS.

T25. Developmental Spontaneous Activity Drives the Maturation of the Calyx of Held Nerve Terminal and Its Synaptic Target in the Medial Nucleus of the Trapezoid Body

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Category: Brainstem: Structure and Function

Background: Synaptogenesis occurs independent of neural activity, but maturation and refinement of nascent connections is an activity-dependent mechanism. Intrinsic patterned spontaneous activity (SA) occurs in several brain regions during development, including the visual and auditory sensory systems. Interestingly, SA in these sensory systems occurs prior to the onset of external stimuli (in mice, ear canals and eyes open after P10), highlighting the importance of stimulus-independent activity during neural circuit formation. In the developing murine auditory system, intrinsic SA originates in inner hair cells in the cochlea, beginning at birth, and propagates throughout the ascending auditory pathway. Globular bushy cells (GBCs) located in the ventral cochlear nucleus (VCN) project contralaterally and innervate principal cells (PCs) in the medial nucleus of the trapezoid body (MNTB) forming the calyx of Held (CH) nerve terminal. The CH:MNTB synaptic connection is utilized as a model system for studying the role of SA during neural circuit formation, in part because growth of the CH occurs rapidly (postnatal day (P)2-P6) resulting in mono-innervation, and key biophysical properties have been characterized. Previous manipulations to eliminate SA at the developing CH have involved genetic strategies that also affect cochlear function and may induce homeostatic compensatory mechanisms in GBCs.

Methods: Direct manipulation of the CH:MNTB synaptic connection to silence activity will be mediated through viral vector, rapid-onset expression of tetanus neurotoxin (TeNT) targeting GBCs. Whole-cell electrophysiological recordings in acute brainstem slices will be utilized to assay the effects of silencing activity on maturation of the MNTB PCs physiological properties. Immunohistochemistry along with high-resolution confocal imaging will be employed to assay structural deficits to the CH following TeNT expression.

Results: Recordings from transduced P6 PCs (n = 11), compared to control, non-transduced ipsilateral MNTB PCs (n = 12), shows a decrease in the frequency (0.7 ± 0.4 Hz vs 3.2 ± 2.1 Hz; p less than 0.05), increase in decay rate (1.2 ± 0.3ms vs 0.7 ± 0.1ms; p less than 0.05), and no change in the amplitude (63.0 ± 18.2pA vs 66.2 ± 15.8pA; p = 0.61) of spontaneous excitatory postsynaptic currents. P6 MNTB PCs innervated by transduced CHs show a delayed transition from tonic to phasic firing (0% phasic/100% tonic vs 93% phasic/17% tonic), where the percentage of phasic PCs at early developmental ages fit to a Boltzmann function resulted in a V50 = P3.5. P9 immunostaining shows impaired growth of the CH expressing TeNT with reduced volume (706 ± 340μm3 vs 1601 ± 294μm3; p less than 0.05) and increased thickness (2.3 ± 0.6μm vs 1.3 ± 0.2μm; p less than 0.05).

Conclusions: This study is ongoing and highlights an important role for SA triggering rapid growth of the CH and the synchronous maturation of the MNTB PC physiological properties.

T26. Differential Cortical Modulation of Inferior Colliculus Sub-Circuits

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Category: Midbrain: Structure and Function

Background: The auditory cortex sends excitatory feedback (corticofugal) projections to the inferior colliculus (IC), a midbrain hub involved in complex sound coding. Corticofugal axons primarily target higher-order dorso-medial and lateral "shell" IC sub-nuclei. We previously characterized corticofugal transmission onto dorsomedial IC neurons, revealing single-cell and network mechanisms enabling non-linear computations in distinct IC cell classes (Oberle et al., 2022; 2023). However, whether corticofugal synaptic activity has similar or divergent effects in the lateral IC is unknown.

Methods: We combined transgenic mouse lines, optogenetics, and patch-clamp electrophysiology in acute IC brain slices to measure corticofugal transmission in the lateral IC (n=55) and compared the results with dorsomedial IC recordings (n=24). We crossed VGAT-ires-cre and Ai14 fl/fl mice to record from GABAergic (VGAT+) and presumptive glutamatergic (VGAT-) neurons. In the auditory cortex we expressed the excitatory opsin Chronos to optogenetically activate auditory corticofugal axons with trains of light flashes.

Results: Nearly all dorsomedial IC neurons tested (22/24) exhibited EPSPs during optogenetic stimulation of corticofugal axons; surprisingly fewer lateral IC neurons in the same slices (20/55) showed EPSPs with the same stimulation. Moreover, corticofugal train EPSPs were significantly smaller in lateral compared to dorsomedial IC neurons, suggesting sparser convergence of corticofugal axons onto lateral IC neurons. Our prior study (Oberle et al., 2023) showed corticofugal signals drive polysynaptic excitation in dorsomedial

VGAT+ neurons but not VGAT- neurons. However, preliminary data suggests this circuit motif is absent in the lateral IC: Majority of VGAT- and VGAT+ corticofugal EPSPs have onset latencies between 2 and 6 ms. The lateral IC has a unique organization characterized by GABAergic "modules": Dense clusters of VGAT+ neurons are targeted by somatosensory inputs, but sparse auditory cortex input, while the surrounding "matrix" zones have a lower density of GABAergic neurons but are densely contacted by auditory corticofugal axons (Lesicko et al., 2016). Interestingly, we find several VGAT+ (4/17) and VGAT- (5/15) cells in the GABA-rich modules respond to optogenetic stimulation of auditory corticofugal fibers despite apparently sparse corticofugal axons in the vicinity. Next, we will determine whether the lateral shell generally receives weaker top-down cortical input or if this is just a phenomenon with descending auditory cortex fibers. To test this, we have begun to apply our Chronos experiments to primary somatosensory cortex. We will optogenetically activate descending fibers from the somatosensory cortex while making slice electrophysiology recordings at the lateral shell. With these new experiments, we will characterize how descending somatosensory input shapes lateral shell signaling, a region known for its multisensory integration, in comparison to the top-down auditory cortical control.
Conclusions: We these experiments, we identified surprising intricacies of the auditory cortico-collicular pathway's impact on distinct IC sub-regions and we will unravel how top-down somatosensory input shapes signaling in the auditory midbrain.

T27. Auditory Hyperexcitability: Central Auditory Processing in the Inferior Colliculus of a Genetic Mouse Model Susceptible to Audiogenic Seizures

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Category: Midbrain: Structure and Function
Background: The auditory system has the ability to sense a large range of sound intensities from whispers to loud alarm sounds, covering 6 orders of magnitude in acoustic pressure. Deficits of sound intensity processing may lead to hearing loss and/or major auditory perception distortions like hyperacusis, a reduced tolerance for moderate sound levels. It has been suggested that hyperexcitability of auditory circuits is a possible mechanism involved in hyperacusis. Audiogenic seizures, reflex seizures induced by loud sounds, are a common feature of many mouse models for central nervous system disorders and may be seen as a model of auditory hyperexcitability. However, the mechanisms underlying these seizures are still poorly understood. They are thought to reflect an imbalance between neuronal excitation and inhibition in the central auditory pathways. Interestingly both peripheral and central hearing impairments, either acquired or congenital, increase the susceptibility to these seizures.
Methods: Here, we report a genetic mouse model for deafness, which shows a particularly high and sustainable susceptibility over development to audiogenic seizures. To decipher the mechanisms underlying the neuronal hyperactivity characterizing the audiogenic seizures, we recorded neuronal activity in the inferior colliculus of these mutant mice.
Results: Neurons of the inferior colliculus in this model had a greater baseline activity level, and were more responsive to acoustic stimuli while being less impacted by the presence of background noise than control neurons.
Conclusions: This model provides new opportunities of deciphering pathophysiological mechanisms of auditory hyperexcitability.

T28. Timbre Encoding in the Inferior Colliculus: Exploring Neural Mechanisms

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Category: Midbrain: Structure and Function

Background: Timbre is the quality that allows sounds that are identical in pitch, duration, and loudness to be distinguished. One aspect of timbre, brightness, is correlated with the spectral centroid of a sound, which is influenced by spectral-peak frequencies. Previous timbre studies focus on vowel-formant encoding, but there is a gap in understanding more generally how spectral peaks are represented in the inferior colliculus (IC). Spectral peaks can saturate inner-hair-cells; when a 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 tuned for amplitude modulation frequency, 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. Here, we investigated IC representation of spectral peaks over suprathreshold levels, using physiological and computational modeling methods to explore mechanisms.
Methods: Extracellular single-unit recordings were made in the IC of awake Dutch-belted rabbits using tetrodes. The reference stimulus was a 300-ms harmonic tone complex (fundamental frequency, F0 = 200 Hz) with a triangular spectral envelope (24 dB/octave) and all components in sine phase (Allen and Oxenham, 2014 JASA 135:1371). The peak of the spectrum was a multiple of F0 nearest to the characteristic frequency (CF) of the neuron. Then, the entire spectrum was shifted upward and downward 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, to test encoding over suprathreshold levels, and were either diotic or contralateral, to investigate the contributions of inputs from both ears. Computational IC models featuring amplitude-modulation sensitivity and broad inhibition were tested against IC responses.
Results: Rate profiles of BS neurons had robust peaks when spectral peaks were near CF, over suprathreshold levels. Rate profiles for binaural stimuli were sharpened compared to the contralateral stimuli, supporting a role of ipsilateral inhibition in shaping responses. However, few BE rate profiles had the hypothesized decrease near spectral peaks. Instead most BE cells had a robust rate increase in response to spectral peaks near CF, or a broad sloping response. An IC model featuring amplitude-modulation sensitivity predicted BS, but not BE, responses. Preliminary inclusion of broad inhibition in the IC model improved predictions of BE responses.
Conclusions: We found that the spectral peak of a tone complex was robustly encoded by the majority of IC neurons. At the level of the IC, a combination of neural mechanisms is involved in the representation of timbre. Future work includes extending this analysis to natural-timbre responses.

T29. Recruitment of Local Inhibitory Circuits in the Inferior Colliculus by Ascending Input From the Cochlear Nucleus

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Category: Midbrain: Structure and Function
Background: The inferior colliculus (IC) is the auditory processing hub of the midbrain and is important for the processing of speech and other vocalizations. Most ascending auditory input converges in the IC, but because it long proved difficult to identify distinct neuron classes in the IC, it has been challenging to determine how specific sources of input to the IC influence IC neuron excitability and signal processing. T-stellate neurons in the ventral cochlear nucleus (VCN) represent one of the major ascending projections to the IC. T-stellate neurons encode information about sound frequency, intensity, and amplitude modulation and have been implicated in the processing of vocalization cues. However, the specific neuronal populations that T-stellate cells target in the IC and how T-stellate cells contribute to IC neuron excitability, remain largely unknown. Our lab recently discovered GABAergic NPY neurons and glutamatergic VIP neurons as two of the first molecularly identifiable neuron classes in the IC.
Methods: With whole-cell path-clamp recordings and optogenetic circuit mapping, we tested the prevalence and synaptic physiology of T-stellate cell projections to NPY and VIP neurons.
Results: We found that while T-stellate neurons provide functional input to both NPY and VIP neurons, T-stellate input to NPY neurons was more frequent than T-stellate input to VIP neurons. EPSPs elicited in NPY neurons were significantly larger than those elicited in VIP neurons, but T-stellate input to both cell classes exhibited short-term synaptic depression. In addition, activation of T-stellate terminals elicited direct excitation and feedforward inhibition in both NPY and VIP neurons.

Conclusions: This data suggests that T-stellate afferents provide excitatory input to neurons in the IC, but the frequency and magnitude of that input differs between classes of neurons. T-stellates may also recruit local inhibitory circuits in the IC. Future steps include in vivo manipulations to determine how T-stellate projections shape auditory receptive fields in NPY and VIP neuron populations.

T30. Recovery of Neural Modulation Tuning in the Midbrain After Kainic-Acid-Induced Cochlear Synaptopathy in the Budgerigar

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Category: Midbrain: Structure and Function

Background: Amplitude modulation (AM) is an important acoustic feature of many communication signals including speech. AM tuning in the auditory midbrain is critical, as the midbrain is the first stage where neurons strongly encode envelope fluctuations of sounds in their average discharge rate. Cochlear synaptopathy is a common pathology in humans often thought to adversely impact AM neural encoding and perception, but few studies had tested this hypothesis. Our model species, the budgerigar, is a small parakeet species that utilizes AM in their vocal communication system and possesses similar behavioral AM detection abilities to humans. To understand the impacts of cochlear synaptopathy on neural processing of AM sounds, we compared modulation transfer functions (MTFs) in the inferior colliculus (IC) of budgerigars with and without cochlear synaptopathy.

Methods: Neural responses in the central IC were recorded in awake budgerigars. Stimuli were amplitude modulated noise- or tone-carrier signals with 1) varying modulation frequency (4 – 1024 Hz; i.e., MTFs), or 2) varying modulation depth (-36 – 0 dB) at best modulation frequency (BMF). Broadband noise carriers were modulated with sinusoidal, raised-sine, or trapezoidal envelopes to characterize MTFs for each envelope shape. Tone-carrier MTFs were measured with sinusoidal AM and with on and off-characteristic-frequency (CF) tone carriers. Rate-modulation-depth functions were measured at BMF with on-CF tone carriers. Cochlear synaptopathy in budgerigars was induced by intracochlear kainic acid infusions (1-2 mM, 1.2-2.5 µL). Auditory brainstem responses and otoacoustic emissions were measured before and after KA exposure to confirm selective AN damage. MTFs were examined from 2 days up to several month after kainic acid infusion.

Results: Average rate responses of budgerigar IC neurons were typically enhanced by modulated tones. For most IC neurons, on-CF MTFs had band-pass tuning, and off-CF MTFs were often high-pass in shape. MTFs measured with noise carriers were often bandpass in shape, with raised-sine and trapezoidal envelopes showing greater variation in discharge rate across modulation frequencies. Immediately after induction of synaptopathy, rate and synchrony representations for all kinds of AM were disrupted. Partial recovery was found 8-10 days after the infusion. However, animals with long-term cochlear synaptopathy (greater than 2 months after infusion) showed prominent rate- and synchrony-based modulation tuning and sensitivity. Noise and tone-carrier MTFs of IC neurons in these animals appeared comparable with controls.

Conclusions: AM tuning in the budgerigar IC was disrupted immediately after KA-induced cochlear synaptopathy, with partial recovery occurring within 10 days. Animals with long-term cochlear synaptopathy showed robust AM tuning comparable to controls. Our results suggest surprising resilience of AM processing in the auditory midbrain in animals with cochlear synaptopathy.

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T31. Brain State-Dependent Modulation of Sound Responses in the Inferior Colliculus

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Category: Midbrain: Structure and Function

Background: Sound responses in auditory thalamus and cortex depend strongly on neuromodulatory brain state, as indexed by the size of the pupil (McGinley et al., 2015). In particular, the gain of responses shows an overall average inverted-U shaped dependence on pupil size. However, at what stages along the auditory

pathway these state-dependent modulatory signals influence sensory processing, and what the pattern of state-dependence is at earlier stages, including midbrain, remains largely unknown. To investigate whether brain state influences sensory processing in midbrain, we performed pupil and sound responses measurements, in the inferior colliculus (IC) in head-fixed awake mice on a cylindrical treadmill.
Methods: To test whether IC is influenced by ongoing pupil-indexed brain state changes, we used Neuropixels probe to record central IC (ICC) responses to pure tones (N=13 mice, n=22 sessions, n=2151 units). Our results indicate a strong state-dependence in the ICC.
Results: The population average response showed an inverted-U shaped relationship. Moreover, we found subpopulations of units that exhibit distinct patterns of state-dependence. The largest group showed an inverted-U shaped relationship while the other major group showed monotonically reduced activity with increasing arousal levels. Interestingly the inverted-U dependent neurons had narrower bandwidth and higher gain than the decreasing neurons, suggesting that low-arousal high-gain neurons play a role in 'detection' of sounds at low arousal. In contrast, the inverted-U neurons may perform finer-grained stimulus analysis at mid-level pupil-indexed state, which is known to be optimal for neural and behavioral sound processing (McGinley et al., 2015). To quantify the degree and pattern of state-dependence of ICC responses, we used a simple pupil-binned mean model to predict single-trial tone evoked firing rate and compared to the grand-average (pupil-independent) response, for each tone carrier frequency. We found that pupil-linked brain state was able to explain a large fraction of variance in the tone-evoked firing rates. Similarly, a four-parameter stimulus frequency response-sorted sigmoidal model showed a large increase in explained variance when brain-state was included in the model.
Conclusions: Overall, our results suggest that sensory processing is strongly modulated by pupil-indexed brain state as early as ICC. Future work using invasive recordings in sub-midbrain auditory structures will be needed to determine at the single-neuron level whether this observed state dependence emerges in ICC or exists in the structures upstream to it.

McGinley, M. J., David, S. V., and McCormick, D. A. (2015). Cortical membrane potential signature of optimal states for sensory signal detection. Neuron, 87(1), 179-192.

T32. Mapping Neural Excitation and Suppression During Sound Processing in the Awake Mouse Inferior Colliculus

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Category: Midbrain: Structure and Function
Background: Sound processing in the central nervous system depends on inhibition to shape neural response patterns and a reduction in inhibition is thought to be a key mechanism underlying changes in brain processing following hearing loss, including those that may underly secondary disorders such as hyperacusis and tinnitus. However, the mechanisms through which inhibition shapes neural processing during normal and impaired hearing in the intact nervous system are poorly understood. This is particularly true in brain regions outside of the cerebral cortex, such as the inferior colliculus (IC) – a hub of the ascending auditory pathway that integrates inputs from upstream brainstem nuclei.
Methods: We used wide-field single-photon and two-photon Ca2+ imaging in awake adult mice to determine the spatial and temporal dynamics of sound-evoked activity in both excitatory (Thy1-jRGECO1a and VGLUT2-IRES-Cre;LSL-GCaMP6s) and inhibitory (VGAT-IRES-Cre; LSL-GCaMP6s) neurons in the IC.
Results: Amplitude-modulated tones evoked ON responses in excitatory neurons along diagonally oriented isofrequency domains in the IC, and suppression of adjacent regions (mid-tone suppression), consistent with the concept of side-band inhibition shaping neural response patterns. Interestingly, OFF responses predominantly occurred at the same location as mid-tone suppression, but not at the location of the ON responses, suggesting different features of the sound stimulus (start and stop) were encoded at different spatial locations of the IC. Acute loud noise exposure – a common model of hearing loss – reshaped excitatory neural response patterns, as mid-tone suppression was absent and OFF responses were significantly reduced in amplitude, consistent with central disinhibition known to follow the reduction in peripheral input and confirming that Ca2+ imaging is sufficient to capture sound evoked inhibition.

To determine how inhibition shapes excitatory neural responses during normal hearing, we assessed whether sound stimulation recruited a local population of GABAergic inhibitory neurons at the location of mid-tone suppression. We found that sound stimulation evoked inhibitory neurons within the same isofrequency domains as excitatory neurons. Surprisingly, the activity of adjacent GABAergic neurons was not enhanced, but suppressed, displaying mid-tone suppression in the same region as excitatory neurons, suggesting that inhibition of GABAergic neurons may be a common feature of sound processing in the intact IC.

Conclusions: We find that sound-evoked neural responses are spatially and temporally dynamic in the intact IC and that the two primary neural classes, glutamatergic and GABAergic, are similarly excited and inhibited in a sound frequency manner. Mid-tone suppression is unlikely to be mediated by local inhibitory neurons and instead may arise from either 1) GABAergic neurons with their somas located in the same isofrequency domain as excitatory neurons, or 2) inhibitory neurons with their somas located in different brain regions (such as the nucleus of the lateral lemniscus or the contralateral IC).

T33. Prefrontal Cortex Inhibition Reduces Prediction Error in the Auditory Cortex

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Category: Primary Auditory Cortex

Background: The Bayesian brain hypothesis proposes that the brain continuously generates a model of the environment based on predictions gained on previous experiences. Incoming sensory information is then compared to this model and either confirms the prediction, or if significantly different enough, prediction errors can be used to update the generative model. Using mismatch negativity, neural correlates of prediction errors are observed as early as the auditory midbrain, with the strength of error increasing up the auditory hierarchy to the auditory cortex (AC) (Parras et al, 2017). The prefrontal cortex (PFC) is involved in planning complex behaviour and decision making, and exhibits strong and long-lasting mismatch responses, consistent with coding of prediction error (Casado-Román et al, 2020). This raises the hypothesis that PFC exerts a top-down control of deviance detection in sensory cortices, and indeed prefrontal inputs have been shown to modulate contextual contrast in the visual cortex (Hamm et al, 2021). The present work advances this research by studying top-down transmission of prediction error information from PFC to AC.

Methods: We injected Female Long-Evans rats with 1 µl AAV5-hSyn-eNpHR3.0-EYFP to the PFC to allow optogenetic suppression of PFC neurons. After 7-11 days of recovery, 64-channel neural recordings were conducted in the AC under urethane anaesthesia. An auditory "oddball" paradigm composed of standard (STD) repeating stimuli, deviants (DEV) and no-repetition controls (CTR) was presented monaurally. This allowed decomposition of the neural mismatch effect into two components: repetition suppression and prediction error. During presentation of these auditory stimuli, we inhibited neurons of the PFC using 595nm LED activation of the eNpHR3.0 channel to assess the top-down transmission of prediction error in the PFC-AC pathway.

Results: Rats showed expression of EYFP in the PFC, demonstrating successful optogenetic virus transfection. Further, neural recordings using a 32-channel optrode in the PFC showed that local LED illumination reduced activity in PFC neurons during spontaneous and auditory-evoked activity.

LFP recordings in the AC during the auditory "oddball" paradigm showed robust neural mismatch, with responses to DEV stimuli greater than CTR stimuli, and limited responses to STD stimuli. PFC inhibition had no effect on LFP amplitudes during STD or CTR stimuli, but reduced LFP amplitudes in response to DEV stimuli.

Conclusions: This study provides evidence for top-down prediction error signalling from PFC to AC. Invasive recordings from animal models such as this allow accurate analysis of prediction error generation and transmission in different brain regions, furthering our understanding of the neural substrate of mismatch negativity.

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T34. Mice Got Rhythm: Sound-Evoked Whisker, Nose and Pinna Movements in the Awake Mouse and Their Relationship to Auditory Cortical Activity

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Category: Primary Auditory Cortex

Background: The auditory system has often been described as an "early warning" system for the brain, optimised for fast detection of sound events occurring far away or outside the focus of attention. A possible behavioural correlate of this “early warning” in awake mice is sound-evoked whisker twitches (Meyer et al. 2018 Neuron) and other sound-evoked body movements (Bimbard et al. 2023 Nat Neurosci). What is the nature of these sound-evoked movements, and how do they relate to auditory cortical activity?

Methods: We used sequences of noise bursts varying in sound intensity, predictability, and duration to analyse sound-evoked whisker, nose, and pinna movements in 7 awake head-fixed mice. In 4 of the animals, we also investigated the relationship between the sound-evoked movements and simultaneously recorded single-unit and multi-unit activity in the auditory cortex.

Results: Sound-evoked whisker, nose, and pinna movements did not resemble startle responses. The movements occurred even for quiet sounds and grew gradually with increasing sound intensity (25 70 dB SPL). Moreover, the movements were minimally affected by stimulus predictability or expectation. In fact, facial movements evoked by 65 dB SPL noise bursts were either similar for regularly and irregularly timed noise bursts (1s versus jittered 0.8-1.2s inter-onset intervals), or slightly but significantly larger for the rhythmic sounds. Further experiments involving long, variable duration noise bursts (0.4-1.6s) showed that while sound onsets evoked robust increases in whisker, nose, and pinna movements, sound offsets only evoked increases in pinna movement. Finally, analysis of auditory cortical recordings revealed a small but significant subset of units (15% for whisker and pinna, 7% for nose) in which trial-to-trial variation in sound-evoked response magnitudes correlated (or anti-correlated) with trial-to-trial variation in sound-evoked movement magnitude.

Conclusions: We conclude that sound onsets reliably evoke whisker, nose, and pinna movements in awake head fixed mice, and sound offsets can also evoke pinna movements. Moreover, trial-to-trial variation in the magnitude of sound-evoked movements is related to trial-to-trial variation in sound-evoked firing rates for a small but significant fraction of neurons in the auditory cortex.

T35. LOXHD1 is Required for TMC1 Localization at the Lower Tip-Link Insertion Area

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Category: Hair Cells: Anatomy and Physiology

Background: Loxhd1 is a large gene (160 kb) associated with age-related and congenital hearing loss in humans, dogs, and mice. It consists of 41 exons encoding 15 repeats of PLATs (Polycystin/Lipoxygenase/Alpha-Toxin), which is known in other proteins to bind proteins and lipids. Our lab previously discovered that the hearing loss caused by mutations in the 10th PLAT repeat results from a hair cell mechanical-electrical transduction (MET) defect onset between postnatal day (P) 7 and P11 (Trouillet et al., 2021). However, the exact mechanism of how LOXHD1 affects MET currents remains unclear, and alternative splicing isoforms may mask additional functions of LOXHD1. In addition, the localization of LOXHD1 in the hair bundle is uncertain.

Methods: To investigate these questions, we generated Loxhd1-Delta, a complete loss-of-function allele, eliminating all Loxhd1 PLAT domain coding sequences. To investigate the localization of LOXHD1, we generated Loxhd1-HA knockin allele. To investigate if the lower tip-link localization of the known MET channel complex components were affected by the absence of LOXHD1, we tracked TMC1, TMC2, TMIE, LHFPL5, and CIB2 using tagged knock-in alleles (Cunningham et al., 2020) or specific antibodies. High-resolution immunofluorescence (IF) imaging and SUB-immunogold SEM, a novel technique to detect sub-membranous proteins at nanoscale (Miller et al., in preparation), were performed to study the distribution of Loxhd1 and MET channel complex proteins.
Results: Different from PLAT10 mutants, Loxhd1-Delta showed ABR/DPOAE thresholds elevated not only in homozygous but also in heterozygous animals. Additionally, earlier MET defect was observed. These findings suggest that Loxhd1 isoforms can partially compensate for mutations affecting PLAT10. HA-tagged LOXHD1 (Loxhd1-HA) was found to be localized at the lower tip-link insertion area. By IF, we detected a reduction of TMC1 signal at the tip of row 2 stereocilia. SUB-immunogold SEM further demonstrated that TMC1 is mislocalized from lower tip-link area of IHCs in Loxhd1-Delta homozygous mice.
Conclusions: Our results indicate the essential role of LOXHD1 in connecting TMC1 to the lower tip-link area, highlighting LOXHD1 as a crucial component of the mature MET machinery.

T36. The Effect of Estrogen on Hair Cells in Larval Zebrafish

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Category: Hair Cells: Anatomy and Physiology
Background: Estrogen can modulate plasticity in the vertebrate auditory system, including the inner ear. For example, estrogen can increase hair cell density in the auditory periphery of some vertebrates, leading to increased auditory sensitivity. We hypothesize that estrogen increases cell proliferation and hair cell addition in zebrafish. Zebrafish have a lateral line system that contains a series of hair cell clusters (neuromasts) located externally along the side of each fish. Here we test how membrane-bound and nuclear estrogen receptors each contribute to estrogen-mediated fluctuations of hair cell turnover.
Methods: 5-day old zebrafish were exposed to either estrogen or estrogen and an estrogen receptor modulator. We then quantified the number of hair cells and proliferating cells in lateral line neuromasts of fixed fish.
Results: Estrogen caused a significant increase in lateral line hair cells. Agonism of G-protein-coupled estrogen receptors (gper) caused a similar increase in hair cell number, while gper antagonism caused a significant decrease. Treatment with fulvestrant, a broad nuclear estrogen receptor antagonist, caused a significant decrease in hair cells. Fulvestrant did not prevent estrogen mediated increases in hair cell number. By contrast, gper antagonism significantly attenuated the effects of estrogen in the lateral line.
Conclusions: Our data demonstrate that estrogen increases the total number of hair cells in the zebrafish lateral line, likely through gper signaling. Future experiments will focus on how estrogen and gper modulation alter cell turnover in the lateral line.

T37. Technical Details of Single-Molecule Microscopy to Visualize Protein Molecule Dynamics in a Stereocilium of Live Hair Cells

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Category: Hair Cells: Anatomy and Physiology
Background: Stereocilia are F-actin protrusions functioning as biological mechanical sensors to detect sound, acceleration and gravity. The stereocilium is a unique environment for protein-protein interactions, where a tightly packed unidirectional F-actin bundle is covered by the plasma membrane. To visualize the behavior of protein molecules in stereocilia, we developed a novel workflow for single-molecule microscopy in live hair

cells. Here, we introduce the technical details of our methodology and demonstrate our application to MYO7A trafficking in a stereocilium.
Methods: We expressed HaloTag-fused proteins in vestibular hair cells using a Helios® gene gun and fluorescently labeled them at a low density using JFX554-conjugated ligands. Vestibular epithelia harboring transfected cells were imaged using the light-sheet illumination of a dual-inverted selective plane illumination microscope (diSPIM). We established single-molecule microscopy using HaloTag-fused human β-actin (HaloTag-actin) and proceeded with MYO7A. To evaluate the activation mechanism of MYO7A in a stereocilium, HaloTag-fused MYO7A head (motor + neck region; HaloTag-MYO7A-HMM) were conditionally dimerized by fusing the p.F36V mutant of FK506-binding protein 12 (FKBP) to the C-terminus and applying an FK506 derivative, AP20187. Anchoring to the plasma membrane or to the F-actin core was achieved by using a ternary of FKBP, the FKBP-rapamycin binding domain (FRB) and a Rapalog, AP21987. We fused either FKBP or FRB to the C-terminus of HaloTag-MYO7A-HMM and fused the remaining one to the anchoring proteins, such as the Tac membrane antigen and the THDII F-actin binding domain of MYO3A, depending on the structure of anchoring proteins.
Results: We expressed HaloTag-actin in vestibular hair cells and labeled it using a different concentration of JFX554-ligand. Fluorescent puncta appeared as the concentration of JFX554 ligand is lowered and became distinguishable at 0.01 nM. We then optimized the imaging condition for myosins using HaloTag-MYO7A-HMM fused with C-terminal FKBP, which is activatable under the AP20187 treatment. Myosins were visualized using 0.3–0.6 nM of JFX554 ligands. Dimerized HaloTag-MYO7A-HMM-FKBP showed processive movements toward stereocilia tips at average 40 nm/sec, consistent with the slow movements of MYO7A. Anchoring to the plasma membrane (via Tac antigen) or to the F-actin (via THDII of MYO3A) did not cause movements of HaloTag-MYO7A-HMM (with FRB or FKBP for anchoring; see Methods) in a stereocilium. However, the positive controls, MYO10 and MYO3A heads, moved in stereocilia with these anchoring machineries. These data suggest that the kinetic properties of MYO7A-HMM is optimized for activation by dimerization.
Conclusions: We developed an experimental workflow to analyze the behavior of single protein molecules in live hair cells and approached the regulatory mechanism of MYO7A trafficking in a stereocilium. We speculate that our technique will be useful to analyze the behavior of wild-type stereocilia proteins and proteins with suspected, but not demonstrated, pathogenic missense variants detected in patients who have a hearing loss.

T38. Sound Encoding in the Cochlear Apex: Is the Place Principle Misplaced?

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Category: Hair Cells: Anatomy and Physiology
Background: The conventional understanding of mammalian cochlear function hinges on the place principle, which posits that each cochlear location resonates most effectively to a unique best frequency. Contrary to this traditional belief, a recent study by Burwood et al., Science Advances 2022 employed optical coherence tomography in vivo on guinea pigs. Measurements taken from two sites, separated by 4300 μm in the low-frequency region—with the most apical site being merely 360 μm from the helicotrema—revealed intriguing findings. The apical location resonated best at 315 Hz, while its basal counterpart had a best frequency of 160 Hz at 64 dB SPL. Even more surprisingly, a midway site between these two points mirrored the frequency of the basal site. This challenges the applicability of the place principle in low-frequency hearing. Nonetheless, more exploration is warranted to decode the enigma of low-frequency sound encoding.
Methods: To delve deeper, we combined time-resolved confocal microscopy with optical flow computation. This allowed us to gauge sound-evoked stereocilia movements of outer hair cells within the cochlear apical turn. Using the guinea pig temporal bone preparation, measurements were taken at two sites approximately 1 mm apart and at an intensity level of about 70 dB SPL in two separate experimental sets.
Results: Intriguingly, our measurements showed that the stereocilia bundle tip and base at the most apical site resonated best at 250 Hz, while the basal site peaked at 200 Hz. Cochlear microphonic data reflected a similar trend with 220 Hz for the apical location and 180 Hz for the basal site. These observations were replicated in four distinct preparations for each site and on multiple cells.

Conclusions: The congruence between mechanical and electrical measurements, as well as the alignment with Burwood et al.'s findings, challenges the conventional understanding of cochlear function. Our results reinforce the emerging view that the place principle may not be prevalent in the cochlear apical region.

T39. Pigment Epithelium-Derived Factor in Human Cochlea, a Study Using IF, RNAscope, Confocal and Super-Resolution Structured Illumination Microscopy

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Category: Inner Ear: Anatomy and Physiology

Background: Pigment epithelium-derived factor (PEDF, Serpin 1) is an endogenous glycoprotein which was recognized as a neurotrophic factor by its roles in protecting cells against oxidative stress, promoting cell survival, reducing inflammation, and deterring pathological angiogenesis. In present study, PEDF was investigated for the first time in human cochlea by using immunofluorescence (IF) and RNAscope® technique. We also observed this interesting and potentially useful protein in guinea pig, mouse and rat.

Methods: PEDF antibody (1:200, Biotechne) specific respectively for human and for rodents, as well as PEDF mRNA transcript probe (1:4000, Biotechne) for human and for guinea pig were used in immunohistochemistry and RNAscope® correspondingly. The archival frozen sections from two human cochleae had been obtained from patients. The cochleae were paraformaldehyde-fixed, EDTA-decalcified and cryo-sectioned. Ethical permission was obtained for the study on both human and animal specimens.

Results: PEDF is differently expressed in human cochlea from cochleae of rodents. While rodents shared a similar PEDF expression pattern, e.g., strong expression in inner hair cells and much weaker expression in the outer hair cells, human inner and outer hair cells showed equal PEDF IF intensity. PEDF-ir (immunoreactivity) was seen in the spiral ganglion neurons (SGNs) of man and rodents. PEDF proteins looked to be mainly cell membrane bounded but also inside cytoplasm. The neuronal PEDF-ir exists in the perikarya, nerve fibers as well as in the nerve terminals beneath the hair cells. PEDF was found in all turns. PEDF mRNA transcript signal, in situ hybridization, by using RNAscope® technique, was strong in SGNs, in cells of the stria vascularis, in the Reissners' membrane and in the spiral limbus of human cochlea. Guinea pig RNAscope showed similar distribution pattern.

Conclusions: With IF and RNAscope®, PEDF could be seen in various cell types in the cochlea. PEDF expression in human outer hair cells appears equally strong comparing with inner hair cells, whereas rodents' outer hair cells display much weaker expression than their inner hair cells. Further analysis is going to compare expression of PEDF between cochlear turns and between different layers of the stria vascularis. The capability of PEDF to promote neurogenesis and to maintain stem cell populations indicates a therapeutic potential for inner ear diseases especially the ones due to degeneration of SGNs and hair cells.

T40. Improvements in the Immunofluorescence Staining of Human Ear Celloidin Sections

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Category: Inner Ear: Anatomy and Physiology

Background: Archival human ear celloidin sections is a very important source to investigate the histopathological changes in the human inner ear. Existing temporal bone laboratories have hematoxylin and eosin-stained (H and E) sections. Only one in ten section is stained and the rest of the sections are stored in 80% ethanol in water. These sections have been used for immunohistochemistry, immunofluorescence (IF), proteomics, and DNA extraction. Our laboratory has developed a protocol for IF of temporal bone celloidin sections (Lopez et al 2016, Hirooki et al 2023). We have recently improved this protocol to quench auto fluorescence, by modifying the pH of the antigen retrieval solution and the use of xylene, and fluorescence quenching solutions.

Methods: We followed the protocol published recently be our laboratory Hirooki et al (2013). We use human celloidin sections containing the cochlea from 10 normal hearing human temporal bone specimens (ages 50-80 years old five male and 5 female). Celloidin is removed from the sections containing the inner ear, then

antigen retrieval solution acidic (pH 2) or basic (pH 9) is used to restore antigen binding, followed by the use of trypsin solution by 7-10 minutes (Vector Trypsin kit), and standard blocking solution is applied (0.5% bovine serum albumin in PBS and 0.1% triton X-100) for 2 hours. We tested primary monoclonal antibodies against acetylated tubulin and pan neurofilaments. Primary antibodies were incubated 72 hrs., following by the incubation secondary antibodies labeled with Alexa 488 or 594. A vector quenching solution is applied for 3 minutes and then DAPI 0.01% is added. To visualize very thin fibers stained with neurofilaments we added an additional step after the use of trypsin. We dehydrate the celloidin sections and immersed them in 100% xylene, sections are rehydrated and then the IF protocol is followed as described thereafter. Sections are coversliped using aqua-soluble mounting media. Laser confocal microscopy is used to analyze the IF-stained sections.
Results: This protocol resulted in successful identification of supporting cells of the organ of Corti and spiral ganglia neurons processes using antibodies against acetylated tubulin and pan-neurofilaments. The use of high pH enhances the immunofluorescent signal and the use of xylene to enhances the visualization of thin fibers in the organ or Corti. Autofluorescence is quenched by the use of xylenes and quenching kit.
Conclusions: The use of an improved standardize methodology to identify different antigens in the human ear allowed the unequivocal identification of spiral ganglia neurons and cells of the human organ of Corti. This method is currently being applied to identify cellular changes in the patients diagnosed with Meniere's disease, noise induce hearing loss and otosclerosis.

T41. Mortui Vivos Docent: A Modern Revival of Temporal Bone Plug Harvests

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Category: Inner Ear: Anatomy and Physiology
Background: Human temporal bones (HTBs) are invaluable resources for the study of otologic disorders and for evaluating novel treatment approaches. Given the high costs and technical expertise required to collect and process HTBs, there has been a decline in the number of dedicated otopathology laboratories. Our objective is to encourage ongoing study of HTBs, beyond the select few dedicated laboratories, by outlining the necessary steps for collection and processing of HTBs.
Methods: We provide the design of a custom-built temporal bone plug cutter to collect an HTB specimen which includes the inner ear, the internal auditory canal (IAC), the medial portion of the external auditory canal (EAC), tympanic membrane (TM), and a portion of the sigmoid sinus. This tool, which was previously commercially available, can be used to efficiently train new users and rapidly collect specimens of interest. We provide detailed figures of the collection process. We then outline microwave decalcification with ethylenediaminetetraacetic acid (EDTA) which can significantly accelerate the decalcification time of a collected specimen, especially when the specimen is trimmed or drilled to the level of the otic capsule. Finally, we provide our approach to embedding in paraffin media for eventual downstream histological and immunohistochemical analyses. This approach is an alternative to celloidin embedding which requires specialized equipment and technical expertise.
Results: To date, we have collected more than 30 temporal bones using our custom-made temporal bone plug cutter. The average post-mortem interval was 52 hours (range: 7-151). To assess decalcification times, eight fixed bones underwent a strict schedule of EDTA changes (twice weekly) and micro-CT imaging (weekly) while being decalcified in the microwave. Of these eight, four were routinely trimmed twice a month until the otic capsule was reached. On average, it took the non-trimmed bone plugs 5.5 months (range: 5.1-5.8) to decalcify. The trimmed bones all decalcified faster, at 3.2 months. Both approaches represent an improvement over the traditionally established 9 month decalcification time. Additionally, these microwave decalcified bones were then successfully immunostained for key inner ear proteins suggesting that this approach does not influence immunogenicity of the sample.
Conclusions: There is no shortage of downstream applications for which HTBs can be used in auditory research. Prior efforts have demonstrated their utility across the spectrum of diagnosis and treatment of

otologic disease. Despite this, procurement and processing of HTBs can be technically challenging and time consuming. We provide a set of protocols and tools that can decrease the barriers to HTB study, and in doing so, hope to encourage continued use of these specimens in the auditory research field.

T42. Haploinsufficiency for the Long Isoform of Myosin XVA Leads to Heightened Susceptibility to Cochlear Insults

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Category: Inner Ear: Anatomy and Physiology

Background: Auditory hair cells form precise and sensitive staircase-like actin protrusions called stereocilia bundles, which mediate mechanotransduction and thus hearing itself. In mammals, these hair cells do not regenerate or renew thus they need to maintain their stereocilia bundles for up to several decades. Myosin XVA, an unconventional myosin, plays a vital role as a molecular motor in stereocilia elongation and maintenance, with its short and long isoforms serving these functions, respectively (Fang, et al., 2015). Mice lacking one allele of the long isoform (Myo15+/ΔN) exhibit normal hearing thresholds and typical stereocilia bundles. However, we recently found that they display reduced mechanotransduction (MET) amplitudes and increased open channel probability at early postnatal stages compared to wild-type littermates. This led us to investigate if Myo15+/ΔN mice might be more susceptible to cochlear insults, testing their response to noise exposure or ototoxic drugs.

Methods: Organ of Corti explants were isolated from Myo15+/ΔN mice and their wildtype control littermates during the first postnatal week to obtain mechanotransducer (MET) currents (via patch-clamp recordings and fluid-jet bundle deflections) or for 24-hour incubation in a medium supplemented with gentamicin or control conditions (cell survival was assessed via immunofluorescence and confocal microscopy). Auditory brainstem responses (ABRs) were measured in 4-week-old mice, before and up to 3 weeks after exposure to broadband noise at 100 dB SPL for 30 min. Temporal bones from mice at ages ~P50 and ~P100 (with or without previous noise exposure) were isolated and either processed for scanning electron microscopy (SEM), or immunostained and imaged with confocal microscopy.

Results: Our noise exposure protocol produced hearing threshold shifts that fully recovered at 8 and 16 kHz but not at 24 and 32 kHz in both Myo15+/ΔN and Myo15+/+ mice. However, the permanent threshold shifts (PTS) were significantly larger (~13 dB) in Myo15+/ΔN mice, indicating that mice lacking just one copy of the long isoform of myosin XVA exhibit an increased sensitivity to permanent noise-induced hearing loss. We are currently processing the temporal bones from these noise-exposed mice to evaluate the integrity of stereocilia bundles and ribbon synapses. Lastly, in vitro, exposure of early postnatal organ of Corti explants to gentamicin led to a higher rate of hair cell death in heterozygous mice when compared to their wild-type littermates.

Conclusions: In humans, mutations on the myosin XVA gene cause the autosomal recessive nonsyndromic deafness DFNB3. Therefore, our data suggests that individuals carrying “recessive” mutations affecting the long isoform of myosin XVA could have increased susceptibility to cochlear insults.

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T43. Sem Analysis of Macrophages Along the Cochlear Spiral in Mice

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Category: Inner Ear: Anatomy and Physiology

Background: The susceptibility of sensory cells to pathological conditions differs between the apical and basal regions of the cochlea, and the cochlear immune system may contribute to this location-dependent variability. Our previous study found morphological differences in basilar membrane macrophages between

the apical and basal regions of the cochlea. However, the details of this site-dependent difference and its underlying structural and biological basis are not fully understood.
Methods: In this study, we utilized scanning electron microscopy to examine the ultrastructure of macrophages and their surrounding supporting structures. Additionally, we examined the phagocytic activities of macrophages and the expression of immune molecules in both apical and basal regions of the cochlea. We employed two mouse strains (C57BL/6J and B6.129P-Cx3cr1tm1Litt/J) and evaluated three experimental conditions: young normal (1-4 months), aging (11-19 months), and noise-induced damage (120 dB SPL for 1 hour).
Results: Using scanning electron microscopy, we revealed location-specific differences in basilar membrane macrophage morphology and surface texture, architecture in mesothelial cell layers, and spatial correlation between macrophages and mesothelial cells in both young and older mice. Observations of macrophage phagocytic activities demonstrated that basal macrophages exhibited greater phagocytic activities in aging and noise-damaged ears. Furthermore, we identified differences in the expression of immune molecules between the apical and basal cochlear tissues of young mice. Finally, our study demonstrated that as the cochlea ages, macrophages in the apical and basal regions undergo a transformation in their morphologies, with apical macrophages acquiring certain basal macrophage features and vice versa.
Conclusions: Overall, our findings demonstrate apical and basal differences in macrophage phenotypes and functionality, which are related to distinct immune and structural differences in the macrophage surrounding tissues.

T44. Dysregulation of Fetal-Derived Resident Tissue Macrophages Mediate CMV-Associated SNHL

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Category: Inner Ear: Anatomy and Physiology
Background: Cytomegalovirus (CMV) is the most common congenital viral infection in the developed world and the leading cause of non-genetic sensorineural hearing loss (SNHL) in children. Congenital CMV can cause progressive SNHL long after resolution of infection, suggesting a lasting effect on the developing host immune response in the cochlea. Resident tissue macrophages (RTMs) are the predominant resident immune cells in the cochlea and a fetal origin has been described in both mouse models and humans. Here, we used a clinically relevant mouse model of CMV-associated SNHL combined with genetic fate-mapping to selectively label fetal-derived cochlear RTMs and investigate the impact of CMV infection on RTM establishment during cochlear development.
Methods: To characterize the fetal origin of cochlear RTMs, we performed fate-mapping of RTMs from the yolk sac (YS) and fetal liver (FL) by crossing Csf1r-Mer-iCre-mer or Cdh5-Cre-ERT2 mice to Ai9 reporter mice and pulse labeling with 4-OHT at embryonic day (E)8.5 or E10.5 respectively to induce Cre-mediated labeling. Depletion of fetal-derived RTMs was performed by treating pregnant dams with anti-CSF1R blocking antibody at E7.5 and E13.5 to investigate the role of fetal RTMs in cochlear development. WT and fate-mapped mice were injected with saline or 200 plaque-forming units of murine CMV via intracerebral injection on postnatal day (P)3. Whole cochlear soft tissue from saline and CMV-infected WT mice was isolated at P6 for single cell-RNAseq analysis. For all other experiments, temporal bones were isolated across multiple postnatal timepoints through adulthood and analyzed by flow cytometry and immunofluorescent staining. Auditory thresholds were assessed using distortion product otoacoustic emission (DPOAE) and auditory brainstem response (ABR) testing at 4 weeks of age.
Results: Our fate mapping of RTMs from YS and FL revealed a highly specific temporal and spatial layering of fetal-derived RTMs across postnatal cochlear development, with replacement of fetal RTMs by bone marrow (BM)-derived RTMs after cochlear maturation at P14. Depletion of all fetal-derived RTMs at birth with an anti-CSF1R antibody led to increased area of the stria vascularis and a trending increase in DCT+ cells at 4-weeks, associated with SNHL. Both sc-RNAseq and flow cytometric analysis demonstrated an early and inappropriate influx of BM-derived infiltrating macrophages in response to CMV, disrupting the normal temporal layering of fetal-derived RTMs. In response to CMV infection, RTMs upregulated inflammation-associated pathways including viral response pathways and lymphoid activation, while downregulating cell signaling pathways critical for inner ear development, such as FGF signaling and TGFb response.

Conclusions: As the most prevalent immune cell in the developing cochlea, understanding the role of fetal-derived RTMs in normal cochlear development and their immune response in congenital CMV infection is essential for elucidating pathological mechanisms of SNHL and developing effective treatments.

T45. Do TRPA1-Deficient Mice Develop Age-Related Changes in Hearing?

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Category: Inner Ear: Anatomy and Physiology

Background: TRPA1 channels are master sensors of tissue damage since they are activated by endogenous compounds generated upon tissue stress or injury. These channels are highly expressed in nociceptive neurons where they trigger pain-like responses. TRPA1 channels are also expressed in the inner ear where they play a role in the regulation of cochlear sensitivity after acoustic trauma (Vélez-Ortega et al., Nat Commun, 2023). Although mice lacking TRPA1 channels have normal hearing thresholds, we uncovered abnormal wave amplitudes in auditory brainstem responses (ABR) as they age. Our histological exploration of the cochlear innervation in adult TRPA1-deficient mice has found abnormalities in fibers presumed to be type II spiral ganglion neurons (SGNs). These unmyelinated afferent fibers innervate the outer hair cells, respond to cochlear tissue damage, activate neurons in the cochlear nucleus following moderate to high sound intensity (greater than 80 dB SPL), and may provide input to trigger the medial olivocochlear (MOC) efferent negative feedback. Here we performed comprehensive hearing testing of TRPA1-deficient mice at several ages to evaluate the protective role of TRPA1 signaling over time.

Methods: We used C57Bl/6 Trpa1 knockout and wild-type littermates at postnatal days 4, 19 and ~40, and at 4-5 months of age. We evaluated ABR to tone burst and click stimuli, distortion product otoacoustic emissions (DPOAE), and the MOC reflex in anesthetized mice. Temporal bones were collected at the specified ages, and the cochlear epithelium was immunolabeled against neurofilament heavy chain (NF-H) and imaged with confocal microscopy. Tone burst and click evoked ABR were used to measure hearing thresholds and auditory nerve activity in anesthetized mice. The MOC reflex was evaluated by measuring DPOAE magnitude changes after the application of contralateral noise.

Results: Hearing thresholds and DPOAE magnitudes of mice lacking TRPA1 were indistinguishable from wild-type mice at all ages tested. However, the ABR wave 1 amplitudes to high intensity click stimuli (greater than 90 dB SPL) were larger in TRPA1-deficient mice compared to their wild-type littermates, and this difference became more pronounced with age. Additionally, although we do observe the presence of an MOC reflex in mice lacking TRPA1, ongoing experiments are exploring whether the magnitude of the reflex is altered.

Conclusions: Our results show that chronic deficiency of TRPA1 channel activity affects the magnitude of auditory nerve activity. However, the precise molecular mechanisms leading to these differences and their functional consequences on hearing are still poorly understood.

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T46. Characterizing the Role of CGRP on OHC Function During Stimulation of Medial Olivocochlear Neurons

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Category: Vestibular: Basic Research and Clinical

Background: Axons of medial olivocochlear (MOC) neurons project from the superior olivary complex across the brainstem and out cranial nerve VIII to innervate outer hair cells (OHCs) along most of the cochlear coil. The predominant MOC transmitter is thought to be acetylcholine (ACh), but other transmitters have been described including gamma-aminobutyric acid (GABA), catecholamines, and alpha-calcitonin gene-related peptide (αCGRP). Electrical stimulation of MOC axons in the floor of the fourth ventricle can both suppress

and enhance distortion product otoacoustic emissions (DPOAEs) and the compound action potential (CAP) by up to 10-20 dB relative to prestimulus conditions. While MOC-mediated suppression occurs with an onset time constant of ~100 ms that quickly returns to baseline following stimulus termination, MOC-mediated enhancement is much slower requiring seconds to peak and persisting for 100's of seconds beyond the stimulus. MOC-mediated suppression, but not enhancement, is attributed to the release of ACh and the activation of postsynaptic α9α10 nicotinic ACh receptors (α9α10nAChRs) and SK2 potassium channels on outer hair cells. Furthermore, MOC-mediated enhancement also operates independently of muscarinic ACh, GABA-A, GABA-B, and dopamine receptors as well as adrenergic signaling. While the slower response kinetics suggest metabotropic receptor signaling, the MOC transmitter and downstream mechanism underlying MOC-mediated enhancement has remained elusive. While previous work concluded that loss of αCGRP does not appear to impact MOC-mediated suppression, its effects on MOC-mediated enhancement effects were not characterized.

Methods: To evaluate if αCGRP plays any role in MOC-mediated enhancement, we recorded DPOAEs in urethane/xylazine mice while stimulating MOC neurons with platinum-iridium electrodes in the floor of the 4th ventricle. Transgenic mice with targeted deletion of αCGRP (CGRP-KO), their littermate controls, and C57Bl/6 mice were employed for these experiments. DPOAE measurements and stimulus generation were done with Tucker-Davis System hardware and a data acquisition card controlled by custom-written software. Stimulus F1 and F2 frequencies were independently presented to Etymotic ER2 earphones in the right ear and distortion products were recorded using an ER-10B+ microphone. In some of the experiments, the α9α10nAChR antagonist strychnine and/or the CGRP antagonist BIBN4096 were administered to anesthetized mice using the intraperitoneal route.

Results: The CGRP antagonist BIBN4096 failed to block either MOC-mediated suppression or enhancement. In agreement with these pharmacological experiments, MOC-mediated enhancement was also observed in CGRP-KO animals. Interestingly, however, a significant reduction of MOC-mediated enhancement in CGRP-KO mice was seen in older age groups of 8-10 months as compared to age-matched wild type and C57Bl/6 controls. Furthermore, the growth of MOC-mediated slow enhancement with decreasing DPOAE stimulus level was also altered in the same group of animals.

Conclusions: In conclusion, MOC-mediated enhancement does not require CGRP, although its loss does appear to impact the amplitude of MOC-mediated enhancement in aging CGRP-KO animals.

T47. Exploring Human Cochlear Masking and OAE Suppression at Different Cochlear Locations Using Frequency Sweeps

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Category: Inner Ear: Cochlear Mechanics

Background: Previous recordings of mouse basilar-membrane (BM) motion in response to exponential frequency chirps have shown that upward sweeps (i.e., from low to high frequency) are more effective suppressors of tonal probe signals than downward sweeps (high to low) (Charaziak, ARO 2022). That study found that the differential suppressive effects of sweep direction depend strongly on sweep rate, being stronger for faster sweeps. These results in mice are reminiscent of findings from human perceptual studies using so-called "Schroeder-phase complexes," which are stimuli that contain multiple harmonic frequency components of equal amplitude but variable starting phase, summing to produce a series of periodic linear frequency sweeps. Behavioral studies find that negative Schroeder-phase maskers (e.g., upward sweeps) are more effective maskers of tonal signals than positive Schroeder-phase maskers (e.g., downward sweeps). In our recent work (Salloom et al, ARO 2023), we measured behavioral masking and OAE suppression as a function of sweep rate and direction in normal hearing humans. Consistent with the sweep direction effects in the Schroeder masking literature, the upward sweeps were more effective maskers/suppressors than downward sweeps. Unexpectedly, we found that the largest up-down differences in both behavioral masking and OAE suppression occurred at sweep rates of approximately ~50 oct/s. This rate of peak effectiveness matches otoacoustic estimates of the group velocity of the traveling wave. These results suggest that both behavioral masking and OAE suppression share common mechanisms, presumably closely related to mechanical suppression in the cochlea.

Methods: The current study builds on our recent efforts investigating the effects of sweep rate and direction in normal hearing humans, both behaviorally and physiologically. Schroeder-phase stimuli are used in both behavioral masking and stimulus-frequency otoacoustic emissions (SFOAEs), either as maskers or suppressors, respectively. We explore the test-retest reliability of our modified SFOAE suppression paradigm to determine the consistency of our estimates of cochlear suppression. In addition, we measure the effects of sweep rate and direction at a higher signal/probe frequency (~4 kHz) to determine whether the dependence on sweep rate and direction varies with location in the cochlea. We hypothesize that the use of these stimuli will provide new opportunities to relate masking and suppression to cochlear processing.
Results: Differences in psychoacoustic masking and OAE suppression for various sweep combinations will be explored and compared. To the extent that the two phenomena share common generation mechanisms, we expect behavioral masking and OAE suppression to depend similarly on sweep characteristics.
Conclusions: The long-term goal of our study is to better understand dynamic aspects of nonlinear signal processing in the cochlea. Here, we expand on previous work in mice (Charaziak 2022) and humans (Salloom et al 2023) to explore the effects of frequency sweep rate and direction on signal suppression and masking in normal hearing humans.

T48. Investigation of the Skull Bone Sound Waves Under Bone Conduction In-Vivo

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Category: Inner Ear: Cochlear Mechanics
Background: The aim is to quantify the surface wave speeds across the skin-covered skull in live humans with previous data in cadaver heads.
Methods: Preliminary tests were conducted on 10 volunteers (awake, no sedation), where the skull was excited transcutaneously via an actuator from a bone conduction hearing aid (BCHA), held by a 5N-steel-band at a location posterior to the forehead. The resultant motion was monitored at ~100 points via a single-sensitivity-axis scanning laser Doppler vibrometer (SWIR Scan-Sense, Optomet, Germany) in the area of the forehead, where the skin was covered with a flexible retroreflective tape. Stimulation was provided at 4 kHz, in order to evoke wave motion with at least half a wavelength within the measurement area (~10-12cm wide), while having sufficiently high output from the BCHA. Signal processing methods have been implemented in order to reduce the effect of motion artifacts from random body movements. In-vivo data is compared with equivalent surface wave measurements of cadaver heads with and without skin.
Results: Measurements on the cadaver heads indicated no major change due to the presence of skin in the spatial distribution (wavelengths) of the wave patterns across the superior skull. In-vivo skull vibration data indicated wave speeds comparable to cadaver head data.
Conclusions: The skull wave motion in patients was similar to previous experimental data in cadaver heads.

T49. Motion of Cochlear Partition Structures of Intact Human Cadaveric Specimens Measured With Optical Coherence Tomography Vibrometry

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Category: Inner Ear: Cochlear Mechanics
Background: Recent work had found substantive anatomical and motion differences between the human cochlear partition (CP) and that of most laboratory animals at the cochlear base. In humans, there is a soft-tissue structure “the bridge” found between the bony osseous spiral lamina (OSL) and the basilar membrane (BM) that may have a profound impact on the motion of structures within the organ of Corti (OoC). Unlike in laboratory animals, the human OSL moves appreciably and the bridge moves as much as the BM (Raufer et al., 2023 PNAS). In the present study, we image through the intact round window with optical coherence tomography (OCT) to visualize and measure the motion of cochlear structures that are beyond the scala-tympani surface of the CP.

Methods: CP motion in response to sound was measured while visualizing structures with OCT vibrometry in six fresh (12-27 h postmortem) human specimens. We made measurements of multiple (up to 27) radial locations within the CP in each specimen. Cross-sectional imaging and vibrometry measurements of the CP were made using a Spectral-Domain OCT system with a 900-nm center wavelength and A line-scan camera frame rate of 46-kHz (GAN620C1, Thorlabs, Germany). We made vibration measurements down to 0.1-0.4 nm, while the image resolution axially was 2.23 μm (in water) and laterally was ~8 μm, using a 36 mm, 0.055NA, 2x objective lens and custom LabVIEW-based software (VibOCT v2.1.5-v2.1.7). SyncAv (v0.47) generated pure-tone sequences from 0.1-21 kHz equalized for constant SPLs ranging from ~80 to 110 dB in the ear canal. Stapes velocity was also measured with a laser Doppler vibrometer and used as a reference for vibrometry.
Results: The upper and lower bony OSL moved together as a flexible beam. The magnitude of OSL motion was relatively similar across specimens whereas BM and bridge magnitudes were more variable. The reticular lamina surface of the OoC and the tectorial membrane generally followed the profile of the BM, but had a more complex motion that varied with frequency and varied across ears. This variation might be related to the slight differences in frequency location measured (9-13 kHz BF region) or the condition of the organ of Corti.
Conclusions: The scala tympani surface of the bridge and BM (which has running continuous collagen fibers) has a simple beam motion independent of frequency. While the general profile of motion was similar across specimens, certain structures moved more consistently across specimens than others. For example, OSL motion was similar, while the BM and bridge differed across ears. The reticular lamina and tectorial membrane motion varied most across ears.

T50. Intracochlear Motion Measurements in the Basal Turn of the Guinea Pig Cochlea: A Comparison Across Species and Cochlear Locations

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Category: Inner Ear: Cochlear Mechanics
Background: Examinations of organ of Corti complex (OCC) motion in the guinea pig cochlea using optical coherence tomography (OCT) have been relatively sparse compared to those in mouse and gerbil. In this study, we examine and compare OCC motion measurements taken through the round window membrane (RWM), as well as through a cochleostomy made in the first turn of the guinea pig cochlea.
Methods: Using OCT vibrometry, we recorded OCC motion in the guinea pig base through the RWM corresponding to a best frequency (BF) of approximately 30-35kHz, and in a more apical region through a cochleostomy with a BF of approximately 20-25kHz. Our recordings were made primarily in the transverse direction for both locations. We assessed the health of the cochlea by measuring distortion product otoacoustic emissions (DPOAEs) before and after the cochleostomy. Recordings through the RWM were performed prior to the cochleostomy. For motion measurements, we stimulated with one second, multitone stimuli with frequencies between 5-40kHz and amplitudes ranging from 20-80dB SPL. We assessed motion at the basilar membrane (BM), reticular lamina (RL), and the outer hair cells (OHCs) which lie between the RL and meet the Deiters cells approximately halfway through the organ of Corti.
Results: In the 20-25kHz region, we found results similar to characteristic traits of OHC motion found in gerbil, including subBF nonlinearity and a region of increased amplitude termed the hotspot. We also observed a related ½ cycle phase shift of the OHCs re: BM and RL. The BM and RL lacked sub-BF nonlinearity. For the 30-35kHz location motion observed through the RWM, we found it more difficult to achieve a sufficient signal to noise ratio to gather data at several depths. Regardless, we found no evidence of strong subBF nonlinearity or a ½ cycle phase shift in the OHC region. BM and RL motion measured through the RWM was comparable to that measured at the 20-25kHz other location.
Conclusions: The subBF nonlinearity in the OHCs of the relatively apical (20-25kHz) location indicates subBF activity that is unique to the OHC region, since it does not appear in the BM or RL motion. Reporting from several experiments, we have found less evidence of OHC activity in the more basal (30-35khz) recordings recorded through the RWM. Overall, we encountered more difficulty observing subBF nonlinearity in the guinea pig than gerbil, which could suggest a more constrained hotspot area across the two species.

T51. Assessment of AAV-Mediated Innate and Adaptive Immunity in the Mammalian Inner Ear

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Category: Gene Therapy

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 is critical to 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 examine the innate and adaptive immune responses triggered by AAV-mediated gene delivery in the mouse inner ear.

Methods: We injected AAV2.7m8-GFP or a 5% glycerol vehicle in PBS into 8-10 weeks-old C57BL/6J mice through the posterior semicircular canal (PSC) approach. ABR tests were performed preoperatively. Cochleae were dissected after ABR evaluations at postoperative day (POD) 28 and immunohistochemically stained with macrophage marker Iba1 and T-cell marker CD3. The number of macrophages and T-cells were quantified using confocal images. Multi-spot cytokine and chemokine assays were used to quantify immune activation in serum and perilymph samples. Neutralizing antibody assay was used to assess the presence of neutralizing antibodies against AAVs. ELISpot assay was used to assess IFNɣ secretion from spleen cells mediated by antigen-specific T cells against AAVs.

Results: The number of macrophages of AAV2.7m8-GFP-injected and vehicle-injected mice at POD28 were statistically significantly increased compared to non-surgery mice. On the other hand, the number of T-cell of AAV2.7m8-GFP-injected and vehicle-injected mice were not statistically significantly increased compared to non-surgery mice. Chemokines and cytokines were detected in perilymph and serum samples of AAV2.7m8-GFP-injected and vehicle-injected mice, though their levels were not statistically significantly different. ABR tests performed preoperatively and at harvest did not show statistically significant differences. Neutralizing antibodies against AAV2.7m8 were detected at POD28 after injections. An increase in IFNɣ secretion against capsid peptide of AAV2.7m8 was not detected in six out of seven mice, and one mouse showed mild increase.

Conclusions: Inner ear gene delivery with AAV2.7m8 can trigger both innate and adaptive immune responses. However, in general, these responses are mild, and did not lead to a hearing loss.

T52. Open Board

T53. Open Board

T54. AAV1-HOTOF Gene Therapy for Autosomal Recessive Deafness 9 (DFNB9)

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Category: Gene Therapy

Background: 1.5 billion people worldwide live with hearing loss, and 430 million of them suffer from disabling hearing loss. Genetic defects cause hearing loss in one of 500 newborns. There is no pharmaceutical or biological treatment for hearing loss. Mutations in the gene OTOF cause autosomal recessive deafness 9 (DFNB9), manifested by the lack of sound detection or speech recognition from birth. Previous studies have demonstrated gene therapy to treat mouse models for DFNB9 with hearing recovery. No such study has been performed in human patients. We conducted a single-arm trial in children with DFNB9 by OTOF gene therapy.
Methods: We used a dual AAV approach in which the human OTOF cDNA was packaged into two adeno-associated virus (AAV) serotype 1 vectors for recombination to produce the full-length OTOF cDNA. A human hair cell promoter, Myo15, was used to drive the OTOF expression in affected hair cells. Five DFNB9 patients (2-6 years of age) with severe-to-complete hearing loss were enrolled in the trial. AAV1-hOTOF was unilaterally injected into the cochlea through the round window membrane (9E11 and 1.5E12 vg). The follow-up studies were performed on the patients for at least 13 weeks.
Results: In the treated patients, no DLT (Dose-Limiting Toxicity) was observed. 39 adverse events were detected, with 95% (37/39) being grade 1 or 2, and 5% (2/39) being grade 3. Hearing was restored in four out of five injected patients. In the low-dose (9E11 vg) group of one patient, hearing recovery was detected at 4 weeks post-injection, with an average ABR (Auditory Brainstem Response) threshold of 64 dB, compared to over 95 dB before treatment. By 26 weeks, the average ABR threshold was further improved to 45 dB. In the best frequency (0.25 kHz and 2 kHz), the ABR thresholds were 35 dB. In the high-dose (1.5E12 vg) group of four patients, hearing was recovered in three patients. At 4 weeks post-injection, the mean average ABR threshold was 74 dB, compared to over 95 dB before treatment. By 13 weeks, the ABR thresholds were further improved to 55 dB. Speech perception testing was improved in 3 out of 4 patients with hearing recovery. Hearing was not recovered in one patient, who had a higher level of the neutralizing antibody before the treatment.
Conclusions: This is the first human study (ChiCTR2200063181) to show gene therapy for DFNB9 is safe and without major adverse effect events. It recovered hearing with increasing efficacy over time in children who were otherwise unable to detect sound. The results support further development of gene therapy in DFNB9 patients, including expanded age groups and long-term observation.

T55. In Vivo Adenine Base Editing Restores Long-Term Auditory Function in a Mouse Model of Recessive Profound Deafness

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Category: Gene Therapy

Background: OTOF is the first gene identified as affecting auditory neuropathy spectrum disorders (ANSD) and accounts for approximately 41-91% of individuals with ANSD, which is a common form of autosomal recessive hearing loss. However, there are no effective drugs for hereditary deafness in clinical practice at present, so it is a major challenge to precisely correct the pathogenic mutation and salvage the damaged hearing in the long term. A common mutation (c.2485C greater than T, p.Q829X) in the OTOF gene induces a prelingual profound hearing loss in patients. Adenine base editors (ABEs) can accurately convert A·T to G·C base pairs and are potential therapeutic tools for G-to-A or C-to-T pathogenic mutations. 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: The mouse model with the humanized homologous mutation (Otof: c.2482C greater than T; p.Q828X) was constructed. The different combinations of three Cas9 variants and two deaminases were

screened. The editing efficiencies were detected by next-generation sequencing (NGS), and the off-targets were detected by GUIDE-seq and transcriptome sequencing. A dual-AAV-PHP.eB packaged with the N-terminal and C-terminal of NG-ABEmax was microinjected into the inner ear of mice. Auditory function, otoferlin expression, and inner hair cell synaptic function were assessed by ABR, immunohistochemistry, and electrophysiology, respectively.
Results: We compared combinations of three Cas9 variants (SpCas9-NG, SpG, and SpRY) that recognize non-classical PAM and two deaminases (ABE8e and ABEmax) in vitro and found that ABE8e activity was superior to ABEmax, while ABEmax had significantly lower bystander editing than ABE8e. However, in SpCas9-NG, the editing efficiency of ABEmax and ABE8e was similar, so NG-ABEmax was used for subsequent in vivo experiments. SpCas9-NG_ABEmax system directly corrected the pathogenic mutation in the mouse model with 89.4% editing efficiency in Otof transcripts and with no significant off-target mutations. The ABE-treated mice showed otoferlin expression in 88% of inner hair cells and showed long-term restoration of near-normal levels of auditory function up to 60 weeks post-transduction. Inner hair cell synaptic exocytosis was also improved.
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 DFNB9, and will facilitate further clinical translation of ABEs in treating hereditary hearing loss.

T56. Targeted Brainstem Delivery of Adeno-Associated Viral Vectors Significantly Improves Gene Delivery to Spiral Ganglion Neurons in Adult Rats

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Category: Gene Therapy
Background: Spiral ganglion neurons (SGNs) are significantly impacted in a number of sensorineural hearing loss (SNHL) disorders. Although gene therapy is a promising approach for SNHL, gene transfer to SGNs is very low following round window delivery of adeno-associated viral vectors (AAVs). Delivery of genes encoding Neurotrophic Factors (NTFs) are of intense interest for preservation and/or restoration of SGNs – studies have shown that despite relatively modest transduction efficiency with intracochlear delivery (5-10%), up to 14% of the SGN population has been rescued with AAV2-NTF. These studies demonstrate the potential of AAV-based NTF gene therapy and make a compelling case for improving SGN transduction efficiency. Our group have shown that delivery route can impact AAV-mediated tropism, and that axonal transport can be utilized to deliver genes to brain regions distal from the injection site, the directionality of which is serotype dependent.
Methods: We hypothesized that targeted delivery of AAV vectors to the brainstem nuclei which innervate the cochlea (cochlea nucleus (CN) and superior olivary complex (SOC)) can be utilized to deliver transgenes to the SGNs in the cochlea via axonal transport. Neurosurgical approaches are considered invasive, however our group has extensive experience in safely targeting AAV-based gene therapies to various subcortical regions in multiple clinical trials utilizing intraoperative MRI guidance and believe the potential improvements in quality of life, present a strong case for development of this approach.
Results: We performed direct intraparenchymal convection enhanced infusion of various AAV serotypes encoding green fluorescent protein (GFP) using an optimized surgical trajectory which was developed to avoid damaging brainstem nuclei important for vital life functions. The surgery was well tolerated by all animals and auditory brainstem response (ABR) did not reveal any threshold shifts related to infusate delivery up to 4 weeks post-surgery. Cochlear transduction was observed with the following major AAV serotypes expressing GFP: AAV2, AAV6, AAV9, AAV-Anc80 and AAV-PhP.B. Quantification of GFP-positive cells revealed cochlear tropism as well as efficiency of gene transfer across the cochlea spiral (apex, middle, base) varied across AAV serotypes, with some serotypes transducing both SGNs and HCs, while others transduced SGNs or HCs exclusively. We also show that secreted transgenic GDNF can be taken up by inner HCs, which contain the cognate receptor. In the brain, most of the AAV serotypes exclusively transduced neurons, which are not antigen presenting. Consistent with our previous findings, AAV9 transduced glial cells in the brain, which are antigen presenting, and lead to an immune response.

Conclusions: This study provides proof of concept that targeted AAV delivery to the brainstem can efficiently deliver transgenes to SGNs in the adult mammalian cochlea via axonal transport, the efficiency of which is serotype dependent.

T57. Rational Design and Predicted Elasticity of Mini-PCDH15 Proteins Used for Usher-1F Gene Therapy

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Category: Gene Therapy

Background: In inner-ear hair cells, two dimeric cadherin proteins called protocadherin-15 (PCDH15) and cadherin-23 (CDH23) form "tip link" filaments essential for hearing and balance. Hair cells respond to sound stimulation or head movements by stretching tip links that gate ion channels and trigger sensory perception in a process known as mechanotransduction. The ectodomain of PCDH15 has 11 extracellular cadherin (EC) repeats interconnected by stabilizing calcium (Ca2+) ions bound to EC inter- linker motifs. A membrane adjacent domain (MAD12) predicted to be mechanically weak (De-la-Torre, et al.,2018) connects the ectodomain of PCDH15 to its transmembrane domain near the mechanotransduction channel. Mutations in the PCDH15 gene cause Usher Syndrome Type 1F, characterized by progressive blindness, congenital hearing loss, and balance deficit.

Adeno-associated viruses (AAV) have been shown to be efficient and effective at delivering small genes (less than 4.7 kb) for gene therapy. However, the PCDH15 coding sequence (greater than 6 kb) is too large to fit in a single AAV vector. Thus, we designed novel, shortened “mini-PCDH15” variants that lack 3-5 EC repeats and can fit in a single AAV. One variant, mini-PCDH15-V4, rescued hearing and balance deficits in newborn Pcdh15-ko mice as well as hearing in Pcdh15fl/fl;Myo15-Cre mice (Ivanchenko et al.,2023). However, two other variants (mini-PCDH15-V7 and -V8), despite sharing similar EC repeat composition and length, were less effective or failed to rescue hearing in vivo. We hypothesize that effective mini-PCDH15s dimerize and have elastic properties similar to wildtype PCDH15, while deficient variants may have elastic properties unsuitable for tip-link function. However, the elastic response of mini-PCDH15s is unknown.

Methods: We combined size exclusion chromatography and negative stain electron microscopy to evaluate mini-PCDH15s ectodomain dimerization. We also characterized the structure of two artificial linkers using X-ray crystallography. In addition, we used experimental structures of native linkers and AlphaFold2 models to predict the structures of the remaining artificial linkers. Using this data, we modeled the structures of the mini-PCDH15-V4, -V7, and -V8 ectodomains and tested their elastic properties in steered-molecular dynamics (SMD) simulations.

Results: We present a structural study of five artificial linkers (EC3-EC7, EC3-EC9, EC4-EC7, EC7-EC11, and EC8-EC11) found in mini-PCDH15-V4, -V7, and -V8. We solved X-ray crystal structures of EC3-EC7 (mini-PCDH15-V7) and EC4-EC7 (mini-PCDH15-V8) showing distinct Ca2+ occupancies and inter-repeat conformations. Equilibrium MD simulations showed that among modeled artificial linkers, EC7-EC11 is the least rigid. Furthermore, SMD simulations of the complete monomeric mini-PCDH15-V4 ectodomain revealed that its elasticity closely mimicked the wildtype PCDH15 elasticity, while the mini-PCDH15-V7 and mini-PCDH15-V8 ectodomains exhibited smaller force peaks and diminished extensibility when compared to the wildtype PCDH15.

Conclusions: Our structures and simulations offer an atomic-level view of the rationally designed mini-PCDH15s used for AAV-therapy, shedding light on mechanistic principles that may guide the optimization of novel mini-PCDH15s for Usher-1F gene therapy.

T58. Stereocilin Gene Therapy Rescues Hearing in Mature STRC Knockout Mice

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Category: Gene Therapy

Background: Stereocilin (STRC) gene variations are the second most common cause of autosomal recessive (AR) hearing loss (HL). This gene, located on chromosome 15q15.3, has posed challenges for research due to its problematic genomic position and duplications, resulting in pseudogenes. The product of the STRC gene serves as an extracellular protein in the stereocilia of outer hair cells (OHC), playing a crucial role in maintaining the proper cohesion and positioning of stereociliary tips relative to the tectorial membrane. Deletion of the genomic region containing the STRC gene has direct links to autosomal recessive nonsyndromic sensorineural deafness-16 (DFNB16) and Deafness-Infertility syndrome.

Methods: We administered a third generation lentiviral vector (LVV) expressing STRC and dtomato driven by an SF promoter to one-month-old homozygous mutant for the STRC gene. We visualized the posterior semicircular canal (PSSC) on the left side and performed a canalostomy using a microdrill and then delivered 1μL of the vector to that side and covered the defect with a muscle or fat graft. Auditory brainstem response (ABR) tests were conducted days after surgery, three and seven weeks delivery. We compared the treated left side to the untreated right side, which served as the control in each mouse. Subsequently, the mice were sacrificed, to assess the gene and its expression via RT-PCR. Additionally, we conducted histological and immunohistochemical evaluations to determine the incorporation of the STRC gene product in the outer hair cells (OHC) with the d-tomato cassette in the vector.

Results: ABR thresholds demonstrated delayed improvements in high frequency hearing in all treated mice. Expression of dTom could be demonstrated in inner and outer hair cells during immunohistochemical evaluation.

Conclusions: The findings hold promise for developing genetic therapy that could effectively treat early-onset hearing loss in patients with STRC gene mutations. The vector technology demonstrated its safety and efficiency in addressing inner ear disorders in mouse models, serving as a foundational step towards translating these discoveries into human clinical trials to evaluate their safety in humans.

T59. Cellular Study of the Protective Effect on Sensorineural Hearing Loss Using Cyclophilin D Inhibitor

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Category: Inner Ear: Damage and Protection

Background: Mitochondria are important organelles that not only produce bioenergy but also control apoptosis and calcium homeostasis. When a stressful environment leads to an imbalance in calcium homeostasis in cells, calcium is transported from the endoplasmic reticulum to the mitochondria. Under conditions of calcium overload and increased reactive oxygen species (ROS) production in the mitochondria, mitochondrial permeability transition pores (mPTPs) open to release mitochondrial calcium as well as proapoptotic factors, leading to cell death. One of the diseases resulting from mitochondrial calcium overload and ROS overproduction is sensorineural hearing loss, which can be an adverse effect of the chemotherapeutic drug cisplatin.

Methods: Each group of HEI-OC1 cells used in in vitro experiments was treated with 20 μM cisplatin with or without cypD inhibitor pretreatment at serial concentration for 1 h. To determine the percentage cell viability, we used MTT assay. We used DCF-DA and MitoSOX to measure intracelluar ROS levels and mitochondrial ROS levels. To measure the mitochondrial calcium levels and cytosolic calcium levels, we used Rhod-2AM and Fluo-4AM, respectively. We performed immunocytochemistry using annexin V and western blot analysis utilizing cleaved caspase-3. Mouse auditory function was assessed by measuring ABR. Auditory function was measuredwith click stimuli and tone burst sound frequencies of 8, 16, and 32 kHz, and acoustic thresholds of SPL were determined using BioSigRP software.

Results: The cypD inhibitor showed a protective effect at a much lower concentration than α-lipoic acid, a drug that has been reported to exert a protective effect against cisplatin. We confirmed that intracellular ROS

was increased in the cisplatin treatment group due to intracellular stress and decreased in the cypD inhibitor treatment group, indicating that cypD inhibitor treatment improved the ROS scavenging ability of the cells. In the cisplatin group, mitochondrial ROS increased due to intracellular stress, while mitochondrial ROS significantly increased in both the pretreatment group and the cypD inhibitor treatment group. The cypD inhibitor treatment group reduced both early and late apoptosis signals compared to cisplatin group. In addition, caspase-3 expression was decreased in drug treatment group compared to cisplatin group. Mice were pre-treated with the cypD inhibitor and their hearing ability was measured using ABR. The protective effects of cypD inhibitor on cisplatin-induced ototoxicity were examied using a mouse model. Pre treatment with cypD inhibitor protected hearing ability against cisplatin-induced hearing loss.
Conclusions: The drug used in this study is different from others used to inhibit cypD because it inhibits a target protein and exerts a protective effect against ototoxic loss, which is difficult to recover. As research in this field continues, we anticipate the possibility of developing a drug that can protect or treat other diseases besides ototoxic hearing loss.

T60. Evaluating the Protective Effects of Raloxifene in a Mouse Model of Noise-Induced Hidden Hearing Loss

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Category: Inner Ear: Damage and Protection
Background: Noise induced hearing loss (NIHL) is one the most common health issues affecting veterans. Exposure to loud noise can lead to permanent elevation of hearing thresholds. Noise induced hidden hearing loss (NIHHL) is a milder form of hearing loss in which auditory thresholds recover but there is permanent damage to or loss of synaptic connections between inner hair cells and afferent nerve fibers (or synaptopathy). Currently there are no FDA-approved therapeutics to treat or reverse NIHL and NIHHL, our laboratory has previously shown that administration of 17β-estradiol (E2) in ovariectomized female mice protects against NIHHL. Additional reports demonstrate that the protective effects of E2 may be mediated through estrogen receptor β (ESR2). Selective estrogen receptor modulators (SERMs) are compounds that exhibit tissue-specific actions through ESR2 or estrogen receptor 1. Raloxifene is an FDA-approved SERM that is approved for the treatment of osteoporosis and risk reduction of certain breast cancers in post-menopausal women. The mechanism of action of Raloxifene in the inner ear, including whether it acts as an ESR2 agonist, is currently unknown. The objective of this study is to evaluate raloxifene as a therapeutic agent for treatment and prevention of NIHHL in intact female mice.
Methods: B6CBAF1/J mice were obtained at 7 weeks of age. At 8 weeks of age, 21-day, slow-release pellets containing placebo, or Raloxifene were subcutaneously implanted in the mice. At 9 weeks of age, baseline auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE) thresholds were established. At 10-weeks of age, mice were noise exposed (97 dB SPL, 8-16 kHz, 2 h). ABR and DPOAE thresholds were quantified 24 hours, 1 week, and 6 weeks post-noise exposure. At each of these timepoints, cochlear tissue was collected for histological analysis of outer hair cell (OHC) loss and cochlear synaptopathy.
Results: Treatment with Raloxifene reduced ABR threshold shifts 6-weeks post-exposure at all measured frequencies. Furthermore, the placebo group showed a statistically significant reduction in wave-I amplitudes 1-week and 6-weeks post-exposure. In contrast, treatment with Raloxifene ameliorated the reduction in ABR wave-I amplitudes. Conversely, DPOAE analysis showed elevated thresholds at 1-day after noise-exposure at 16, 24, and 32 kHz, however, we did not observe any differences between Raloxifene- and placebo-treated groups at any timepoint. Similarly, histological analysis revealed loss of synapses at 24 and 32 kHz after noise-exposure, but no significant differences between Raloxifene- treated and placebo-treated groups were detected.
Conclusions: These data indicate that Raloxifene may be used to partially protect from NIHHL in gonadally intact female mice. Based on our findings, systemic administration of Raloxifene could be considered for pre-clinical studies examining hearing preservation in females.

T61. Predicting Strial Atrophy From Audiogram Shape

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Category: Inner Ear: Damage and Protection

Background: Prior animal work and human otopathology suggest that steeply down-sloping audiograms are "sensory", i.e. caused by hair cell degeneration, while flatter audiometric losses are "metabolic", i.e. caused by strial atrophy, but this concept has never been rigorously tested in human specimens. In our prior study of age-related hearing loss in humans, a multivariable regression suggested that strial degeneration was not a significant contributor to threshold shifts. Here, we expanded that analysis by adding cases specifically selected for their "flat" and "gently sloping" audiograms, regardless of the otologic history.

Methods: 160 temporal bone cases from the Mass Eye and Ear archives were selected for study. All had audiogram-death interval less than 6 yrs (mean = 1.6 ± 0.16) and minimal air-bone gap (mean = 3.8 ± 0.62 dB). Cases were categorized into five groups based on audiogram shape, using the standard deviation (SD) of thresholds across all frequencies and the low-frequency pure tone average (PTA): Normal, Flat Moderate and Flat Severe had low SDs (less than 16) with PTAs of less than 25, 25 to 50, and greater than 50, respectively. Sensory and Descending had high SDs (greater than 16) with PTAs less than 25 and 25 to 50, respectively. In each case, fractional hair cell survival was assessed in all cochlear sections, peripheral axons were fluorescently labeled and counted in each half turn where the osseous spiral lamina is cut tangentially, and strial area was measured at 14 equally spaced locations along the spiral. In isolated cases, we verified that the strial areas measured corresponded to the regions still expressing Na+/K+ATPase, a key component of the stria's ion-pumping machinery.

Results: Strial atrophy was worse in the cochlea's apical half, where "Flat Severe" audiograms showed the most strial atrophy, while the "Sensory" group had the least. "Flat Moderate" and "Descending" groups were indistinguishable with respect to strial atrophy, despite the marked differences in the flatness of their audiograms. A strong correlation was seen between the low-frequency PTA and strial atrophy in the apical cochlea (p less than less than 0.001), but no significant correlation between strial atrophy and audiogram flatness per se. There was significantly more strial atrophy in females vs. males (p less than 0.005), with no significant difference in age. A multivariable regression suggested that strial atrophy only contributes significantly to the audiometric threshold in the apical half of the cochlea.

Conclusions: Humans lose high-frequency hearing as they age due to basal turn loss of inner and outer hair cells. When there is also apical strial atrophy, it adds to the low-frequency PTA, leading to flatter audiograms. Although the correlation between strial atrophy and low-frequency PTA was highly significant, the variance explained was low (13%), thus it cannot be used to diagnose strial degeneration on a case-by-case basis.

T62. Repair of the Stereocilia F-Actin Core is Mediated by the Mechanosensitive Protein XIRP2

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Category: Inner Ear: Damage and Protection

Background: Hair cells are under constant mechanical stress which can lead to sublethal structural damage. The contributions of structural damage to hair cell dysfunction and ultimately hearing loss is an area of active investigation. Our lab previously found that intense noise exposure can induce lesions in the F-actin cores of stereocilia. Interestingly, lesion frequency decreases back to pre-noise exposure levels one week later, indicating that there is likely an active repair mechanism. What are the underlying molecular mechanisms by which hair cells might sense and respond to actin damage? The protein Xin actin binding repeat containing 2 (XIRP2) is enriched in the hair bundle and we have shown that XIRP2 global KO mice exhibit increased stereocilia lesions following noise exposure compared to WT controls, as well as progressive hearing loss. Additionally, XIRP2 was found to be enriched in F-actin lesions. These data indicate that XIRP2 could mediate stereocilia repair and that this process plays a role in preventing age-related hearing loss. We are currently investigating the potential mechanisms by which XIRP2 mediates this repair.

Methods: We have employed two established in vitro models of mechanical stress to determine how XIRP2 is recruited to F-actin lesions. First is a laser photoablation system which locally induces a strain site in the stress fibers of fibroblast cells transfected with GFP-XIRP2 or different truncated versions of XIRP2 fused to

GFP. The recruitment indices of each XIRP2 subfragment were compared to determine their mechanosensitivity.
The second model exposes fibroblasts transfected with the same constructs to a regimen of cyclical uniaxial stretch, followed by immediate fixation and imaging. Colocalization of each protein with stress fibers was compared before and after stretch.

We have also generated mice with endogenous XIRP2 tagged with GFP, using the so-called split GFP approach, allowing us to image the movement of XIRP2 in stereocilia.

Results: From our laser photoablation studies, we found that full-length XIRP2 is not recruited to stress fiber strain sites, but that the truncated C-terminal domain is recruited. We are currently investigating the minimally mechanosensitive region of the XIRP2 C-terminal domain.
We also confirmed that the split GFP approach successfully tags endogenous mouse XIRP2 with GFP, and its expression and localization mirrors the pattern found in WT mice. We are in the process of preparing organ of Corti explants from these mice and performing laser photoablation to visualize the recruitment of GFP-XIRP2 to damaged F-actin in hair bundles.

Conclusions: The recruitment of the truncated C-terminal domain, but not the full-length protein, indicates that its mechanosensitivity is likely regulated in vivo. Additionally, the XIRP2 C-terminal domain is a novel mechanosensor domain, indicating a molecular mechanism by which XIRP2 may sense and respond to damaged F-actin in hair cells following loud noise exposure.

T63. Screening of FDA-Approved Drug Library Against Acquired Hair Cell Loss in Zebra Fish (Danio rerio)

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Category: Other, Hair cell loss
Background: Cisplatin is the most widely used anti-cancer drug of all age groups. Likewise, aminoglycosides (AGs) are frequently used to treat a variety of life-threatening infections. Unfortunately, they can both cause permanent sensorineural hearing loss. Ototoxicity, neurotoxicity, and nephrotoxicity limit the use of these therapies. The incidence of cisplatin induced hearing loss (CIHL) is about 36% for adults and 40-60% for pediatric cancer patients. Similarly, 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 cisplatin and AGs, there is no FDA approved drugs except sodium thiosulfate for pediatric cancer patients. Our project aims to screen FDA-approved drugs (approved to treat metabolic disease and that are in wide use) against CIHL and AGIHL in Zebra fish. So that the selected candidate drug(s) could be fast tracked for its use as a cotreatment to prevent acquired hearing loss.
Methods: About 60 drugs that blocks ion channels, vasodilators, agonist and antagonist of adrenergic and angiotensin receptors, lipid regulators, alpha and beta agonist, that are widely used to treat metabolic disease for a long period of time were used in this study. 6-8 of 5 pdf Zebra fish were pretreated with 10 nM, 100 nM, 1 μM, or 50 μM of each drug for 30 min at 28°C, followed by co-treatment with cisplatin/AGs and the respective drug concentrations for 6 hours at 28°C. Then, the fish were left to recover in embryo medium for 30 mins, fixed and immunolabelled against otoferlin. The number of hair cells per neuromast was quantified using a florescence microscope. Fish co-treated with cisplatin and statins served as a positive control for the CIHL experiments.
Results: Totally, 50 drugs that are already in use for hypertension and/or lipidemia were screened. Among them, Valsartan, Losartan, and Bepridil, showed significant hair cell protection compared to ototoxin-alone treatment. The effect of Losartan against cisplatin mediated hair cell loss correlates with the work by Xu et al., 2021 suggesting the use of Losartan against tumor induced hearing loss.
Conclusions: This study allowed us to shortlist the angiotensin 2 receptor blocker drug candidates for further screening in a mouse model of sensorineural hearing loss.

T64. Evaluation of the Effects of Rock Inhibitors on Auditory Damage Model Caused by Blast Exposure in CBA/J

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Category: Inner Ear: Damage and Protection

Background: Cochlear synaptopathy (CS) is considered the characteristic pathology behind "hidden hearing loss." Experiments with chinchillas have shown that CS can occur after blast exposure (Hickman TT et al. Sci Rep. 2018). Inhibitors of ROCK have been reported to have multifaceted effects, including improved blood flow, neuroprotection, and even nerve fiber elongation and synapse regeneration. We previously reported the effects of ROCK inhibitors on auditory nerve injury using in vitro and in vivo mouse models (Koizumi Y et al. Front neurosci. 2020, Mol brain, 2021). In this study, we generate a mouse model of CS by varying blast peak pressures and investigate the auditory nerve and synapse benefits of post-blast administered ROCK inhibitors.

Methods: Wild type male CBA/J mice aged 6 weeks or older were assigned to one of the following 5 groups: (A) no blast control, (B) blast exposure only, (C) post-blast sham control (PBS), (D) post-blast ROCK inhibitor (10 mM), (E) post-blast ROCK inhibitor (100 mM). A shock tube described by Hickman et al. 2018 that can reliably generate blast peak pressures between 160-180 dBP SPL was used. Blast pressure was measured with two 1/8" condenser microphones at mouse ear level. Cochlear function was assessed by ABR and DPOAE pre-exposure, 24-hours and 2-week after exposure. ROCK inhibitor and PBS were administered at the round window via a posterior ear incision 48-hours after exposure. All procedures were approved by the Mass Eye and Ear IACUC.

Results: Blasts of approximately 165-175 dBP SPL were delivered to mice 1-10 times for a total of 14 different combinations to titrate blast intensity. We focused on 4 blasts at 166.48±1.49 dBP SPL that yielded reproducible temporary threshold shifts (TTS). In the blast exposure only group, DPOAEs showed a threshold shift of about 15-20 dB 24-hours after blast exposure that returned to pre-exposure levels after 2-week. Additionally, 2-week post-blast ABR thresholds were no different from pre-exposure thresholds. ABR wave I amplitudes were significantly reduced compared to that pre-exposure. These changes in hearing function occurred over almost the entire frequency range (5.66-45.24 kHz). The PBS group showed similar results as the blast exposure only group, but the ROCK inhibitor groups showed a concentration-dependent improvement in ABR wave I amplitude.

Conclusions: In CBS/J mice, 4 exposures blast of 166.48±1.49 dBP SPL produced a temporary threshold shift and reduced ABR wave I amplitude. We find round window administration of ROCK inhibitors improve wave I ABR amplitude. Histological evaluation of the inner ear is currently underway. This study demonstrates the successful development of a useful mouse model of blast-induced CS and demonstrates preliminary efficacy of post-exposure administered ROCK inhibitors in mitigating blast induced CS.

T65. Endothelin a Receptor-Mediated Induction of Laminin α2 and Collagen α1(III) in Strial Capillary Basement Membranes Directly Damage Strial Cells Contributing to Progressive Loss of Strial Function in Alport Mice

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Category: Genetics A: Genomics and Gene Regulation

Background: In earlier work, we demonstrated that induction of endothelin-1 and activation of endothelin A receptor (ETAR) signaling plays a major role in regulating strial pathology in the COL4A3 knockout mouse model for Alport syndrome. Blocking the receptor with the ETAR small molecule inhibitor sitaxentan

prevented thickening of the strial capillary basement membranes (SCBMs) and the accumulation of extracellular matrix in the SCBMs including collagenα1(III) and laminin 221.
Methods: We explored whether the receptors for collagenα1(III) and laminin 221 (α-dystroglycan and integrin α7β1 (for laminin α2) and α2β1 integrin and DDR1 (for collagen α1(III)) are present on conditionally immortalized strial pericytes, marginal cells and intermediate cells as well as in the intact stria vascularis. We performed comparative analysis of gene expression in isolated glomeruli compared to isolated stria vascularis from wild type and Alport mice using RNA-seq. We tested transcriptional regulation and cell signaling processes involved directly by overlaying strial pericyte, marginal, and intermediate cell cultures with recombinant laminin 221. We examined the effects of endothelin treatment of pericytes on cytoskeletal dynamics and CDC42 activation using immunofluorescence, RNA-seq analysis, and on cell signaling using Full Moon Bio cell signaling microarrays.

Results: Receptors for collagenα1(III) and laminin 221(α-dystroglycan and integrin α7β1 (for laminin α2) and α2β1 integrin and DDR1 (for collagen α1(III)) are present in RNA in all three strial cell lines as well as RNA from microdissected stria vascularis. These same receptors were confirmed to be present on marginal or intermediate cells by immunofluorescence. RNA-seq analysis of RNA from strial cell lines overlayed or not with recombinant laminin 211showed differential regulation of genes implicated in strial pathology confirming that these cell lines are different from one another. RNA-seq analysis of RNA from isolated wild type and Alport glomeruli and stria vascularis revealed similar regulation of genes implicated in the pathogenesis of both organs. Treatment of pericytes with ET-1 resulted in changes in cell signaling consistent with observed reduction of filamentous to globular actin ratios and stabilization of microtubules observed by immunofluorescence.
Conclusions: The induction of collagenα1(III) and laminin α2 likely contributes to strial pathology in Alport syndrome via aberrant signaling through α-dystroglycan and integrin α7β1 (for laminin α2) and α2β1 integrin and DDR1 (for collagen α1(III)). Endothelin-1 activation of ETARs directly contributes to altered cell signaling resulting in changes in actin and microtubule cytoskeletal dynamics.

T66. Quantitative Imaging of the Healthy and Pathological Tympanic Membrane With a Hand-Held Optical Coherence Tomography (HHOCT) Device

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Category: Clinical Otolaryngology and Pathology
Background: Patients with conductive hearing loss commonly present with tympanic membrane (TM) perforations, TM retractions, and cholesteatomas. Otoscopy, the gold standard for visual assessment of these pathologies, is qualitative and only provides a 1D view. This limited view of the TM surface can impede accurate diagnosis, for instance not being able to clearly differentiate conditions such as TM retractions from perforations. Point-of-care depth resolved 3D imaging of the TM and middle ear (ME), combined with quantitative image analysis, could improve the diagnosis and management of these patients, ultimately improving patient outcomes. This study aims to demonstrate the ability of a hand-held OCT (HHOCT) device to provide depth resolved, volumetric imaging of the TM and ME structures for the quantitative assessment of TM perforations, TM retractions, and cholesteatomas.
Methods: We imaged the middle ear of 31 patients with initial complaints of varying degrees of hearing loss. Patients were first seen by an otologist for clinical evaluation and subsequently imaged using the HHOCT device with integrated live video feed in the clinic. Both ears of each participant were imaged, providing OCT volumes at less than 0.5 seconds per volume. Healthy volunteers were also imaged to provide a baseline for quantitative metrics. Images were post processed using Amira and MATLAB to perform segmentation of the TM and create thickness maps of the TM. The thickness at any point (voxel) was defined as the minimum of the line integral on the line segments passing through the voxel on a binary map of the TM.
Results: From the 31 patients imaged we found 5 cholesteatomas, 11 TM perforations, and 15 retractions. We segmented a representative TM from each condition along with a normal TM and found differences in TM thickness across these samples. The OCT volumes allowed for clear distinction of intact TM retraction pockets

from a perforation, which can sometimes be difficult just using an otoscope. We were also able to characterize the depth of the retraction pocket and determine if the TM was in contact with middle ear structures like the incus and promontory.
Conclusions: Our HHOCT device can readily be integrated into a busy clinic for noninvasive, real-time, volumetric imaging of the TM and middle ear. Quantitative metrics derived from the images can be used to characterize TM pathologies and potentially aid in diagnosis and management. OCT has the potential to become a routine clinical tool, promoting better patient outcomes.

T67. Pharmacological Chaperones Attenuate Sustained PERK Activation, Providing Protective Effects Against Noise-Induced Hearing Loss

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Category: Inner Ear: Damage and Protection
Background: Noise-induced hearing loss can result in either temporary or permanent effects, depending on the exposure's intensity and duration. Currently, no study has thoroughly investigated the temporal alterations of cochlear transcriptome following exposure to temporary threshold shift (TTS)- or permanent threshold shift (PTS)-inducing noise to identify pathogenic mechanisms and therapeutic targets for noise-induced hearing loss.
Methods: We conducted a longitudinal transcriptome analysis of adult C57BL/6 mice for up to two weeks following TTS and PTS, respectively.
Results: The endoplasmic reticulum (ER) stress and unfolded protein response (UPR) were induced one day after TTS- and PTS-inducing noise exposure. Western blot showed the activation of IRE1α and PERK branches of the UPR after exposure to TTS and PTS-induced noise. And it demonstrated that the activation of the PERK branch persisted only after 2 weeks of exposure to PTS noise. Furthermore, the expression of CHOP, a proapoptotic factor in the PERK downstream signaling, increased significantly in both inner hair cells and outer hair cells in response to PTS-inducing noise exposure, but not TTS-inducing noise exposure. To elucidate the role of PERK in noise-induced hearing loss, the effect of the PERK inhibitor GSK2656157 on TTS was investigated. Intriguingly, GSK2656157 inhibited hearing recovery in TTS, suggesting that early PERK activation is required for hearing recovery in TTS. Furthermore, administering GSK2656157 three days after PTS-inducing noise exposure partially rescued hearing loss, suggesting that the sustained activation of PERK contributed to noise-induced hearing loss. We demonstrated that chemical chaperones, tauroursodeoxycholic acid and 4-phenylbutyric acid, protect against noise-induced PTS.
Conclusions: In summary, we conducted a longitudinal study of the cochlear transcriptome after TTS- and PTS-inducing noise exposure, identifying the role of UPR in response to noise, and proposing new mechanisms and therapeutic targets for noise-induced hearing loss.

T68. Open Board

T69. Loss of Synaptic Ribbons is an Early Cause in ROS-Induced Acquired Sensorineural Hearing Loss

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Category: Inner Ear: Damage and Protection
Background: Sensorineural hearing loss (SNHL) was considered to result from cell death, including hair cell (HC) death, in the cochlea. Recently, synaptopathy between cochlear HCs and spiral ganglion neurons has been gathering attention as a cochlear HC loss precursor not detectable by normal auditory evaluation (called “hidden hearing loss”).
Previous studies have convincingly argued that reactive oxygen species (ROS) contribute to the development of the acquired SNHL triad—that is, age-related hearing loss (ARHL), noise-induced HL, and drug-induced

HL. We have previously reported the mechanism of HC loss by induced NADPH oxidase (NOX) 3 expression in outer HCs and supporting cells in aging, after noise exposure (NE), and cisplatin treatment. However, the underlying molecular mechanism of HC loss due to ROS remains unknown. To advance our understanding of the molecular mechanisms involved in HC loss in ROS-induced acquired SNHL, we focused on and evaluated the effects of ROS on the synaptic ribbon.

Methods: We established a mouse model of ROS overproduction in cochleae by creating transgenic (TG) mice that express the gene for human NOX4 (NOX4-TG mice). To screen for genes that play an important role in synaptopathy, DNA microarray analysis was used. We examined the number of synaptic ribbons in IHCs and assessed hearing function in 1-, 1.5-, 2-, 4-, and 6-month-old WT and NOX4-TG mice. CtBP2, a major component of the synaptic ribbon, immunostaining was used for this purpose. Furthermore, to confirm whether the synaptic ribbon is a target of ROS-induced acquired SNHL, we administered cisplatin and intense noise, which induce ROS production, in 2-month-old WT and NOX4-TG mice.

Results: We found that mRNA levels of Piccolo 1, another major component of the synaptic ribbon, were decreased in postnatal day 6 NOX4-TG mice cochleae compared to those in WT mice; they were also decreased by NE in 2-week-old WT cochleae. The level of CtBP2 was significantly lower in NOX4-TG mice cochleae of 1-month-old and 4-month-old mice compared to that in WT mice, although no significant differences were noted at 1.5- and 2-months. The decrease in CtBP2 plateaued in 4-month-old NOX4-TG, while it gradually decreased from 1 to 6 months in WT mice. Furthermore, CtBP2 level in 2-month-old NOX4-TG mice was significantly decreased after exposure to cisplatin and noise compared to that in WT mice.

Conclusions: We found that Piccolo 1 and synaptic ribbons are targets of ROS. Additionally, we demonstrated that ROS are at least one of the causes of synaptopathy induced by aging (ARHL). Furthermore, we demonstrated that cisplatin and intense noise, both of which induce ROS production and aggravate ARHL, cause synaptopathy. We propose that protection of synaptic ribbons and reduction in ROS levels are promising approaches to developing novel therapeutic strategies for acquired SNHL.

T70. miR-431 Secreted by Human Vestibular Schwannomas Increases the Mammalian Inner Ear's Vulnerability to Noise Trauma

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Category: Inner Ear: Damage and Protection

Background: Vestibular schwannoma (VS) is an intracranial tumor that arises on the vestibular branch of cranial nerve VIII and typically presents with sensorineural hearing loss (SNHL). The mechanisms of this SNHL are postulated to involve alterations in the inner ear's microenvironment mediated by the genetic cargo of VS-secreted extracellular vesicles (EVs). We aimed to identify the EV cargo associated with poor hearing in VS and determine whether its delivery caused hearing loss and cochlear damage in a mouse model in vivo.

Methods: VS tissue was collected from routinely resected tumors of patients with good (VS-GH, n=6) or poor (VS-PH, n=7) pre-surgical hearing measured via pure-tone average (PTA) and word recognition (WR) scores. PH was defined as PTA greater than 30 dB and a WR score of less than 70%; otherwise, patients were classified as GH. Next-generation sequencing was performed on RNA isolated from cultured primary human VS cells and EVs from VS-conditioned media, stratified by patients' hearing ability. microRNA expression levels were compared between VS-PH and VS-GH samples to identify differentially expressed candidates for packaging into a synthetic adeno-associated viral vector (Anc80L65). Viral vectors containing candidate microRNA were infused to the semicircular canals of mice to evaluate the effects on auditory brainstem response thresholds at 6-weeks post-injection and after noise exposure (8–16 kHz for 2 hours at 100 dB SPL). Immunohistochemistry of the mice cochleae was used to compare the number of inner hair cells (myosin 7A-positive) and pre-synaptic ribbon density (CtBP2-positive).

Results: Differentially expressed microRNAs included hsa-miR-431-5p (enriched in VS-PH) and hsa-miR-192-5p (enriched in VS-GH). Newborn mice receiving intracochlear injection of viral vectors over-expressing hsa-miR-431-GFP (n=6 mice), hsa-miR- 192-GFP (n=6), or GFP only (control, n=7) had similar hearing 6 weeks post-injection. However, after acoustic trauma, the miR-431 group displayed significantly worse

hearing, and greater loss of synaptic ribbons per inner hair cell, in the acoustically traumatized cochlear region in comparison with the control group.
Conclusions: Our results suggest that miR-431 contributes to VS-associated hearing loss following cochlear stress. Further investigation is needed to determine whether miR-431 is a potential therapeutic target for SNHL.

T71. CC Chemokine Receptor 2 Protects Outer Hair Cells in CSOM Mouse

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Category: Inner Ear: Damage and Protection
Background: Chronic Suppurative Otitis Media (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. We have generated a validated CSOM mouse model with Pseudomonas Aeruginosa (PA). Our data revealed that the outer hair cell (OHC) loss occurred in the basal turn of the cochlea. Macrophages are the major immune cells associated with OHC loss. Macrophage-associated cytokines are upregulated. Specifically, CCL-2, an important member of the monocyte chemoattractant protein (MCP) family, is elevated over time following middle ear infection. CCR2 is a common receptor of MCP family and the unique receptor of CCL2. CCR2-/- mice have been used extensively in studies of monocyte activation in neurodegenerative diseases. In the current study, we investigated the role of CCR2 on hair cell survival in CSOM cochlea.
Methods: We generated CSOM in CCR2-/- mouse comparing with CCR2+/+ CSOM mouse. We assessed OHC loss and immune responses in the cochlea at 10 days following the middle ear infection.
Results: OHC survival rates were 84 ± 12.5% in the basal turn of CCR2+/+ CSOM cochleae, whereas 63± 19.9% in the basal turn of CCR2-/- CSOM cochleae. There were significant differences (P=0.036). Macrophage numbers were significantly reduced in CCR2-/- CSOM cochleae compared with CCR2+/+ CSOM cochleae (P=0.001). In addition, the overall of cytokines profile was reduced in CCR2-/- CSOM cochleae.
Conclusions: Taken together, the data suggests that CCR2 plays a protective role in the CSOM cochlea. The possible mechanisms underlying the protective effect of CCR2 need to be further elucidated.

T72. Dynamics of Tissue-Resident Macrophages in the Acoustic Overstimulation in Mice Cochlae

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Category: Inner Ear: Damage and Protection
Background: Macrophages are essential immune cells in the cochlea that contribute to inflammation, tissue repair, and homeostasis. They also play important roles in local cochlear immunity. As a result of acoustic injury, the immune system in the cochlea is activated, leading to the production of inflammatory molecules and the infiltration of immune cells. However, the molecular mechanisms responsible for initiating immune responses remain unclear. We investigated the functional roles of the tissue macrophages which were stimulated by interleukin 6 (IL-6) and C-C chemokine receptor type 2 (CCR-2), pro-inflammatory molecules, in the regulation of cochlear responses to acoustic overstimulation.
Methods: To investigate the distribution of macrophages in the cochlea, C57BL/6J mice were performed immunohistochemical analyses of cochlear tissue. The cochleae after acoustic overstimulation (120 dB, 3 hours) were compared to those of controls. We used the modified Kawamoto's film method for thin cryosection of frozen blocks. Immunohistochemistry for F4/80 was performed to demonstrate the existence of resident macrophages in the cochlea. We also investigated the IL-6 and CCR-2 expressions after acoustic overstimulation.
Results: This study revealed that tissue macrophages were present especially in the basilar membrane and the lateral wall under control conditions. Immunohistochemistry showed an increase in the number of activated cochlear macrophages especially in the basilar membrane and the lateral wall after acoustic overstimulation.

Conclusions: The present findings suggest that the IL-6 and CCR-2 are involved in macrophage activation after acoustic overstimulation. Therefore, these responses may be a potential therapeutic target for treatments preventing the damage caused by acoustic trauma in the cochlea.

T73. The NLRP3 Inflammasome in Macrophages Causes Sensory Hearing Loss in Chronic Suppurative Otitis Media (CSOM)

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Category: Inner Ear: Damage and Protection

Background: CSOM is a global disease and affects 300 million people worldwide. It is the most common cause of permanent hearing loss in children in the developing world. We previously showed that sensory hearing loss (SHL) in CSOM is associated with macrophages and not due to direct bacterial invasion or direct ototoxin exposure.

We aimed to investigate the macrophage associated mechanism that drives hearing loss in CSOM. The NLRP3 inflammasome is an innate immune sensor and is expressed in monocytes and macrophages. It can be activated via multiple different pathways, including many direct pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) that are toxic.

Methods: We investigated in our validated pseudomonas aeruginosa CSOM mouse model. We measured the gene expression of components of the NLRP3 pathway via qPCR in CSOM. We assessed hair cell loss in cochlear whole mount samples and macrophages numbers in cochlear cryosections at 7 and 14 days during CSOM. We measured protein levels of the NLRP3 inflammasome and its downstream cytokines (IL-1b, IL-18) in CSOM with ELISA immunoassays. We used C57BL/6 wild type (WT) and NLRP3 knockout mice (NLRP3 -/-) in our experiments.

Results: We found that the relative mRNA levels of components of the NLRP3 pathway (NLRP3, PYCARD, Caspase 1, IL-1b) were significantly increased at 7 days in CSOM without depletion of cochlear macrophages. We then used a NLRP3 knockout mouse model (NLRP3 -/-) to study the inflammasome function in CSOM. We found that the knockout condition was protective for HC loss in the cochlea and showed significantly better outer hair cell (OHC) survival at 14 days compared to the WT control ($p = 0.0393$). The cochlear macrophages numbers did not differ between the WT and NLRP3 -/- mice at 7 ($p = 0.5573$) and 14 ($p = 0.7459$) days in CSOM. The protein levels of NLRP3 ($p = 0.0018$) and its downstream cytokines IL-1b ($p = 0.0004$) and IL-18 ($p = 0.0129$) were significantly increased at 7 days in CSOM compared to the non CSOM control.

Conclusions: The NLRP3 inflammasome in macrophages causes SHL in CSOM and could be a potential target for future therapeutics development to prevent NLRP3 associated hearing loss.

T74. Hyaluronic Acid Conjugates for Therapeutic Management of Noise Induced Hearing Loss Specific Inner Ear Inflammation

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Category: Inner Ear: Damage and Protection

Background: Noise-induced hearing loss (NIHL) is a prevalent concern in modern society. The intense or prolonged noise exposure can trigger inflammatory signaling cascades in the cochlea, resulting in irreversible damage to the inner ear structures and hearing loss. There is an increasing need for treatments, necessitating the development of innovative therapeutic agents that can be delivered to the inner ear structure for the treatment of inflammation as well as the protection of auditory cells. In previous studies, we found that drug delivery across ear-specific anatomical barriers was significantly improved by hyaluronic acid (HA) conjugation. The present study aimed to build on our previous data by developing novel anti-inflammatory

therapeutics to modulate inflammatory responses and the inner ear cells from noise induced hearing loss specific inflammatory damage.
Methods: HA-drug conjugates (HAC) were synthesized and characterized for the confirmation of drug conjugation and its efficiency. To test the anti-inflammatory potential of the conjugates, an in vitro inflammatory model using mouse macrophages (RAW264.7) was developed, wherein lipopolysaccharide (LPS) was used as an inflammation inducer. The inflammatory cytokine release upon treating the RAW cells with the HACs under inflamed conditions was examined using ELISA and V-PLEX Proinflammatory Panel 1 Mouse Kits. Conjugate cytocompatibility was assessed using MTS and LDH cytotoxicity assays. The inner ear cell (HEI-OC1) survival under inflamed conditions was evaluated using MTS and LDH cytotoxicity assays. Tissue cytocompatibility and permeation were assessed in RWM and TM in vitro models.
Results: The most prominent inflammatory cytokines TNF-α, IL-6, IL-12p70, and IL-10 were released under inflammatory conditions. A statistically significant decrease in the levels of these cytokines was observed with the treatment of HACs under LPS-stimulated conditions. These conjugates were also confirmed as cytocompatible with cell viability studies conducted on RAW264.7 and HEI-OC1 cells. The HAC was also able to permeate the RWM and avoid the cytotoxic effects seen by the unconjugated drug.
Conclusions: The findings of this study could help in the development of topically deliverable new therapeutics for noise-induced hearing loss associated inflammation. These hyaluronic acid-based conjugates show promise as a safe and effective alternative for protecting inner ear cells, potentially transforming drug development for noise induced hearing loss. Further validation of these conjugates using clinical trials would broaden the scope of the new discoveries in auditory research.

T75. After the Music: Factors Affecting Concert-Induced Temporary Threshold Shift

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Category: Inner Ear: Damage and Protection
Background: Noise exposure can cause both temporary and permanent threshold shifts (TTS/PTS). Vulnerability to each differs between individuals. The factors determining these individual differences have not been conclusively established. This may have important implications for occupational and recreational noise standards, which are "one-size-fits-all" and generally based on data from listeners who are (a) white, (b) male, and (c) exposed to occupational noise.
There is increasing evidence from animal - and some human - studies that the factors determining individual vulnerability may include sex, melanin levels, and the pattern of prior noise exposure. Improved understanding of these factors should support identification of at-risk individuals and more sophisticated hearing-loss-prevention strategies. Although vulnerability to PTS is ultimately of greater importance, investigating individual susceptibility to TTS is an important ancillary research goal.

The present study investigates whether extent of TTS is predicted by sex, skin tone (as a proxy for melanin levels), and prior noise exposure. It uses TTS induced recreationally, by gathering hearing data immediately after a noisy concert. It also includes some secondary subclinical measures of temporary auditory shift.

Methods: We recruited 50 normally hearing adults aged 18 to 22 who don't use hearing protection in noisy environments. They attended two sessions: one in the days before a scheduled concert, one immediately after it.
We tested for TTS principally using pure-tone audiometry (mean of 3-6 kHz thresholds). Exploratory outcome measures included shifts in extended high-frequency audiometry, tinnitus, and otoacoustic emissions. Sex, Fitzpatrick-Scale skin-tone category, and noise exposure were obtained via self-report.

Results: Analysis via multiple linear regression is ongoing. We will report whether extent of TTS is related to past-10-weeks noise exposure (as a measure of potential "conditioning" effects of noise) and to sex and skin tone. (Greater energy of noise exposure, female sex, and darker skin tone are all expected to be associated with lower TTS.) Exploratory analyses will include alternative outcome measures, as well as lifetime noise exposure and baseline auditory measurements.

Given the novel methods used to obtain TTS in this study, we will also report descriptive data on the feasibility of this approach and on the extent and variability of resulting TTS.

Conclusions: Improving understanding of individual differences in susceptibility to TTS may serve wider efforts towards a more personalised approach in hearing protection and care. Though the present study is small, it should cast light on the value of future larger investigations using this paradigm.

T76. A Comprehensive Characterizations of Zebrafish Rheotactic Behaviors and Its Application to Otoprotective Drug Screening

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Category: Inner Ear: Damage and Protection

Background: Aquatic animals have rheotaxis that maintains a balance in response to water flow. They sense water flow through hair cells in lateral line, thereby leading to behavior changes relevant to damages on hair cells, the primary sensory receptor cells within auditory and vestibular systems. Zebrafish are efficient animal models for high-throughput drug screening with human-like hair cells along the lateral line.

Methods: Zebrafish rheotactic behaviors could be assays for hair-cell-targeted drug screening. However, knowledge and tools for rheotaxis analysis along the extent of hair-cell damage have not been fully investigated. This article aims at characterization of rheotactic behaviors identifying lateral line states via an analysis platform that simultaneously examines multiple zebrafish larvae.

Results: we developed an automated framework that incorporated animal test hardware equipment and real-time analysis software for monitoring aquatic behaviors of multiple larvae. Through this framework, a commensurable measure for one-dimensional characterization of rheotactic behaviors was consolidated so that its linear changes could be associated with the population of hair cells remaining intact.

Conclusions: These findings satisfied requests for an automated analysis platform to conduct large-scale screening and a bio- marker that discriminate the seriousness of hair cell damage to screen candidates having significant effects i- n otoprotective drug discovery. This study was published in expert systems with applications.

T77. AC102 Outperforms Dexamethasone in the Treatment of Functional Hearing Loss and Synaptopathy in a Guinea Pig Model of Noise-Induced Hearing Loss

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Category: Inner Ear: Damage and Protection

Background: There is currently no approved medication for the treatment of Sudden Sensorineural Hearing Loss (SSNHL). Clinical practice guidelines often recommend the use of corticosteroids. Unfortunately, there is, if at all, only weak evidence that this standard-of-care (SoC) results in a clinically relevant benefit. AC102, a pyridoindole, is in development to fill this unmet medical need. Therefore, to understand how the efficacy of AC102 compares to the SoC, we investigated both AC102 and the corticosteroid dexamethasone (DEX) separately and in combination in a well-established model of noise-induced hearing loss (NIHL).

Methods: In vitro, HT22 cells were treated with either AC102 or DEX alone, or AC102+DEX. Neurite outgrowth was assessed 24h after treatment start. In vivo proof-of principle-studies were performed in a guinea pig model of NIHL. After noise overexposure (4kHz, 125dB SPL, 1h), 47 animals received either a mono-treatment with AC102 or vehicle (formulated in thermosensitive gel; single local application), DEX alone (4 mg/kg daily over 4 days) or a combined treatment with AC102+DEX. Functional hearing was assessed by ABR measurements 21 days after noise trauma followed by histological evaluation of outer hair cells (OHCs) and ribbon synapses.

Results: Treatment with AC102 significantly increased neurite outgrowth in vitro compared to controls. In contrast, DEX (0.01-1µM) had no impact on neurite outgrowth. AC102+DEX reduced the effect of AC102

although this effect was mitigated when DEX was delayed by 4h after AC102. In vivo findings demonstrated that DEX alone did not have any therapeutic effect on functional hearing compared to vehicle-treated controls 21 days post-trauma. In strong contrast, AC102 resulted in a significant recovery of hearing thresholds across the tested 2-32kHz frequency range. However, the therapeutic effect of AC102 was significantly attenuated when combined with DEX. AC102+DEX also significantly decreased ribbon synapse counts compared to AC102 treatment alone, while AC102 mono-treatment restored synapses near levels of naïve animals. Compared to vehicle-treated animals, AC102 significantly increased OHC counts to a similar extent, while DEX alone had no measurable impact on OHC or ribbon synapse numbers.
Conclusions: In vitro, AC102 demonstrated a pronounced capacity to enhance neurite outgrowth while in vivo, it effectively restored ribbon synapses and increased OHC survival following noise trauma. Importantly, AC102 restored functional hearing thresholds across a broad frequency range providing compelling evidence of its therapeutic potential for the treatment of hearing loss. These findings underscore the limited therapeutic efficacy of corticosteroids as a SoC treatment as DEX did not show any notable improvements in hearing outcomes. Furthermore, the combination of AC102 with DEX displayed a negative effect on AC102's therapeutic actions, emphasizing the importance of monotherapy with AC102. These findings formed the Phase 2 clinical trial AC102-201 currently underway across Europe in which subjects with ISSNHL receive either AC102 or oral corticosteroids.

T78. Subclinical Effects of Noise Exposure: A Pre-Registered Longitudinal Study

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Category: Inner Ear: Damage and Protection
Background: Findings on the subclinical effects of noise exposure have often been inconsistent and inconclusive, likely due to (a) small samples, (b) inaccurate retrospective estimates of noise exposure, (c) post-hoc data dredging, and (d) individual variability in outcome measures unrelated to noise exposure. The most persuasive evidence comes from studies with large samples, large differences in noise exposure, and within-subject difference measures. However, all are limited by their cross-sectional designs. Difference measures at a single timepoint are inevitably less powerful than differences in the same measure over time. Purely retrospective estimates of lifetime noise exposure are problematic, even when participants with high and low exposure diverge greatly. None of the extant work has been pre-registered, leading to potential type I error from exploration of rich data sets.
Some longitudinal research over short time periods has been reported. However, studies spanning years and encompassing a substantial portion of lifetime noise exposure are required. In the UK, the optimal period includes the late teenage years, when concert and nightclub attendance become commonplace.

The present study on subclinical effects uses: a prospective approach with regular follow-ups (to improve estimates of noise exposure); repeated measures (to minimise between-subject variability); and pre-registration (to guard against data dredging).

Methods: A cohort of 220 teenagers with normal hearing, aged 16-17, underwent 3.5 hours of physiological, perceptual, and self-report measures. At age 19-20, the test battery will be repeated. The participants self-report noise exposure at 18-month intervals.
Planned analyses will test for relations between noise exposure and changes in: extended high-frequency audiometry; distortion-product otoacoustic emissions; auditory brainstem response wave I amplitude; and the middle-ear muscle reflex.

Exploratory analyses will incorporate: changes in speech perception and tinnitus; effects of sex and skin tone; effects of pattern of noise exposure; effects of nicotine, alcohol, and deprivation; and relations between personal characteristics, physiology, and perception.

Results: At the mid-point of the study, retention is high (95%). The sample skews male (56%) and is highly diverse in terms of skin tone, deprivation, and educational attainment.

The main research questions will be addressed in 2026, but baseline data are available. In particular, the initial homogeneity of the cohort (young, otologically normal, with little noise exposure) allows examination of extraneous variability in our measures.

Conclusions: A large-scale longitudinal study in this population is challenging but feasible. We will present baseline data, including non-auditory contributors to speech perception, and effects of sex, skin tone, and ear-canal size on auditory measures. Follow-up data will be analysed primarily in line with the pre-registered protocol, but extensive (and openly disclosed) exploration will also be possible. The data set will be shared openly, and our team welcomes suggestions and collaborations to support exploitation of the data.

T79. Pesticide Induces Hearing Impairments in Exposed Rural Inhabitants

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Category: Inner Ear: Damage and Protection

Background: Hearing impairment is a major global concern affecting 466 million individuals, including 34 million children. The prevalence of hearing loss and auditory processing disorders has increased due to the use of novel products, industrialization, noise, and exposure to ototoxic agents. Pesticides, among these agents, have emerged as significant contributors to hearing loss, especially in those who have occupational exposure or reside near agricultural areas.

Methods: Our study included 63 young volunteers, all Chilean residents and native Spanish speakers. Participants were categorized into two groups: exposed (E) and unexposed (UE), based on surveys covering social, economic, educational, medical, and hearing history. Inclusion criteria for the E group were as follows: a) Verifiable history of working on farms or orchards using pesticides; b) Co-residence with family actively involved in monoculture agriculture; c) living to 500 meters or less from monoculture areas. Age, education, tobacco and alcohol habits, medical history, and noise exposure **were** considered and balanced between groups. Participants performed clinical and high-frequency audiometry, Auditory Brainstem Responses (ABR), Distortion Product Otoacoustic Emissions (DPOAEs), and measurement of the strength of auditory efferent reflex through DPOAEs with contralateral auditory stimulation (CAS). Additionally, a digit-in noise task assessed the functional impact on auditory processing.

Results: Audiometric analysis revealed statistically significant differences in the mean threshold at 14 kHz (E: 16.61 ± 16.45 dB; UE: 7.75 ± 11.06 dB; $p = 0.032$) and 16 kHz (E: 21.90 ± 19.66 dB; UE: 11.25 ± 13.94 dB; $p = 0.021$). ABR positive peaks analysis in Wave I, III, and V showed reduced amplitudes in Wave III in E, with no significant differences in Wave I and V (Wave I: E: 0.13 ± 0.47, UE: 0.34 ± 0.19; Wave III: E: 0.42 ± 0.26, UE: 0.56 ± 0.23; Wave V: E: 0.64 ± 0.25, UE: 0.68 ± 0.29; Wave I; $p = 0.05$; Wave III; $p = 0.0274$). DPOAE suppression induced by CAS at f2 frequencies ranging from 1 to 6 kHz demonstrated a significant reduction in DPOAE suppression in E group at 1.5 kHz ($p = 0.0407$). Importantly, our statistical analysis shows a significant interaction between stimulation and the percentage of absent emissions when comparing the groups ($p = 0.0099$). The digit in the noise task did not reveal significant differences between the non-exposed (15.50 correct responses) and exposed (14.00 correct responses) ($p = 0.35$).

Conclusions: In summary, our data reveal pesticides exposure effects on auditory function in young, which is independent of noise exposure or occupational factors. These findings suggest impairments in high-frequency auditory perception, alterations in ABR Wave III amplitudes, and changes in the strength of the efferent reflex measured by DPOAEs. Future studies will help elucidate the mechanisms associated with these effects.

T80. Hearing Impairment and Cochlear NLRP3 Inflammasome Activation Induced by High-Fat Diet Consumption in Mice

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Category: Inner Ear: Damage and Protection

Background: Obesity affects the auditory system, altering its functionality and auditory processing. However, there is very little research related to the molecular and physiological mechanisms underlying this relationship. Inflammation and the NLRP3 inflammasome activation, a multiprotein platform that responds to metabolic damage signals, may play a role in the development of sensorineural hearing loss (HL) induced by obesity. Our research aimed to evaluate the effect of obesity induced by high-fat diet (HFD) consumption on the functionality of the auditory system, hair cell survival and inflammation, and NLRP3 inflammasome activation in the cochlea.

Methods: 7 weeks old male C57BL/6J mice (n=40) were fed a control diet (CD) containing 10% fat, 20% protein, and 70% high-fat carbohydrates or a HFD, with 60% fat, 20% protein, and 20% carbohydrates, for 12 or 16 weeks. Body and adipose tissue weight, glucose tolerance, adipocytes size and lipid infiltration in liver (histology), distortion product otoacoustic emissions (DPOAEs), cochlear hair cells numbers and integrity (by IF), caspase 3 levels (IF), inflammation markers levels (cytokines: interleukin (IL)-6 and TNF-α by PCR), and NLRP3 inflammasome activation pathway (NLRP3, ASC, Caspase-1, IL-1β and IL-18 levels by IF and qPCR) were measured.

Results: Animals fed a HFD significantly (p less than 0.05) increased body weight (56% at 12 weeks and 84% at 16 weeks of treatment), developed hepatic steatosis, adipose tissue hyperplasia, and glucose intolerance compared to CD. In addition, HFD consumption increased the 2F1-F2 DPOAE threshold by 13,75 dB (p less than 0.05) after 16 weeks of treatment compared to CD but did not affect DPOAEs at 12 weeks. Cochlear mRNA levels of NLRP3 inflammasome components (NLRP3, ASC, Caspase-1, IL-1β, and IL-18) and IL-6 and TNF-α were altered at 16 weeks but not at 12 weeks. Caspase-3 presence was affected by diet at the studied times.

Conclusions: This data suggests a role for inflammation and NLRP3 inflammasome in hearing impairment induced by obesity, after the chronic consumption of a high-fat diet in C57BL/6J mice. These results contribute to explaining the sensorineural hearing loss related to obesity. To our understanding, this is the first work describing the participation of NLPR3 inflammasome in the cochlea related to obesity and metabolic alterations of nutritional origins. These data contribute to both interdisciplinary research and discussion in the hearing loss field. It also highlights the relevance of developing epidemiological studies with a more metabolic and nutritional point of view to understand the mechanisms underlying the effects of obesity and related metabolic alterations in hearing processing.

T81. Examining the Therapeutic Potential of Cell-Derived Exosomes in Hair Cell Protection

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Category: Inner Ear: Damage and Protection

Background: Aminoglycoside antibiotics cause permanent hearing loss by killing mechanosensory hair cells (HCs) in the inner ear. We have recently shown that exosomes derived from heat-shocked utricles protect against neomycin-induced HC death in cultured utricles from adult mice. Exosomes are small (~50-150 nm) extracellular vesicles that mediate intercellular communication. They are generated by most cell types and deliver proteins, metabolites, and nucleic acids to recipient cells to induce functional changes. Our data suggest that exosomes carry cargo from supporting cells to HCs to activate a pro-survival response. Mass spectrometry analyses of exosomes derived from heat-shocked utricles identified 235 unique proteins, including HSP70, which was critical for the protective effect. The goal of this study is to determine additional key cargo molecules that promote HC survival. Since exosome yield from mouse utricles is very low, we have utilized immortalized cell lines, which release large numbers of exosomes containing HSP70. We hypothesize that similar to utricle-derived exosomes, cell line-derived exosomes carry molecular cargo that protect HCs from aminoglycoside-induced death.

Methods: Exosomes from cell lines were purified via differential ultracentrifugation and characterized using nanoparticle tracking analysis and Western blot analysis. Isolated exosomes were applied to utricles in the absence or presence of neomycin. Quantitative mass spectroscopy will be used to examine the proteomes of both protective and non-protective exosomes.

Results: Exosomes released by heat shocked colorectal carcinoma cell line (CT26) were isolated and applied to utricles; however, CT26-derived exosomes were not protective against neomycin-induced HC death. In contrast, exosomes derived from HEI-OC1 cells were protective against neomycin-induced HC death.
Conclusions: This study uses protective and non-protective exosomes produced by cell lines as a tool to examine exosomal cargo that contribute to protection against neomycin-induced HC death. While CT26-derived exosomes did not show a protective affect against neomycin-induced HC death, we will examine the proteome of these non-protective exosomes and compare that to the proteome of protective exosomes in order to begin to identify the full list of protective exosomal cargo to guide the development of designer exosomes for inner ear therapeutics.

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T82. Mild Frequency-Selective Synaptopathy After Multiday Gentamicin Treatment

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Category: Inner Ear: Damage and Protection
Background: Previous studies indicated that systemic treatment with a combination of gentamicin and furosemide (G/F), using various dosing combinations at multiple post-treatment time points, resulted in a rare occurrence of aminoglycoside-induced ototoxic synaptopathy. In comparison, general synaptic damage and substantial hair cell loss were readily observed in the cochlea. To thoroughly assess the health risks associated with synaptopathy and hidden hearing loss caused by ototoxic aminoglycosides, we conducted a multi-day gentamicin treatment in CBA/CaJ mice and evaluated peripheral auditory functions, including outer hair cells (OHC), inner hair cells (IHC), and ribbon synapses.
Methods: Nine-week-old CBA/CaJ mice were selected and intraperitoneally injected with 100 mg/kg of gentamicin daily in the morning for 14 consecutive days, while littermates in the control group were administered PBS. Hearing functionality was assessed using auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE), which indirectly measure outer hair cell (OHC) function. The assessment was conducted before and immediately after the multiday treatment, as well as at 6 weeks after the cessation of the treatment. ABR thresholds and ABR wave-I amplitudes were both extracted to evaluate inner hair cell (IHC) and ribbon synaptic functions.
Results: Our data suggest that multiday gentamicin treatment induced mild synaptopathy in a restricted cochlear region. Treated mice did not display any gentamicin-induced ABR threshold shift up to 6 weeks after the therapy. In contrast, the click ABR wave-I amplitude input-output (I/O) function indicated a mild but significant suprathreshold amplitude reduction. This reduction was initially observed following the multiday therapy and persisted even after the treatment had ended. With pure tones, such an amplitude reduction was observed at the frequencies of 12 kHz and 16 kHz. Ototoxic effects of gentamicin were also observed with DPOAE measurements. However, it is worth noting that this effect was typically detected at lower sound levels of the DPOAE primary tones and at frequencies that did not match the gentamicin effects depicted by the compromised ABR wave-I. The frequency-selective reduction of wave-I amplitude induced by gentamicin potentially indicates a specific inner hair cell (IHC)/synaptic malfunction, which has not been observed with combined G/F treatment.
Conclusions: Electrophysiological measurements suggest mild synaptopathy after multiday gentamicin treatment, and histological work is warranted to confirm the anatomical synaptic defect. We suspect that, unlike the single G/F treatment, multiday treatment causes repetitive insults to the inner ear's sensory and neural compartments, disrupting potential inflammatory resolution that could occur with single G/F treatment. Without swift and effective resolution of inflammatory events in the cochlea, activated macrophages linger in the synaptic zone, impeding normal synaptic function.

T83. Regulators of Phagocytosis in the Vestibular Maculae of Mice

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Category: Inner Ear: Damage and Protection

Background: Homeostasis of the inner ear requires the quick removal of dying cells from its various epithelia. This task is accomplished by both macrophages and supporting cells, but the signals that initiate phagocytosis are not known. Membrane externalization of phosphatidylserine (PtS) is a highly conserved signal that targets a dying cell for engulfment. Externalized PtS is detected by members of the TAM kinase family (Tyro3, Axl and MerTK), whose activation triggers the formation of a phagosome and the engulfment of targeted cells. This study examined the role of MerTK in inner ear function and injury response.

Methods: In vivo experiments: MerTK-null and WT mice were given a single injection of IDPN (3 mg/gm), which caused a significant hair cell lesion in the vestibular maculae. Fixed specimens were used to characterize and quantify phagosomes. In vitro experiments: organotypic cultures of mouse utricles were treated with inhibitors of Rho-family GTPases (which regulate the formation of phagosomes), and the presence of phagosomes and active phagocytosis of dying hair cells was quantified. Additional studies used immunohistochemistry to identify which cells in the inner ear express MerTK.

Results: Consistent with genomic data, we observed immunoreactivity for MerTK in supporting cells of the cochlea and utricle. This suggests that activation of MerTK (by hair cell death) may trigger phagocytic behavior in supporting cells. Prior studies have also described filamentous actin phagosomes in the lower stratum of the mouse utricle (e.g., Bucks et al., eLife 2017). We observed these structures in both uninjured utricles and saccules, but not in the cristae. Actin phagosomes were not present at P0, but develop during the first post-natal week. Notably, genetic deletion of MerTK did not affect the numbers of these structures in either normal mice or after IDPN-induced ototoxic injury. Finally, we did not observe any clear interaction between these actin structures and dying hair cells. In order to identify the signals that initiate the formation of actin phagosomes, we treated cultured utricles with inhibitors of Rho-family GTPases. Treatment with ML141 (a small molecule inhibitor of Cdc42/Rac1) did not affect the numbers of phagosomes, and current experiments are testing whether other inhibitors influence phagosome assembly. Finally, the mouse utricle contains a resident population of macrophages, which are normally confined to the stromal tissue that underlies the sensory epithelium (e.g., Kaur et al., Front Cell Neurosci 2015). IDPN-induced hair cell injury caused macrophages to migrate into the sensory region and engulf cellular debris.

Conclusions: Recruited macrophages remove some hair cell debris from the injured utricle. The maculae also possess presumptive phagosomes, but the precise role of these structures and the signals that initiate their formation remains unclear.

T84. Rapid Cochlear Hair Cell Loss in Adult Mice After Single Aminoglycoside Infusion

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Category: Inner Ear: Damage and Protection

Background: To advance the development of new treatments for hearing loss, it is important to understand potential signaling between dying hair cells and adjacent supporting cells. A feasible strategy for investigating dying cochlear hair cells and surviving supporting cells is the induction of hair cell death with aminoglycosides. We propose that single-cell -omic investigations require synchronized and robust hair cell loss in the cochlea of adult mice. We noted a lack of adult mouse aminoglycoside-induced hair cell loss models compatible with single-cell temporal trajectory analysis.

Methods: We infused a single dose of a hyperosmotic sisomicin solution (ho-sisomicin) into the posterior semicircular canal of one-month-old FVB mice to elicit consistent and synchronous damage in the cochlea. Immunohistochemistry was conducted at different time points post-infusion to characterize the molecular characteristics of the emerging damage. Cell type-specific antibodies were used to identify and quantify hair cells and supporting cells. Apoptotic cells were identified with TUNEL assay. To characterize structural abnormalities in the stria vascularis (SV) we measured the cross-sectional area of SV using F-actin labeling. To reveal functional deficits associated with the combination of sisomicin with hyperosmotic stress, we recorded Auditory Brainstem Responses (ABRs) and Distortion Products of Otoacoustic Emissions (DPOAEs).

Results: We observed robust outer hair cell loss 14 hours following the administration of ho-sisomicin. Outer hair cells underwent apoptosis, with the highest number of apoptotic cells occurring between 5 to 7 hours after ho-sisomicin infusion. The loss of outer hair cells was nearly complete and uniform throughout the cochlea, except for the most apical region. Interestingly, there were no notable alterations in the number of inner hair cells 24 hours after infusion. However, a delayed but complete loss of inner hair cells was observed at the 72-hour mark. Hair cell loss was accompanied by a complete and permanent absence of ABR thresholds and the disappearance of DPOAEs as early as 3 hours after injection. We did not observe any significant changes in the cross-sectional area of the stria after 24 hours following the infusion. However, after 7 days, we noted a reduction in the SV area. Importantly, the number of supporting cells were unaffected by the injection.
Conclusions: We established a novel, fast, and reproducible adult cochlear hair cell damage mouse model without affecting supporting cells. We argue that our approach overcomes limitations of systemic administration, because pathological changes are robust and temporally well-defined. The ho-sisomicin-induced hair cell loss paradigm will provide the framework for collecting transcriptomic and epigenetic data and the basis for future gene therapy approaches in the adult mammalian cochlea.

T85. New Molecular Entities Protect Hair Cells Against Aminoglycosides-Induced Ototoxicity

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Category: Inner Ear: Damage and Protection
Background: Aminoglycosides are one of the most commonly-used classes of antibiotics worldwide. They are a highly effective treatment against sepsis and chronic infections, particularly among cystic fibrosis and tuberculosis patients. However, permanent hearing loss and/or balance disturbance occurs in up to 20% of patients taking aminoglycosides. Despite this debilitating side effect, currently there are no FDA-approved therapies to prevent aminoglycosides ototoxicity.
Methods: A novel chemical entity (DXU656683) and its analogs have been identified in our hit-to-lead expansion drug screening campaign. Preclinical characterization of their otoprotective efficacy was carried out using in vivo zebrafish assays. DXU656683 and analogs are tested against the entire aminoglycosides drug class, including gentamicin, neomycin, kanamycin, tobramycin, amikacin, streptomycin, sisomicin, ribostamycin, and geneticin (G418) in acute and chronic exposure models. Additional experiments such as MET channel function, Texas Red-conjugate gentamicin uptake, ROS and inflammatory responses, and TUNEL hair cell apoptosis assays are also performed to elucidate the mechanisms of DX656683 otoprotective actions. E. coli growth inhibitory assays were also performed to test its effect on aminoglycosides antibacterial activity.
Results: Our in vivo zebrafish assay results show that DX656683 offers substantial otoprotection against the entire class of aminoglycosides and it does not attenuate aminoglycosides antibacterial activity in vitro. DX656683 and its analogs mitigate aminoglycosides-induced hair cell damage via both MET channel blockade and intracellular mechanisms. The combination of the two modes of action are dose-dependent.
Conclusions: We have demonstrated the potential otoprotective efficacy of DX656683 and its analogs in our preclinical studies and determined the optimal dose against the entire class of aminoglycosides. In vivo rodent ABR/DPOAE studies are underway to establish its efficacy in mammalian models. Given its broad protection against aminoglycosides in clinical use, DX656683 holds the promise to be an effective drug candidate for aminoglycosides ototoxicity prevention.

T86. Time-Course of MHCII Expression in Rat Spiral Ganglion After Hair Cell Loss

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Category: Inner Ear: Damage and Protection
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 and die 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 in the spiral ganglion following deafening, including MHCII, a molecule involved in antigen presentation to lymphocytes and adaptive immune response. Preliminary data showed the MHCII knockout prevents SGN degeneration, indicating a causal role of MHCII-mediate antigen presentation and adaptive immune response for SGN loss.
SGN degeneration is a delayed process, and its pattern consists with adaptive immune response, which requires antigen presentation in prior. We investigated if the temporal expression of MHCII corresponds to SGN degeneration and if MHCII-mediate antigen presentation occurs before SGN deg Here we show by immunofluorescence that the MHCII expression is not comparable between deafened and hearing rats before SGN loss but increase after SGN death.

Methods: Male and female Sprague-Dawley rats were injected with kanamycin in the second postnatal week to kill hair cells and euthanized at postnatal day 21, 32, 45 and 70. Sections were immunolabeled with 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 ImageJ and IMARIS software with custom-written macros. The outline of Rosenthal canal for each turn was manually traced to measure cross-sectional area and to calculate macrophage, MHCII+APC which were counted in every fourth near-midmodiolar section
Results: In deafened rats at P21, the number of MHCII+ macrophage is not significantly different from hearing rats. At P70 and P32, deafened rats showed upregulation of MHCII. The number of MHCII+ macrophages does not change temporally in hearing control.
Conclusions: Previous findings from RNA sequencing showed the upregulation of MHCII genes after SGN death at P32 and P60. Consistent with that, immunohistology shows the increase of MHCII+ macrophages after P32. These results implicate the upregulation of MHCII-mediated antigen presentation immune response after deafening, suggesting a significant role of adaptive immune response in SGN degeneration.

T87. Open Board

T88. Detection and Quantification of Gentamicin in the Inner Ear Using Different Liquid Chromatography Methods

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Category: Inner Ear: Drug Delivery
Background: Quantifying drugs in the inner ear is challenging due to its relative inaccessibility. The ototoxic drug gentamicin, used to treat vestibular disorders like Meniere's disease, consists of 4 major C-subtypes - C1, C1a, C2, and C2a. Simultaneous detection of these subtypes is difficult because gentamicin lacks UV-absorbing chromophores. Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) can quantify gentamicin due to its high sensitivity. High-performance liquid chromatography with fluorescence (HPLC-Fl) can also quantify derivatized gentamicin. We present new LC-MS/MS and HPLC-Fl protocols to detect multiple gentamicin subtypes simultaneously in microliter sample volumes.
Methods: Gentamicin was separated on an Acquity UPLC BEH C18 column (Waters, 2.1 x 50 mm) using a gradient of 0-100% acetonitrile in water containing nonafluoropentanoic acid (100 mM) at 0.2 mL/min. Gentamicin standards (2.5-1,000 ng/mL) were prepared in artificial perilymph, with amikacin (500 ng/mL) as an internal standard. MS/MS analysis was performed using electrospray ionization in positive mode and fragmented with stepped collision energy.
Alternatively, gentamicin was derivatized with ortho-phthaldehyde (OPA) and ethanethiol before being eluted from a Poroshell 120, EC C-18 column (50 x 4.6 mm), using an isocratic mobile phase of 0.02 M sodium heptanesulfonate monohydrate in methanol: water: acetic acid (80:18:2 v:v:v) at 1 mL/min. OPA reagent solution was composed of 630 µL of OPA solution (360 mg OPA dissolved in 25 mL methanol), methanol (7.3 mL), 0.4 M boric acid, pH 10.4 (2 mL), ethanethiol (52 µL) and stored for 60 minutes before use. A solution of kanamycin in water (5000 ng/mL) served as an internal standard. Gentamicin standards (1 µL) prepared in artificial perilymph were added to a mixture of water (369 µL), internal standard (5 µL), and OPA

reagent (125 µL). OPA-derivatives of gentamicin were detected at 337 nm-454 nm (excitation-emission wavelengths, respectively).

Results: Using LC-MS/MS, the LOD and LOQ for gentamicin are 5 ng/mL and 25 ng/mL, respectively. The standard curve obtained was linear with R2 greater than 0.99. Fragment ions were seen for each major C-subtype of gentamicin and amikacin. Using HPLC-Fl, OPA-derivatives of the four major C-subtypes of gentamicin were successfully separated. The LOD and LOQ for gentamicin are 0.1 ng/mL and 10 ng/mL, respectively. This method was validated as per USP specifications and was linear (R2 greater than 0.99) over a concentration range of 10-400 ng/mL. The %RSD of precision and accuracy are 4.03 ± 2.42 and 5.43 ± 2.93, respectively.

Conclusions: A new sensitive LC-MS/MS protocol and a novel HPLC-fluorescence method were developed to detect and quantify low levels of gentamicin C-subtypes in small sample volumes in vitro. These methods will be used to determine gentamicin concentrations in perilymph samples from the inner ear.

T89. Novel Transcanal Device for Delivery of Cochlear Hypothermia

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Category: Inner Ear: Drug Delivery

Background: Mild therapeutic hypothermia (MTH) has been found to be neuroprotective against cochlear trauma. MTH modulates biological pathways which lead to the reduction of interleukin and oxygen-based free radicals. In an effort to circumvent secondary effects stemming from systemically delivered MTH, our research has demonstrated the feasibility, safety, and effectiveness of localized MTH. However, the initial approach utilized was an invasive probe placed in the facial recess during cochlear implant surgery. This approach, which successfully demonstrated the applicability of MTH, is unfortunately less practical as a day-to-day clinical therapeutic approach. With translational application and the operating room as our focus, we designed and assessed the efficacy of a noninvasive balloon catheter placed transcanal. The primary objective of this study was to evaluate the feasibility of achieving MTH within the cochlea of cadaver heads by employing this approach.

Methods: Temperature recordings from thermistors placed at the base of the cochlea, ear canal, scalp, and bead bath, were gathered from four half-cadaver heads (2 females and 2 males) during 16 rounds of experimentation. Specimens were pre-warmed and consistently maintained within a temperature range of 35.5-38°C to replicate typical human conditions. Once stable temperatures were achieved in the whole specimen, a cooling device featuring a liquid-flowing catheter probe with a balloon tip was employed. This probe, connected to a cooling machine, was positioned in the ear canal near the eardrum for periods of 15 or 30 minutes.

Results: Thermistors inserted through the round window indicated a mean temperature decrease of 3.5 – 4°C during a 15-minute cooling period and 4 – 5°C within a 30-minute cooling period. Female and male cadaver heads exhibited a mean of 4.3°C and 4.9°C temperature drop at 30-minute cooling periods, respectively. Baseline cochlear temperatures were fully restored within 15 minutes in both female and male cadaver heads after the device was removed. Thermistors placed on the scalp recorded no temperature fluctuations throughout any of the experiments. Importantly, no morphological changes were observed in the ear canals and eardrums of all specimens during the trials.

Conclusions: Our results indicate that cochlear hypothermia can be effectively accomplished using an external cooling system positioned at the ear canal. This recent data provides significant support for implementing a more accessible and simplified clinical approach to mitigate potential cochlear damage during middle and inner ear surgeries.

T90. Membranous Labyrinth Alteration and Functional Evaluation After a Posterior Semicircular Canal Injection in Adult Mice

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Category: Inner Ear: Drug Delivery

Background: Viral-mediated cochlear gene delivery is a promising therapeutic strategy for the treatment of inherited and acquired deafness. Different cochlear and vestibular surgical approaches are used to infuse the viral solution into the inner ear. Among the proposed approaches, posterior semicircular canal (PSCC) infusion is considered as a safe and efficient technique for transducing both cochlear and vestibular epithelium in adult mice.

However, the inner ear is limited by an inextensible bone box, containing the delicate membranous labyrinth, opened only in the cisternial space by the cochlear aqueduct. The high volume and speed of PSCC injection raise the question of the morphological consequences on the membranous labyrinth.

Methods: Three FVB adult mice underwent a left PSCC injection. A polyimide tube with an external diameter of 124µm was inserted into the PSCC and fixed using a biological glue. Sterile iso-osmolar saline of a total volume of 1.1µL was infused at a speed of 4 nL/s. The tube was closed and left in place after injection.

Hearing and the vestibular function were assessed with auditory brainstem responses (ABR), distortion product otoacoustic emissions (DPOAE), and vestibular comportment check-list at 24 hours and 3 weeks. The temporal bones were extracted, fixed, and stained with phosphotungstic acid for one week.

Non-destructive 3D imaging was done by µ-Computed Tomography (EasyTom 150, 1601 shadow images recorded over 360°, pixel size of 3.8µm, controlled and back-projected). The right ear served as control.

Results: Our results demonstrated that a single PSCC injection, with the same parameters used in viral gene therapy, induced alteration of the morphology of the vestibular membranous labyrinth, 10 minutes after injection. The membranous labyrinth of the PSCC, the three ampullae, and the utricule were detached from the bony labyrinth. Only a limited detachment of the PSCC was visible 24 hours after injection. The vestibular membranous labyrinth was strictly comparable with control at 3 weeks. The injection site was however filled with connective tissue. The cochlear function and morphology were normal at the different time points.

Conclusions: Our results showed that PSCC injection induced critical but transient alterations of the structure of the vestibular membranous labyrinth, but the vestibular function was not altered. As expected, the cochlear anatomy and function were similar to control. The mechanism involved in the transient detachment of the vestibular membranous labyrinth and the restoration of a normal anatomy are to be explored.

Taken together, these results reveal the histological consequences of the PSCC injection on the resilient vestibular membranous labyrinth. Despite transient alteration of the vestibular architecture, PSCC is safe for both the cochlear and vestibular function.

T91. Liposomal Nanoparticle for Non-Invasive in Vivo Drug Delivery to the Inner Ear

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[1]*Stanford University School of Medicine*

Category: Inner Ear: Drug Delivery

Background: Otitis media (OM) is a common middle ear disease that affects millions of children worldwide every year. OM is often associated with an accumulation of middle ear fluid behind the eardrum. Delivering drugs to the middle ear require systemic administration or placement of tympanostomy tubes. Tympanostomy tubes and removal of middle ear

mucoid effusion is the most common procedure in pediatric patients. N-acetylcysteine (NAC) has been shown to have positive effects on reducing OM fluid accumulation. Furthermore, rhodamine is an ototoxic dye that has potential to be used as a non-invasive hearing loss model.

Methods: We have developed murine models for OM fluid buildup using the CBA/CaJ strain at 4-6 weeks of age, with a sample size of n = 5. We delivered liposomal nanoparticles containing NAC and measured the effects on OM post-delivery. We conducted auditory brainstem responses (ABRs). Additionally, we have a

separate model of liposomal nanoparticles containing rhodamine to induce hearing loss in mice of strain CBA/CaJ. Finally, we perform histology to show liposomal delivery reaching the inner ear.
Results: The ABR results show that there is no hearing loss on any frequency (4kHz, 5.7 kHz, 8Hz, 11.3 kHz, 16 kHz, 22.6 kHz, 32 kHz, and 43.5 kHz) caused by the liposomal nanoparticles and NAC. We also see improvement in OM symptoms post-liposomal NAC delivery. Lastly, we see hearing loss occur in all frequencies after liposomal rhodamine delivery.
Conclusions: We have successfully developed liposomal nanoparticles that can be used as a vessel to deliver a variety of drugs. Our OM model indicates that NAC can be encapsulated by liposomes to help reduce OM inflammation and fluid buildup. Furthermore, rhodamine can be encapsulated by liposomes to create an alternative hearing loss murine model. These nanoparticles have the ability to cross the tympanic membrane without perforation and deliver to the middle and even as far as the inner ear. Therefore, the future implications of liposomal nanoparticles are vastly unexplored.

T92. Outer Hair Cell Innervation by Type II Spiral Ganglion Neurons Depends on Eph/Ephrin Signaling

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Category: Development: Cellular/Systems
Background: The molecular mechanisms dictating type II spiral ganglion (SGN) neuron growth and outer hair cell (OHC) innervation must be determined to understand normal auditory development. SGNs are bipolar neurons that relay auditory input to the cochlear nuclei after receiving glutamatergic input from mechanosensitive receptor hair cells. Type II SGNs represent a fascinating subdivision of SGNs in that they have a highly stereotyped projection pattern whereby they bypass the IHCs, make a 90° turn toward the cochlear base, then synapse with 10-15 outer hair cells (OHCs). Planar cell polarity (PCP) proteins have previously been shown to mediate type II SGN turning (Ghimire et al., 2018, 2019), but whether additional axon guidance mechanisms are involved remains unknown. In this research, I am investigating the involvement of Eph/Ephrin signaling in type II SGN turning and OHC innervation, as well as how Eph/Ephrin and PCP signaling possibly interact in this process.
Methods: To accomplish this, I generated Efna3 and Vangl2 null mice possessing Neurog1CreERT2 and R26RtdTomato, a combination permitting SGN sparse labeling. Conversely, Efna3; Vangl2 double knockouts (DKOs) were examined using anti-NF200. Immunostaining, confocal imaging, and 3D rendering in Imaris software were used to quantify type II SGN turning, branching, and other navigation characteristics. Cochlear explants were used to determine the temporal effects of exogenous Ephrin-A3 on type II SGN navigation.
Results: Compared to controls, Efna3 null mice showed a small but significant increase in type II SGNs incorrectly turning toward the apex. Both Efna3 null and heterozygous mice showed increased proportions of type II SGNs with abnormal navigation behaviors. In addition, Efna3 nulls displayed decreased branch numbers, suggesting EPHRIN-A3 may normally serve as a positive growth cue. As predicted, Vangl2 nulls displayed an immense rise in type II SGNs incorrectly turning toward the apex. Interestingly, Vangl2 null cochleae displayed a lower number of branches per type II SGN compared to control littermates, suggesting VANGL2 may also act as a positive growth cue. At E16.5, WT cochleae show EPHRIN-A3 immunoreactivity on membranes of Deiters' and pillar cells. However, EPHRIN-A3 protein appears reduced and more diffuse in Vangl2 nulls, suggesting that EPHRIN-A3 acts downstream of PCP signaling. In cochlear cultures, EPHRIN-A3-Fc (a gain-of-function manipulation) led to type II SGN collapse at E15.5, indicating a repulsive function. However, at P0, EPHRIN-A3-Fc treatment led to type II SGNs with elevated branch numbers, indicating a positive growth function. We are currently investigating the nature of this possible functional switch of EPHRIN-A3 at different developmental stages, as well as the role of EPHRIN-A3 in OHC synapse formation.
Conclusions: Taken together, our findings suggest that EPHRIN-A3 may act downstream of PCP signaling to mediate type II SGN turning during development, and that EPHRIN-A3 is an important component of OHC innervation.

T93. Lef1 and Tcf7l2 are Wnt Signalling Effectors With Contrasting Functions During Inner Ear Sensory Organ Formation

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[1]*UCL Ear Institute*

Category: Development: Cellular/Systems

Background: The inner ear is composed of several sensory organs responsible for the detection of sound, head position and acceleration. During embryonic development, these organs originate from neurosensory-competent domains within the otocyst, but the molecular signals controlling their formation remain unclear. The transcription factor Sox2 is required for neurosensory specification since its deletion abolishes the differentiation of sensory organs and their associated neurons. Sox2 is initially present throughout the otocyst, then it becomes restricted to its ventro-medial aspect. Our recent work suggests that this restriction is regulated in a dose-dependent manner by a dorso-ventral gradient (high to low) of canonical Wnt activity. High levels of Wnt signalling antagonise sensory organ formation dorsally, while low levels are needed to maintain prosensory specification ventrally. To find out how Wnt signalling can exert these two contrasting functions, we analysed the expression and function of four members of the Tcf/Lef family of transcription factors in the chicken otocyst.

Methods: To assess the role of each Tcf/Lef transcription factor in sensory organ formation, we analysed their gene expression pattern using RNA-scope and immunohistochemistry. For functional validation we implemented both gene over-expression and CRISPR/Cas9 mediated knock-out by in ovo electroporation in the embryonic chicken inner ear.

Results: We found that Lef1, Tcf7, Tcf7l1 and Tcf7l2 exhibit distinct expression patterns in the chicken otocyst. Lef1 and Tcf7 expression was found in the dorsal regions specific also for high Wnt reporter activity, Tcf7l1 was expressed in the entire otocyst and Tcf7l2 was detected in the Sox2-positive neurosensory domain localised in the ventral otocyst. Our functional experiments showed that over-expression of Lef1 antagonises Sox2 expression in the developing inner ear, whereas Tcf7l2 gain-of- function induced ectopic Sox2-positive domains. Overexpression of Tcf7 and Tcf7l1 had no impact on prosensory specification.

Conclusions: All members of Lef/Tcf family are active in the otocyst but are differentially distributed. The expression pattern of Lef1 and its gain of function reflect high levels of Wnt activity, while distribution of Tcf7l2 and the effects of its over-expression resemble those of low levels of Wnt activity. Hence, our results suggest that these two transcription factors are key effectors of the Wnt activity gradient during inner ear sensory organ formation.

T94. PRDM16 Deletion Reveals Novel Roles for Kölliker's Organ in Mouse Cochlear Development and Hearing

Hongji Zhang[1], Timothy Papiernik[1], Amal Yaghmour[1], Ahmad Alzain[1], James Benjamin Lennon[1], Rahul Maini[1], Xiaodong Tan[2], Ava Niazi[3], Joosang Park[3], Sungjin Park[3], Claus-Peter Richter[2], Michael Ebeid*[1]

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Category: Development: Cellular/Systems

Background: PR domain containing 16 (PRDM16) is a key transcriptional regulator in the development of multiple tissues including craniofacial, adipose, and neural tissues. Our lab has recently identified PRDM16 expression within Kölliker's organ (KO) cells starting around E13.5. A gene trap model of Prdm16 deletion showed defects in KO, spiral limbus (SL) and tectorial membrane (TM) during development. Because Prdm16 null mice die at birth, we generated an inner ear specific Prdm16 conditional knockout (cKO) mouse model that enabled characterizing postnatal cochlear development.

Methods: Whole mount and cochlear sections were used to characterize cochlear phenotype in Prdm16 cKO mice by immunolabelling, trichrome and H and E staining at birth as well as postnatal time points (P7, P14 and P21). Proliferation of SL mesenchymal cells was analyzed using the 5-ethynyl-2′-deoxyuridine (EdU) cell proliferation assay injected 2 hours before sacrificing mice. P28 inner ears were harvested and imaged using synchrotron X-ray phase-contrast and micro-computed tomography at Argonne National Laboratory (resolution of 1.7 μm). Images were visualized and analyzed using Amira software (ThermoFisher Scientific).

Transmission electron microscopy (TEM) was used to analyze TM defects. Hearing functions at P-21, -60 and -90 were assessed by auditory brain stem response (ABR) at 4, 8, 16, and 32 kHz.
Results: Prdm16 cKO at P0 showed hypoplastic KO, shortened cochlear duct, and multiple isolated ectopic sensory epithelial cells within the KO domain. This phenotype persists throughout the first week (P7) of postnatal cochlear maturation. Analysis of P14 and P21 cochlear sections revealed hypoplastic spiral limbus, lack of inner sulcus, lack of interdental cells and detached tectorial membrane. TEM analysis of tectorial membrane shows absent limbal domain and delayed collagen fibril development. Analysis of SL mesenchymal cell proliferation showed an increased percentage of proliferating mesenchymal cells in the apical turn in Prdm16cKO compared to control at E18.5 and P5. Immunostaining for SL extracellular matrix proteins at P0 and P5 showed reduced COLII, TGFBI and COCHLIN staining in the middle and apical turns in Prdm16 cKO. Finally, Prdm16-deficient mice showed a hearing deficit, as indicated by elevated auditory brain stem response thresholds at all frequencies, consistent with the cochlear structural defects.
Conclusions: This work establishes Prdm16's necessity for developing normal hearing in mice via its regulation of tectorial membrane, spiral limbus, and interdental cellular development. Such understanding will help recognize Kölliker's organ contribution to cochlear development and the pathophysiology of hearing deficits involving Prdm16 haploinsufficiency, such as 1p36 deletion syndrome.

T95. Incorporating Novel Audiogram Classification Strategies to Identify Genes and Pathways Involved in Subtype Components of Age-Related Hearing Loss

Samah Ahmed*[1], Kenneth Vaden[2], Judy R. Dubno[2], Britt Drogemoller[1]

[1]*University of Manitoba,* [2]*Medical University of South Carolina*

Category: Genetics A: Genomics and Gene Regulation
Background: Age-related hearing loss (ARHL) affects one-third of the population over 65 years. The diverse pathology that underlies this heterogeneous group of phenotypes likely involves distinct biological mechanisms. Although genome-wide association studies (GWAS) have uncovered genetic variants underlying self-reported ARHL, there are large gaps in our understanding of the specific genetic factors involved in distinct hearing loss phenotypes. This may be due, in part, to challenges associated with accurate phenotyping for older adults with hearing loss. In this study, we used a mathematical model fitted to individual audiograms to estimate the magnitude of subtype components of ARHL.
Methods: We have obtained genomic and audiologic data from 26,622 healthy older individuals participating in the Canadian Longitudinal Study on Aging. By adopting a novel approach developed by Vaden et al., we derived metabolic and sensory estimates for each audiogram. Predicted line fit error was used to exclude audiograms that were not representative of ARHL. We identified ears with better and worse hearing thresholds based on the calculated estimates. SNP-heritability testing was used to investigate the degree of variability that could be explained by genetics for each phenotype. GWAS was performed by linear regression, including significant clinical variables and the first ten genetic ancestry-related principal components as covariates. We performed functional enrichment analysis to identify biological pathways underlying hearing loss phenotypes.
Results: We found that metabolic estimates were higher for older compared to younger individuals, with no noticeable difference between males and females. Although sensory estimates were significantly higher in older individuals, the magnitude of the association was minor, with males showing more sensory hearing loss compared to females. For both metabolic and sensory estimates, ears with less severe hearing phenotypes showed higher heritability ($h2 = 0.064$ and 0.106, respectively) and were included in downstream analyses. GWAS revealed that rs6453022, a missense variant in ARHGEF28 gene, was significantly associated with the metabolic phenotype ($P=2.67x10-9$); while rs36062310, a missense variant in KLHDC7B gene, was significantly associated with the sensory phenotype ($P=2.37x10-12$). Sex-stratified analyses also revealed key differences in the GWAS results. Enrichment analyses revealed differences in the biological pathways underlying the two hearing phenotypes, with the RhoA activity regulation pathway implicated in the metabolic phenotype, and pathways relating to sensory processing of sound by hair cells and the calcium/calmodulin signalling implicated in the sensory phenotype.
Conclusions: In this large-scale genetic study, we have identified differences in the associations observed for two distinct subtype components of age-related hearing loss. Identifying specific processes involved in sensory and metabolic hearing loss has improved our understanding of the biological mechanisms underlying

different components of ARHL. This information can, in turn, guide the development of targeted treatment strategies for the more precise treatment of ARHL.

T96. The Importance of Phenotyping in Large-Scale Genomic Analyses of Hearing Impairment in Older Adults

Morag Lewis*[1], Bradley A. Schulte[2], Judy R. Dubno[2], Karen P. Steel[1]

[1]*King's College London,* [2]*Medical University of South Carolina*

Category: Genetics B: General

Background: Age-related hearing loss (ARHL) is a common, heterogeneous disease with a heritability of 70%, but the genetic landscape underlying this form of hearing impairment remains largely unknown. Studying such a condition requires large cohorts, detailed genotyping, and deep phenotyping. However, while large cohorts with next-generation sequence data are becoming increasingly common, the challenge of administering individual audiometric tests to so many people has meant that in-depth auditory phenotyping is rarely included in these projects.

Methods: Here we present our analyses of three cohorts with different forms of phenotype data. The first cohort, an ongoing study at the Medical University of South Carolina (MUSC), has detailed audiometry. The second cohort was selected from the UK Biobank, where the only useable auditory phenotype data are the participants' responses to questions about difficulty hearing. The third cohort was chosen from the NIH All of Us Research Program, where the auditory phenotype data is based on electronic health records and clinical diagnoses. Only people aged 55 and older were included in these analyses. For each cohort, we defined a group of people with normal hearing and at least one hearing loss group, and compared variant load per gene between phenotype groups to identify genes with more or fewer variants than expected in people with hearing impairment.

Results: From the MUSC cohort, we obtained multiple gene lists associated with different components of ARHL derived from the audiogram. Nearly half of the genes linked to hearing ability were also identified in the UK Biobank analysis, and this list of common genes includes multiple known deafness genes as well as candidates found in other large-scale analyses, giving confidence that the results were robust. However, the gene lists from the NIH All of Us cohort had few genes in common with the previous cohort lists, and were not enriched in known deafness genes. We suggest the lack of replication in the NIH All of Us cohort may be because many people with ARHL do not obtain medical diagnoses, especially when hearing loss is mild-to-moderate, and thus our "normal hearing" group in the NIH All of Us cohort probably included people with ARHL. The relatively small size of the hearing loss group in the NIH All of Us cohort (15%) bears this out; in the UK Biobank, 48% of the cohort reported difficulty hearing.

Conclusions: In conclusion, for a common condition like ARHL, subjective questions are useful (as in the UK Biobank), but the ideal phenotyping would be full audiometry (as in the MUSC cohort), which allows different forms of hearing loss to be analysed in detail. The nature of the phenotyping data available must be considered alongside the disease being studied when planning analyses on general cohorts.

T97. Open Board

T98. Open Board

T99. Epigenetic Characterization of Vestibular Hair Cells and Type II-To-Type I Hair Cell Conversion

Sarath Vijayakumar*[1], MI ZHOU[1], Amanda Ciani Berlingeri[2], Neil Segil[3], Jennifer Stone[2], Litao Tao[1]

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Category: Genetics A: Genomics and Gene Regulation

Background: Sensory hair cells (HCs) in mammalian vestibular organs are essential for motion detection and body movement. Type I and type II HCs in vestibular organs are categorized based on their distinct

morphology, physiology and innervation. A thorough characterization at the epigenome level of adult vestibular HC subtypes is warranted to better understand HC phenotypes and function, and to gain insights relevant to HC regeneration. Sox2 is a key transcription factor maintaining the type II HC fate in vestibular organs of adult mice; deletion of Sox2 from type II HCs causes them to convert to type I-like HCs. In addition to studying the epigenome of mature type I and type II hair cells, we are exploring epigenetic changes following Sox2 deletion from type II HCs, to define the mechanisms that control the type I and II fates.
Methods: Atoh1-CreER:Rosa26TdTomato:Sox2flox/flox (Sox2 cKO) animals were used to label type II hair cells and to induce type II HC-specific Sox2 deletion by injecting tamoxifen at six weeks of age; and Sox2wt/wt littermates (Sox2 WT) were used as wildtype controls. One week, 1 month, 2 months and 4 months after tamoxifen injection, animals were sacrificed, and utricles were dissected out for single cell multiome analysis with 10X Genomics platform. Single cell multiomic data were analyzed using R packages including Seurat, Monocle3, Signac and ArchR; pseudo-bulk data derived from single cell datasets were analyzed by DeepTools and HOMER.
Results: Utilizing the correlation of chromatin accessibility between elements and the correlation between chromatin accessibility and gene expression (described in an accompanying poster), we identified putative distal enhancers for genes that are HC subtype-specific or common to both subtypes. We also found different binding motifs enriched in subtype-specific elements. During type II-to-type I HC conversion after Sox2 deletion, enhancers for many type I HC specific genes gain accessibility, correlating with the transcriptional upregulation of type I HC specific genes.
Conclusions: Similar to transcriptomes in utricular HC subtypes, type I and type II HCs share many common accessible elements and have their own unique chromatin accessibility profiles. The expression changes of subtype-specific genes during subtype conversion were accompanied by changes of chromatin accessibility at distal enhancer regions.

T100. Transcriptomic Characterization of Vestibular Hair Cells and Type ll-to-Type l Hair Cell Conversion in Adult Mice

Amanda Ciani Berlingeri*[1], Sarath Vijayakumar[2], Mi Zhou[2], Neil Segil[3], Litao Tao[2], Jennifer Stone[4]

[1]*The 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*

Category: Genetics A: Genomics and Gene Regulation
Background: The sensory organs of the mammalian vestibular system contain specialized mechanosensory hair cells (HCs) that respond to head motions. Mammalian HCs are categorized as either type I or type II, which differ in morphology, physiology, and innervation. Mature HCs die due to various causes, and regeneration is limited to type II-like HCs, resulting in no restoration of vestibular function. Little is known about the genes controlling the mature phenotypes of type I and II HCs. Tamoxifen-induced deletion of Sox2 from type II hair cells of adult Atoh1-CreER:Rosa26TdTomato:Sox2flox/flox (Sox2 CKO) mice leads to their conversion into type I-like cells, with significant morphological and molecular changes. Identifying crucial genes for this conversion may enable more comprehensive HC regeneration and functional restoration.
Methods: We analyzed the transcriptomes of vestibular HCs in Sox2 CKO and wildtype (WT) mice at 1 week, 1 month, 2 months, and 4 months post-tamoxifen treatment. Single-cell multiome sequencing was performed on whole utricles or isolated utricular sensory epithelia to collect mRNA data from individual HCs and supporting cells, using the 10X Genomics platform. Datasets were analyzed using Seurat Library, creating cell clusters based on transcriptional similarities, and verified using known marker genes. Monocle3 library was used to assess the effects of Sox2 CKO on type II HCs over time compared to WT.
Results: In the WT data, we observed three common HC subtypes: type II (e.g., Calb2-positive), type I (e.g., Spp1-positive), and striolar type I (e.g., Ocm-positive). To validate novel gene expression, we identified genes with greater than 2X enrichment in one subtype versus another based on an average read count greater than 2. We utilized the gEAR portal to cross-reference other HC RNAseq datasets and conducted GO analysis using DAVID and ShinyGO. We are currently validating the expression of select genes enriched in specific clusters through hybridization chain reaction/fluorescent in situ hybridization or immunolabeling. We seek to verify their spatial and cell type-specific expression, evaluate their suitability as markers for adult murine vestibular HCs, and explore the presence of similar HC subtypes in other vestibular organs.

In the Sox2 CKO datasets, we identified a persistent cluster of cells with intermediate transcriptional features between type I and type II HCs. These cells displayed reduced expression of type II marker genes and increased expression of type I marker genes. However, even at 4 months post-tamoxifen, this cluster remained, indicating incomplete transition to the type I fate.

Conclusions: Our findings reveal genes that are downregulated and upregulated as type II HCs transition into type I-like HCs. Further exploration of these genes will offer new insights into requirements for regenerating both type I and type II HCs after damage.

T101. Open Board

T102. A New Allele of Otoferlin Allows Reversal of Hearing Loss

Elisa Martelletti*[1], Nina Treder[2], Alishba Saleemi[2], Darcey. A Kirwin[2], Neil J. Ingham[2], Karen P. Steel[2]

[1]*King's College London,* [2]*Wolfson Centre for Age-Related Diseases, King's College London*

Category: Genetics B: General

Background: Otoferlin is a critical protein for proper synaptic functioning in inner hair cells (IHCs). It is highly expressed at the ribbon synapses of IHCs, where it serves as a Ca2+ sensor. The absence of Otoferlin results in the failure of signal transmission from IHCs to spiral ganglion neurons due to impaired synaptic vesicle release. Mutations in this gene have been identified and described in both humans and mice, leading to severe hearing loss. In mice, hearing loss associated with Otoferlin mutations was successfully reversed through gene therapy within 30 days after birth (Akil et al 2019).

Methods: We characterised a new mouse allele of the otoferlin gene, Otoftm1a. Auditory Brainstem Responses (ABRs) were recorded in ketamine/xylazine-anesthetized mice at various ages, in response to click stimuli and tone pips from 3 to 42 kHz. Ribbon synapses in inner hair cells were labelled using Ribeye and GluR2 antibodies, followed by manual quantification based on confocal imaging at cochlear best-frequency regions of 12 and 30 kHz. To restore Otoferlin gene activity, a genetic approach was employed, involving tamoxifen injection to activate Flp recombinase that recognizes FRT sites in the Otoftm1a allele, resulting in the removal of the mutagenic targeted insertion (Martelletti et al., 2023).

Results: Mice homozygous for the Otoftm1a allele, bred on a C57BL/6N background with the Cdh23ahl allele repaired, displayed no ABR responses up to 95dB SPL from 2-14 weeks old compared to their wild-type (Otof+/+) and heterozygous (Otof+/tm1a) littermates. Quantification of ribbon synapses at 12 and 30 kHz regions at 2 weeks revealed a significant reduction in co-localised pre- and post-synaptic components in Otoftm1a/tm1a mice compared to wild-type counterparts. These findings align with previous studies demonstrating a decrease in IHC synapses in different Otoferlin mutants (Stalmann et al., 2021; Roux et al., 2006). Following tamoxifen injection at 4 weeks old and activation of Otof transcription, some reversal of hearing impairment was observed between 3 and 24 kHz in Otoftm1a/tm1a mice carrying the Flp recombinase Flpo, with some variability among mutants.

Conclusions: We have characterised a novel mouse mutant for Otoferlin, which exhibits severe hearing loss starting at 2 weeks and a decreased number of ribbon synapses. Upon activation of the Otof gene at 4 weeks, a reversal of hearing impairment was observed in the 3 to 24 kHz range, although some variability was noted among individual mice. In summary, this study confirms the potential for gene therapy interventions to mitigate hearing loss associated with Otoferlin mutations.

T103. Zfp719 is a Transcription Factor Important for Maintenance of Hearing in Mice

Morag Lewis*[1], Jing Chen[1], Neil J. Ingham[1], Maria Lachgar-Ruiz[1], Karim Boustani[1], Karen P. Steel[1]

[1]*King's College London*

Category: Genetics B: General

Background: Background: Mice carrying a targeted mutation disrupting translation of Zfp719 showed elevated auditory brainstem response (ABR) thresholds in a high-throughput targeted mutagenesis

programme. Zfp719 encodes a zinc finger transcription factor whose function is largely unknown. Here we present our study characterising the development and function of the inner ear of these mutant mice.
Methods: Methods: We have carried out longitudinal ABR measurements to determine when these mice first demonstrate hearing impairment. We have measured DPOAEs and normoxic and anoxic endocochlear potentials, and have examined the hair cells using scanning electron microscopy, all at several ages. We have carried out RNA-seq at three stages; before (postnatal day (P)4) and after onset of hearing (P14), and after hearing loss is evident (P21). Expression of Zfp719 was studied using the lacZ reporter gene.
Results: Results: Zfp719 is expressed in multiple locations in the inner ear, including the hair cells, stria vascularis, and spiral ganglion neurons. Mice carrying the mutant allele exhibit rapidly progressive hearing loss between two and three weeks old, and by 6 months old homozygotes are completely deaf. DPOAEs showed impaired outer hair cell (OHC) function at 4 weeks old but not at 2 weeks, correlating with the progressive change in ABR thresholds, while the normoxic endocochlear potential was unaffected at 3 weeks old. Scanning electron microscopy revealed that some OHCs exhibited abnormalities such as missing or fused stereocilia at 3 weeks old, and extensive OHC degeneration was observed at 8 weeks old, along with some inner hair cell abnormalities. Transcriptome analysis showed many more significantly misregulated genes at three weeks old compared to earlier stages, including several genes known to be important for hearing, including Kcnj13, Ceacam16, and Prdm16.
Conclusions: Conclusions: Zfp719 is expressed throughout the cochlea, but the main effect of this mutant allele were the fused and missing hair cell stereocilia. However, at three weeks old, when thresholds were highly raised, these morphological defects were comparatively mild and only observed in a minority of outer hair cells, suggesting that the underlying pathology remains to be fully understood. The development of normal hearing at an early age prior to rapid progressive hearing loss suggests that Zfp719 is not critical for development of the inner ear and auditory function, but is necessary for maintaining hearing.

T104. Open Board

T105. A Variant in MUC5B, a Gene Which is Upregulated in the Middle Ears of Transgenic or Infected Mice, is Associated With Increased Biodiversity in the Middle Ears of Children With Otitis Media

Helen Gomez*[1], Christina Elling[1], Nam Lee[2], Elizabeth Davidson[1], Mahmood F. Bhutta[3], Scott D. Hirsch[1], Wasyl Szeremeta[4], Melissa A. Scholes[1], Jennifer M. Kofonow[1], Harold S. Pine[4], Sven-Olrik Streubel[5], Elisabet Einarsdottir[6], Todd Wine[1], Juha-Pekka Vasama[7], Jeremy D. Prager[1], Patricia J. Yoon[1], Charles E. Robertson[1], Kenny H. Chan[8], Petri S. Mattila[9], Norman R. Friedman[1], Daniel N. Frank[1], Lena Hafren[9], David A. Schwartz[1], Tasnee Chonmaitree[4], Ivana V. Yang[1], Allen F. Ryan[10], Regie Lyn Santos-Cortez[1]

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Category: Genetics B: General
Background: The MUC5B promoter variant rs35705950 is the strongest risk factor for developing idiopathic pulmonary fibrosis (IPF) among adults greater than 60 years old. Minor allele frequency is on average 8% across multiple populations, suggesting that it might have a protective effect especially in childhood. MUC5B is the predominant mucin in middle ear (ME) effusions in chronic or mucoid otitis media (OM).
Methods: To further investigate the role of the rs35705950 variant in OM, we performed: logistic regression analysis using IPF diagnosis and history of OM in adult patients from the COMPASS database; family-based association studies using genotypes from 1,020 multi-ethnic trio families; genotype-based analysis of 16S rRNA gene sequence data from nasopharyngeal (NP) and ME samples of Coloradan children with OM; and Muc5b expression after inoculation of non-typeable Haemophilus influenzae (NTHi) into mouse ME.
Results: IPF was negatively associated with history of OM (p=0.004) after adjusting for age, sex, ethnicity, and smoking in 47,163 adults. Transmission disequilibrium testing showed that the rs35705950 variant was

not significantly associated with OM in multi-ethnic trios. In Coloradan children with OM and who carry the rs35705950 variant, alpha-diversity was increased in the ME, but not in the NP. Muc5b was upregulated after NTHi inoculation of mouse ME, with specific expression in epithelial cells. In previous studies, ME effusion was observed in Muc5b-overexpressing and knockout mice, consistent with OM and known effects of the rs35705950 variant in human lung.
Conclusions: Future work involves ME inoculation of Muc5b-overexpressing mice to determine the effects of mouse genotype and infection in the ME. In conclusion, the enhanced ability to identify at-risk individuals through genetic risk variants, coupled with a comprehensive understanding of the interplay among pathogens, host genetics, and the environment, not only contributes to improving patient care but also plays a pivotal role in significantly diminishing the overall disease burden of OM.

T106. Identification of a Novel Copy Number Variant in EYA4

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Category: Genetics B: General
Background: Autosomal dominant nonsyndromic hearing loss (ADNSHL) is a genetically heterogeneous disorder, accounting for 15% of cases of genetic hearing loss with a discernable etiology. One such cause is pathogenic single nucleotide variants (SNVs) in EYA4, which have been reported in over 50 ethnic groups with a variety of auditory phenotypes. Copy number variants (CNVs) in EYA4, by contrast, are rarer. Herein, we describe a novel CNV in EYA4.
Methods: A retrospective review of patients referred for targeted genomic enrichment and massively parallel sequencing (TGE + MPS) using the OtoSCOPE panel was completed. Genetic results and available phenotypic data were reviewed by a multidisciplinary team to assess variant pathogenicity. Haplotypes were reconstructed using variants within EYA4 identified in sequencing data.
Results: We identified a novel pathogenic complex CNV in EYA4 (NC_000006.11:g.133845845_133851724delins133859363_133859438inv) in five unrelated probands of European descent with a family history of ADNSHL. This CNV removes a 5.8kb region of EYA4 that encompasses the last three exons (18-20) and inserts an inverted downstream sequence. The protein, if produced, would lack part of the highly conserved C-terminal eyaHR domain, which mediates interactions with SIX and DACH protein families. The complexity of this CNV coupled with haplotype reconstruction suggests a founder effect rather than a recurrent event.
Conclusions: This finding expands the mutational spectrum of EYA4-associated deafness and highlights the importance of state-of-the art bioinformatic analysis of next generation sequencing data to identify complex CNV events.
This project was supported in part by NIH-NIDCD grants DC002842, DC012049, and DC017955 (RJHS) and NIH-NIDCD grant 5T32DC000040 (MJK and JKD).

T107. Tonsil-Derived MSC`s Anti-Oxidative Response Against Magnetically Charged Iron Oxide Nanoparticle

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Category: Regeneration
Background: Mesenchymal stem cell therapy and diagnosis are emerging study fields in various areas due to the highly proliferative, self-renewal, differentiation potential, high capability for scavenging oxidative stress, and free of ethical issues rather than embryonic stem cells and induced pluripotent stem cells. However, there are limitations including immune response, prooncogenic, differentiation capacity, and reduction of migration with undesired areas. Studies show that the underlying cytotoxicity of nanoparticles is unbalanced reactive oxygen species, oxidative stress, and ferroptosis which likely depend on their morphology, size, dosage, and coatings. We improved mesenchymal stem cells (MSCs) homing phenomenon with high anti-inflammatory

effects for restoring damaged hair cells. This study aims to determine the antioxidative response of tonsil-derived hMSC after labeling positively charged iron oxide nanoparticles with and without n-acetyl cysteine (NAC) pretreatment.
Methods: Previously isolated and maintained tonsil-derived MSCs were cultured and labeled with PYRB (positively charged iron oxide NP) with different doses and certain time points. Cells were cultured overnight and labeled with PYRB 5,10, 20ug/ml doses under 24-72 hours. Cell viability was measured with an enzyme-linked immunosorbent assay, ROS (reactive oxygen species) was measured using a DCFDA cellular ROS detection kit, and antioxidant activities (catalase, SOD, GSH) were measured with a colorimetric microplate reader to determine the oxidative stress on MSCs. Intracellular iron concentration and magnetic attraction were used with Ferene-S and crystal violet staining. Ferroptosis downstream gene PTGS2 and transferrin receptor TFRC were analyzed with a quantitative polymerase chain reaction.
Results: Cell viability showed dose and time-dependent decrease, ROS level increased after labeling positive charged IONP, and pretreatment of NAC can rescue cells due to ROS decrease. Catalase and SOD activity significantly diminished, GSSG accumulated, and GSH/GSSG ratio decreased at 10 and 20ug/ml positive charged IONP, however no significant difference in GSH level, which means MSC responded against IONPs primarily through catalase activity. Pretreatment of NAC-rescued cells through SOD and GSSG level, not catalase activity. Intracellular iron concentration increased dose-dependently but no difference with NAC pretreatment. The ferroptosis downstream gene was significantly increased at high concentrations which means cell death due to iron accumulation.
Conclusions: Collectively, these results indicate that hMSC can maintain redox homeostasis via their catalase activity primarily when labeled with PYRB varied on their dosage and period, and antioxidant supplements such as NAC can decrease oxidative stress after labeling NP. This study can be an important concept for diminishing oxidative stress in mesenchymal stem cells through increasing enzymatic activity and a more effective translational approach in regenerative medicine.

T108. Rho Kinase or DNA Methyltransferase Inhibitors Produce Ectopic Hair Cells After Aminoglycoside-Induced Damage in Neonatal Mouse Cochlear Explants

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Category: Inner Ear: Damage and Protection
Background: The replacement of mechanosensory hair cells (HCs) lost due to noise, aging, or ototoxic drug exposure has long been a focus of auditory research and drug development. Aminoglycoside antibiotics are commonly used in the clinic despite their known ototoxic effects on HCs. Previous studies have implicated inhibitors of DNA methyltransferase (DNMT) or the Rho kinase pathway as potential drivers of HC regeneration or protection from aminoglycoside-induced damage, respectively. However, direct evidence is lacking because no fate-mapping was involved in the previous studies. Here, we utilized a cochlear explant model of HC loss combined with fate-mapping of the neighboring supporting cells (SCs), the progenitors of regenerated HCs, to investigate whether these compounds stimulate HC regeneration.
Methods: We permanently labeled the SCs that surround the outer HCs (the pillar and Deiters' cells) with a red fluorescent marker (tdTomato) prior to HC damage using Prox1CreERT2::Rosa26loxP-stop-loxP-tdTomato reporter mice. Mouse pups were injected with tamoxifen at postnatal day (P)0, and cochlear explant cultures were established at P2. Explants were equilibrated overnight followed by treatment with neomycin for 24h to induce HC death, and then treatment with the compounds of interest for 96h. Explants were then fixed, immunostained with anti-myosin VIIa antibodies, and analyzed via confocal microscopy to quantify the number of regenerated (tdTomato-positive) HCs or to identify myosin VIIa-positive ectopic HCs in each explant.
Results: The number of regenerated (tdTomato-positive) HCs in the middle or basal regions was very low (less than 5 cells) in cochlear explants treated with Fasudil (Rho kinase inhibitor) or Decitabine (DNMT inhibitor) 24h after neomycin-induced damage. Preliminary data show no significant differences in the number of HCs between neomycin only and the treatment groups. Additionally, we identified a significant amount of ectopic HC formation in the middle or basal turns for both treatment groups. In both cases, the ectopic HCs were located lateral to the third row of outer hair cells, in the Claudius cell or outer sulcus cell regions.

Conclusions: Based on these preliminary results we conclude that both compounds could induce formation of HCs from non-sensory cells. However, the cells lateral to the Organ of Corti had a much greater response than the pillar and Deiters' cells.

T109. Neural Correlates of Memory-Guided Attention in Age-Related Hearing Loss

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Category: Aging

Background: Age-related hearing loss (ARHL) is well known to be associated with cognitive decline, but the reason for this relationship is not yet resolved. Some hypotheses suggest that reduced sensory input may strain limited cognitive resources or produce cortical reorganization that attempts to remediate auditory function (Slade et al., 2020, Trends in Neurosci. 43(10):810-21), but at the expense of other key cognitive functions such as long-term memory. 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 ARHL is associated with deficits in behavioural and neural correlates of memory-guided attention.

Methods: Participants aged 40 to 80 with pure-tone thresholds greater than 40 dB HL (hearing loss group) or less than 30 dB HL (typical hearing group) and without evidence of cognitive impairment will participate. During training, both groups learn to associate 60 background sounds (e.g., ocean shore) of 3-second duration with the spatial location of a probe tone target (left or right ear) that occurs after the background. As a test of memory-guided attention 1 hour after training, presentation of the same acoustic backgrounds will cue participants to attend to the learned location, and we will record EEG and reaction times as they respond to probes that either happen on the learned side or the opposite side. Evidence of memory-guided attention is shown by faster reaction times, earlier evoked potentials, and modulation of 8-12 Hz alpha oscillations for probes on the learned side relative to probes on the opposite side. Explicit memory for probe–background associations and auditory spatial attention will also be tested.

Results: Data collection is ongoing.

Conclusions: We expect that the hearing loss group will show weaker evidence of memory-guided attention compared to the age-matched typical hearing group. Since brain regions that support attention–memory interactions are known to decline with both cognitive impairment and ARHL, results could clarify how these conditions co-occur. Findings may also be used in healthcare settings to inform clinicians about the risk of dementia or Alzheimer's disease that are known to correlate with hearing loss in late life (Lin et al., 2011a; Livingston et al., 2017).

T110. 5xFAD-Induced Hearing Loss in AHL-Corrected C57BL/6 Mice (FAD2) Does Not Correlate With Amyloid Deposition

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Category: Aging

Background: Case-controlled and longitudinal population-based studies have established a strong correlation between age-related hearing loss (ARHL) and dementia. However, no evidence demonstrates a causative link between Alzheimer's disease (AD) and auditory deprivation.

Methods: We have developed ahl-corrected C57BL/6 (B6)-background 5xFAD mice (designated as FAD2), expressing five AD-linked mutations with human APP and PSEN1 transgenes, and assessed hearing, loss of sensory hair cells, and amyloid deposition by immunohistochemistry.

Results: Ahl-corrected B6 wildtype mice (WT2) did not show increased thresholds from 3 to 9 months in either sex at 8, 16, and 32 kHz or loss of sensory hair cells. However, compared to WT2 and 3-month-old FAD2 mice, 9-month-old FAD2 mice showed a significant elevation of thresholds at all frequencies with wide variability between individuals, and 80% of females suffering ≥ 55 dB SPL compared to 40% of their male

littermates. Importantly, all FAD2 mice showed amyloid plaques in brain tissues with strong staining in the hippocampus subiculum region and sensory and association cortices, without deposition in the cochlea. Additionally, wave I amplitudes of WT2 were unchanged, but those of FAD2 at 3 months were significantly higher than WT2 mice, and were reduced by 9 months, at which time FAD2 males were approaching WT2, and FAD2 females were significantly lower than WT2 mice. FAD2 females showed longer wave I latencies and increased wave IV:I amplitude ratios at 9 months. There were no differences in ABR wave IV amplitudes or the wave I-IV inter-peak latencies between WT2 and FAD2 mice. Only 4 out of 25 FAD2 mice displayed outer hair cell loss at 9 months.
Conclusions: Our results demonstrate that FAD2 mice have significantly decreased hearing sensitivity at 9 months, with females showing more severe deficits than males. Amyloid deposition in the brain does not correlate with the severity of hearing impairment.

T111. Open Board

T112. Gap Detection Ability Declines in an Early Stages of Age-Related Hearing Impairment

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Category: Aging
Background: Age-related hearing impairment (ARHI) is often associated with decreased auditory temporal resolution and is thought to be involved in cochlear synaptopathy. However, an examination to detect the early manifestations of ARHI due to cochlear synaptopathy has not yet been established. The gap detection test has been reported to be a behavioral examination for evaluating temporal auditory processing. In this study, we aimed to investigate whether gap detection ability declines in the early stages of ARHI, and its usefulness in detecting age-related cochlear synaptopathy.
Methods: Young-aged (1-month old) and middle-aged (1-year old) CBA/J male mice were used. Auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE) were measured as auditory function. Then, the ASR, prepulse inhibition (PPI) of ASR, and gap prepulse inhibition of ASR (GPIAS) were investigated to evaluate auditory processing performance and tinnitus perception. Finally, cochleae were examined to confirm cochlear pathology including the loss of hair cells (HCs), spiral ganglion neurons (SGNs), and their synapses, and transverse sections of the brainstem and brain, through the cochlear nucleus (CN) to the auditory cortex (AC), were examined for the expression of synaptic markers.
Results: Although hearing thresholds in middle-aged mice were not significantly different from those in young mice, the amplitude of ABR wave I was significantly decreased in middle-aged mice, and the relative ABR amplitude from waves 2 and 5 to wave 1 was significantly increased. Next, regarding the gap detection ability, a significant increase in gap detection thresholds was observed in middle-aged mice compared to young mice. Histological assessment revealed that cochlear synapses were significantly deteriorated in middle-aged mice, and their degeneration was correlated with an increase in gap detection thresholds. In the cochlear nucleus and auditory cortex, the inhibitory synaptic expression of GAD65 and the expression of parvalbumin, a GABAergic inhibitory interneuron marker, were significantly decreased in middle-aged mice, consistent with central auditory degeneration.
Conclusions: In middle-aged mice, in which hearing thresholds were not elevated, cochlear synaptic dysfunction, central hyperactivity, and decline in gap detection ability were observed, suggesting that evaluating gap detection performance may enable the identification of decreased auditory temporal resolution in the early stages of ARHI, which is involved in both peripheral and central synaptic degeneration.

T113. Abnormal Cholesterol Metabolism and Lysosomal Dysfunction Induce Age-Related Hearing Loss by Inhibiting mTORC1-TFEB-Dependent Autophagy

Seongjun So*[1], Jungho Ha[1], Yun Yeong Lee[1], Young Sun Kim[1], Sivasubramanian Ramani[1], Siung Sung[1], Jeong Hyeon Yun[1], Miyoung Lee[1], Oak-Sung Choo[2], Jeong Hun Jang[1], Yun Hoon Choung[1]

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Category: Aging

Background: Cholesterol is a risk factor for age-related hearing loss (ARHL). However, the effect of cholesterol on the organ of Corti during the onset of ARHL is unclear.

Methods: CBA/J male mice (age, 6 months; n=20) were maintained under standard animal house conditions. Auditory thresholds were measured by ABR (8, 16, 32 kHz) at pre-6 months and post-24 months. We established ARHL at 24 months. The 6 months male mice (n=20) were used for young control. Then, mice were sacrificed and analyzed by histology: transmission electron microscopy (TEM), H and E, Sudan Black B (SBB) and Filipin staining and biochemical assay: IHC, enzymatic analysis and immunoblot. In addition, prepared mRNA from young and aged cochlea were performed RNA Seq (Illumina). The in vitro studies using the HEI-OC1 cells were prepared by treating with low dose Doxorubicin (DOXO, 100 ng/mL) or cholesterol (1 mM).

Results: RNA-sequencing showed a positive correlation with increased expression of genes related to metabolic diseases, cholesterol homeostasis, and target of rapamycin complex 1 (mTORC1) signaling in the ARHL group, as compared to the younger group. In addition, ARHL tissues exhibited increased cholesterol and lipofuscin aggregates in the organ of Corti, lateral walls, and spiral ganglion neurons. Autophagic flux was inhibited by the accumulation of damaged lysosomes and autolysosomes. Subsequently, we observed a decrease in the level of transcription factor EB (TFEB) protein, which regulates lysosomal biosynthesis and autophagy, together with increased mTORC1 activity in ARHL tissues. These changes in TFEB and mTORC1 expression were observed in a cholesterol-dependent manner. Treatment of ARHL mice with atorvastatin, a cholesterol synthesis inhibitor, delayed hearing loss by reducing the cholesterol level and maintaining lysosomal function and autophagy by inhibiting mTORC1 and activating TFEB. The above findings were confirmed using stress-induced premature senescent House Ear Institute organ of Corti 1 (HEI-OC1) cells.

Conclusions: The findings implicate cholesterol in the pathogenesis of ARHL. We propose that atorvastatin could prevent ARHL by maintaining lysosomal function and autophagy by inhibiting mTORC1 and activating TFEB during the aging process.

T114. Neural Correlates of Temporal Processing Improve Following Treatment With Aldosterone: A Behavioral and Neurophysiological Approach

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[1]*University of South Florida*

Category: Aging

Background: Age-related hearing loss (ARHL), 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. The cumulative degradation of auditory sensitivity and perceptual aptitude leads to severe declines in workplace productivity, quality of life, cognition, and communication abilities. Aldosterone (ALD) is a mineralocorticoid hormone secreted by the adrenal cortex and plays a role in the maintenance of critical ion pumps, including the Na-K+-Cl co-transporter 1 (NKCC1), which is essential in the homeostatic maintenance of the endocochlear potential. ALD levels decline with age and appear to be associated with the degree of hearing loss both in humans and in animal models. Here, we utilized the CBA/CaJ mouse model and investigated the therapeutic effect of long-term systemic treatment of ALD on neural response properties and temporal coding in the inferior colliculus (IC), a major midbrain convergence site critical for processing complex sounds such as speech.

Methods: To assess the effects of ALD on sound-evoked activity in the IC, multi-channel arrays were placed in the IC central nucleus and neural activity was acquired from twelve 16-month-old CBA/CaJ mice which were used as either a control or administered a 120-day slow release (1.67 μg ALD/day) subcutaneous pellet. The IC was mapped and receptive fields were acquired to determine the best frequency at which each unit responded to and its minimum threshold. Recording sites were characterized into low, mid, or high frequency regions based off IC tonotopy. Temporal coding was assessed via a gap-in-noise (GiN) paradigm, where minimum gap detection thresholds and temporal response patterns were measured. The GiN stimulus consisted of a broadband carrier noise with silent gaps embedded within it at various durations. Behavioral

gap detection was measured using the gap-prepulse inhibition of the acoustic startle response (GPIAS) using the GiN paradigm with the addition of a startle elicitor. Auditory brainstem response (ABR) audiograms were used to assess peripheral sensitivity.
Results: ALD treatment resulted in significantly reduced minimum gap thresholds, indicating improved neural correlates of temporal processing. Following ALD treatment, receptive field thresholds were significantly improved across all frequency regions. Tuning properties were also affected, as receptive fields were broadened. An improvement in GPIAS properties were also observed in ALD mice, along with stable hearing thresholds over time relative to the control indexed by ABRs.
Conclusions: Changes in mineralocorticoid hormone receptor (MCR) expression may be a contributing factor to age-related central auditory processing deficits. These results are the first to indicate a role of MCR in the neural correlates of temporal processing. The results point to a potential target for future therapeutic interventions to prevent or slow the progression of ARHL.

T115. Loss of POU4f3 From Adult Vestibular Hair Cells Leads to Reduced Vestibular Function and Hair Cell Death

Kendra Stansak[1], Tianwen Chen[1], Tian Wang[2], Caroline Nall[1], Tierah Macon[1], Wu Zhou[1], Hong Zhu[1], Alan Cheng[2], Brandon Cox[3], Brad Walters*[1]

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Category: Aging
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 among aged individuals. 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 whether it is necessary for maintenance of vestibular hair cell health during adulthood and aging.
Methods: To test whether POU4F3 changes during aging, vestibular organs from mice and from human donors were immunolabeled with antibodies against POU4F3, SOX2, and MYO7A. In mice, Pou4f3 was conditionally deleted from type I or type II hair cells in 8-week old male and female mice using Atoh1-CreER:Pou4f3loxP/loxP and Fbxo2-CreER; Pou4f3loxP/loxP mouse models, respectively. Vestibuloocular reflexes were recorded at either 6 weeks or 6 months after tamoxifen induction. Tissue was collected and POU4F3 expression, stereocilia bundles (phalloidin), and hair cell numbers (MYO7A) by type (SOX2) were examined in utricles, anterior cristae, and horizontal cristae.
Results: In human samples, a number of MYO7A+ vestibular hair cells lacked POU4F3 immunoreactivity at all ages investigated. Quantification of POU4F3-; MYO7A+ hair cells across age in wildtype mice suggested moderate loss of POU4F3 from cristae hair cells with age. VOR assessments showed that conditional deletion of Pou4f3 from either type I or type II hair cells led to deficits primarily in rotational performance. VOR performance largely recovered in Atoh1-CreER:Pou4f3loxP/loxP conditional knockout mice 6 months after induction, however when the VOR was conducted in the dark, deficits again became apparent. Atoh1-CreER:Pou4f3loxP/loxP conditional knockout mice exhibited decreased numbers of phalloidin labeled stereocilia bundles and MYO7A+ hair cells in the horizontal and anterior cristae.
Conclusions: The data suggest that Pou4f3 plays a role in vestibular hair cell survival in adult mammals, and that loss of Pou4f3 from either type I or type II hair cells can lead to reduced vestibular function, bundle degeneration, and impaired hair cell survival particularly in the cristae. Studies are ongoing to examine potential sex differences and to test whether Pou4f3 overexpression can promote hair cell survival and retention of vestibular function with advanced age.

T116. Understanding Cochlear Implants Using Machine Learning

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Category: Auditory Prostheses

Background: Current cochlear implants (CIs) fail to restore fully normal auditory perception in individuals with sensorineural deafness. Several factors may limit CI outcomes, including suboptimal algorithms for converting sound into electrical stimulation, plasticity limitations of the central auditory system, and auditory nerve degeneration. Models that can predict the information that can be derived from CI stimulation could help clarify the role of these different factors and guide development of better stimulation strategies.

Methods: We investigated models of CI-mediated hearing based on deep artificial neural networks, which have recently been shown to reproduce aspects of normal hearing behavior and hierarchical organization in the auditory system. To model normal auditory perception, we trained a deep neural network to perform real-world auditory tasks (word recognition and sound localization) using simulated auditory nerve input from an intact cochlea. We modeled CI hearing by testing this same trained network on simulated auditory nerve responses to CI stimulation. To simulate the possible consequences of learning to hear through a CI, we retrained this network on CI input. Further, to model the possibility that only part of the auditory system exhibits this plasticity, in some models we retrained only the late stages of the network.

Results: When the entire network was reoptimized for CI input, the model exhibited speech intelligibility scores significantly better than typical CI users. Speech recognition on par with typical CI users was achieved only when just the late stages of the models were reoptimized. However, for sound localization, model performance remained abnormal relative to normal hearing even when the entire network was reoptimized for CI input.

Conclusions: Overall, this work provides initial validation of machine-learning-based models of CI-mediated perception. Our results help clarify the interplay of impoverished peripheral representation from CI stimulation and incomplete central plasticity in limiting CI user performance of realistic auditory tasks.

T117. Machine Learning and Cochlear Implant Performance Outcome Predictions: A Systematic Review

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Category: Auditory Prostheses

Background: Cochlear implant (CI) user speech and sound perception outcomes are difficult to predict due to the variability inherent in CI user demographics, device characteristics, and prior linguistic and listening experience. Robust and accurate predictions of CI user performance outcomes are valuable because they can inform providers and patients about candidacy, treatment optimization, and likely outcomes. Machine learning (ML) techniques are uniquely poised for this predictive challenge because they can analyze non-linear interactions using an immense amount of multi-dimensional data. Due to the recent surge in the utilization of ML methods, we systematically reviewed the breadth and efficacy of current ML models for predicting CI performance outcomes.

Methods: We conducted a systematic literature search with Scopus, Web of Science, EMBASE, MEDLINE, CINAHL, and CENTRAL from the date of inception through July 2023. We included studies with ML models that predict a CI outcome and excluded those that involve patients with hearing aids (HA). Using PRISMA guidelines, we extracted data from 15 included studies with a total of 4873 pediatric and adult participants (sample size 4 - 2489). Risk of bias was assessed using the NIH Quality Assessment tool and Prediction model Risk Of Bias Assessment Tool (PROBAST).

Results: Fifteen out of 1174 studies met inclusion criteria. Studies predicted heterogeneous outcome measures including sound perception (8 studies), speech production (3), summative auditory indices (3), cochlear nerve function (1), social deficit scores (1), and quality of life measures (1). While the number and types of features used to build ML models varied extensively, implantation age (9 studies), sex (7), speech reception thresholds (5), pre-operative HA use (4), and duration of deafness (4) were most frequent. Commonly used models were tree-based methods (7 studies), support vector machines (6), k-nearest neighbor (3), logistic regression (3), and neural networks (2). Various statistical measures evaluated model performance, however for the 11 studies reporting classification accuracy, the median accuracy was 90.83% (range 76 - 98%). For model validation, studies employed k-fold cross-validation (11), train/test split (4), and validation with outside hospital datasets

(2). Common variables driving model performance were implantation age (3 studies), pre-operative audiological measures (3), CI wear time (2), pre-operative HA use (2), and duration of deafness (2).
Conclusions: CI outcome predictions using ML models demonstrate high predictive performance and elucidate novel factors that contribute to CI speech performance, sound perception, and quality of life outcomes. While many models showed high accuracy and other favorable evaluation statistics, the majority were not adequately reported with regard to dataset characteristics and model creation. This suggests the need for greater clarity and standardization in reporting outcomes, with the ultimate hope that the iterative improvement of these models can allow for their use as a powerful clinical tool.

T118. Predicting Cochlear Implant Outcomes Using Resting-State Functional Near-Infrared Spectroscopy Recordings

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Category: Auditory Prostheses
Background: Cochlear implants (CIs) have greatly improved hearing restoration for individuals with severe or profound hearing loss. However, a considerable variability in CI outcomes remains unexplained by subject-specific factors such as age and deafness duration. This study investigates the use of resting-state functional near-infrared spectroscopy (fNIRS) recordings to predict speech understanding outcomes before and after CI implantation. Our hypothesis is that resting-state functional connectivity reflects brain plasticity following hearing loss and implantation, and that the average clustering coefficient in brain functional connectivity networks can capture this variation in CI users.
Methods: Twenty-two cochlear implant candidates participated in this study. Resting-state fNIRS data were collected pre-implantation and at one month post-implantation. Speech understanding performance was assessed using BKB sentences in noise. Resting-state functional connectivity (rsFC) networks were constructed using regularized partial correlation, and the average clustering coefficient was measured in the signed weighted networks as the predictive measure for implantation outcomes.
Results: We found a significant correlation (p less than 0.01) between the average clustering coefficient in resting-state functional networks and speech understanding outcomes. Importantly, our analysis reveals that this measure provides unique information not accounted for by subject-specific factors like age and duration of deafness.
Conclusions: Our proposed approach uses an easy-to-setup resting state functional brain imaging metric to predict speech understanding outcomes in implant recipients. The results show that the average clustering coefficient, both pre and post implantation, correlate with speech understanding outcomes.

T119. Temporal Acuity of Electric Hearing Enhanced by a Chronic Penetrating Auditory-Nerve Electrode

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Category: Auditory Prostheses
Background: Cochlear-implant (CI) users show only limited sensitivity to the temporal fine structure (TFS) of sounds, including the periodicity that supports perception of pitch in speech and music. In cats, we previously demonstrated that low-frequency brainstem pathways are specialized for transmission of TFS. We evaluated the range over which neurons in the inferior colliculus synchronized to electrical pulse trains at various rates. Conventional CIs that stimulated primarily the basal cochlea produced a limited range of phase locking in the midbrain, whereas an intraneural electrode that penetrated the auditory nerve could activate restricted neural populations from the cochlear apex and elevated the upper limit of phase locking by about an octave. Here, we examine the feasibility of long-term implantation with an intraneural electrode and utilize non-invasive EEG to investigate temporal acuity by selective apical nerve stimulation.

Methods: Cats were deafened bilaterally and implanted in one ear with either an 8-channel CI electrode array or an iridium-wire intraneural electrode. The intraneural electrode targeted cochlear apical fibers exiting the base of the cochlea in the trunk of the auditory nerve. At two-week intervals thereafter, cats were sedated and the electric Auditory Brainstem Response (eABR) and Frequency Following Response (eFFR) were recorded with scalp electrodes. The eABR measured auditory thresholds to single pulses varying in current level. The eFFR measured neural phase locking to unmodulated pulse trains that varied in rate from 43 to 643 pulses per second (pps).
Results: Chronic implantations in four cats are showing stable performance of the intraneural electrode, presently from 4 to 13 weeks post-implantation. eABR thresholds have been consistent or decreased slightly over experimental sessions and are ~7 to 10dB lower than those measured in six CI-implanted cats. eFFR recordings show robust phase-locked responses for both intraneural and CI cats, often up to the highest tested pulse rates. Neural latencies are given by the group delay, computed from the rate of change in eFFR phase lag across pulse rates. Those latencies are consistent with the composite activities of multiple neural generators. Typically, we observe a transition from lower stimulus rates, at which thalamocortical or midbrain sources show relatively long latencies (greater than ~4ms), to higher rates at which only shorter-latency brainstem or auditory nerve responses are present. Importantly, that transition indicates stimulus-synchronized activity at midbrain and/or thalamocortical levels up to ~350-500pps for the intraneural cats compared to less than 280pps for CI cats.
Conclusions: Our ongoing results show the feasibility of chronic implantation with an intraneural electrode in an animal model. The scalp-recorded eFFR permits the non-invasive tracking of temporal processing in electric hearing and, consistent with our previous single-unit studies, suggest that selective stimulation of apical cochlear nerve fibers elevates the rates over which electric TFS can be transmitted to midbrain or higher auditory pathways.

T120. Relationship Between Electrode Position, Electrode Impedance and Electrophysiological Thresholds in an Animal Model of Cochlear Implantation

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Category: Auditory Prostheses
Background: It was earlier reported that the electrode position can influence stimulation thresholds and the spread of excitation (Basta et al., 2010, Hughes, 2006, 2010). However, current models suggest also a correlation between intraoperative electrode impedances or eCAP thresholds and the electrode position. However, the accuracy of this correlation is widely unknown.
Methods: To investigate this in more detail, adult guinea pigs (Dunkin Hartley) were mechanically deafened by a repeated insertion of a clinical cochlear implant electrode just before a unilateral implantation of a guinea pig scala tympani electrode array. Six electrode contacts were inserted into the cochlea. Electrode impedances were determined in the monopolar (MP1) and the common ground (CG) mode just after the electrode insertion. Thresholds of electrically evoked compound action potentials from the auditory nerve (eCAPs) were also determined. After the histological tissue fixation by a perfusion with paraformaldehyde (4%), µCT-scans of all cochleae were performed with an isotropic voxel size of 10.5 µm. The µCT used a high voltage of 70 kVp, a current of 114 µA, and an integration time of 381 ms to minimize electrode artifacts. The position of the electrode contacts in relation to the modiolar wall and the lateral wall was determined to calculate the intra cochlear position index (ICPI) for the basal, medial and apical part of the electrode array.
Results: The following statistically significant results were found. There was a strong positive correlation between the electrode impedances in the medial part measured in the CG-mode and the distance to the modiolar wall (r=0.986). A strong positive correlation could also be found for impedances and the distance to the modiolar wall measured in the MP1-mode at basal (r = 0.829), medial (r = 0.943) and apical (r = 0.886) electrodes. The correlation between the impedances measured in the MP1-mode and the ICPI-values was statistically significant for apical electrodes only (r = 0.928).
Surprisingly, the correlation between eCAP-thresholds and the distance of the electrode contacts to the modiolar wall or the ICPI was not significant.

Conclusions: The results suggest that the position of the electrode could be determined precisely by the measurement of electrode contact impedances in the MP1-mode. eCAP-thresholds seems not to be well suited for the prediction of the cochlear implant electrode position.

This research was funded through a grant provided by Cochlear Research and Development Ltd under reference number IIR-1296.

T121. Frequency Modulated Phase Coding – Patient Performance

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Category: Auditory Prostheses

Background: The auditory system performs a spectral analysis of sound using an array of overlapping auditory filters. The output of each filter contains two forms of information: the relative slow variation in amplitude over time (E, envelop) and the rapid oscillations with the rate close to the center frequency of the filter (TFS, temporal fine structure). E cues alone can lead to high intelligibility for speech in quiet. However, normal hearing subjects take advantage of TFS in noisy listening environments and for music recognition. TFS cues are not considered in most of today's cochlear implant (CI) coding strategies. This study aims to test a novel CI coding strategy that implements TFS information.

Methods: The code: Pre-emphasis balances the frequency content of the recorded audio signal. A gamma tone filter extracts the acoustic information in 12- or 16-frequency bands. Timing (phase) information was derived from the best frequency in each frequency band. Sentence recognition has been tested in all participants with sentences from the Hearing In Noise Test (the noise not played). Loudness was adjusted to "comfort level". A set of 10 sentences was played via the RIB2 or the HRStream research interface to the participant's cochlear implant. The subjects were asked to repeat their understanding and describe the listening experience. The number of correct words was counted.

Results: Ten subjects (9 female and 8 male) using a CI from company A and 7 from company B participated. Their age varied between 34 and 82 years (average 62.7 ± 13.6). The time of CI use ranged between 2 months and 4 years. To habituate to the new code, the subjects were allowed to read along the sentences of one set. Next, the patients were asked to repeat what they heard and to describe the listening experience. When the performance with the novel code and the company A processor is directly compared, the scores with the novel code are, on average, 24.8 percent points lower than those obtained with the company's processing code after years of use. Note that the novel code has only been used for a short time. For company B's code, the data show that 50% of the test subjects reached greater than 80% in their speech recognition scores, one 100% and one 96%.

Conclusions: The advantages of the novel coding strategy include (1) auditory nerve stimulation can be done simultaneously at all contacts; (2) the average repetition rates are 100-300 pulses per second; (3) loudness is encoded by the number of pulses in each channel; (4) timing information is added to the pulse pattern.

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T122. Dexamethasone-Eluting Cochlear Implants Reduce Intracochlear Foreign Body Response and Electrical Impedance for Extended Period Following Surgery in a Murine Model

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Category: Auditory Prostheses

Background: The inflammatory foreign body response (FBR) following cochlear (CI) can negatively impact the outcome of CI including raised electrode impedances. The aim of this study is to investigate the long-term

effect of dexamethasone, a potent anti-inflammatory glucocorticoid on the intracochlear FBR and CI electrical impedance after cochlear implantation in a murine model.
Methods: Using a round window approach, the left ear of 10-12-week-old CX3CR1+/GFP Thy1+/YFP hearing mice on C57B6 background were implanted with a 3-channel CI (dexamethasone-eluting or standard implant). The right ear served as unoperated control. The functionality of the CIs was tested with serial measurement of electrical impedance and neural response telemetry (NRT). Between 7- and 28 days post-CI, 5 hrs/day, and 5 days/week electrical stimulation was delivered with a threshold level of 30 CL below the NRT threshold and comfort level determined by behavioral response. Another group of age-matched CX3CR1+/GFP Thy1+/YFP mice was implanted with standard CI followed by injection of dexamethasone (10mg/kg) in the middle ear to mimic current clinical practice. 10, 28, 56, or 112 days post-operatively, mice were euthanized, implants were explanted and tested for dexamethasone elution with HPLC. Harvested cochleae were fixed with 4% PFA, processed, and sectioned at 30µm thick cryosections parallel to the mid-modiolar plane. Sections labeled with anti-α-Smooth Muscle Actin (α-SMA) antibody were used to quantify intracochlear fibrotic response. Following manual tracing of the outlines of scala tympani, Rosenthal canal, modiolus, and lateral wall for each turn, the volume of each area was measured. The density of nuclei, CX3CR1+ macrophages, and Thy1+ spiral ganglion neurons (SGNs) were calculated. The ratio of the volume of scala tympani and of α-SMA-positive fibrotic tissue was calculated to quantify fibrotic response.
Results: Lowered electrical impedance was observed in the dexamethasone-eluting group. Transimpedance matrix measures showed decreased access and polarization resistance in dexamethasone-eluting groups compared to control groups. HPLC reveals that dexamethasone elution continues until 112 days post-CI. Cochlear implantation resulted in the infiltration of cells, macrophages, and fibrotic tissue into the cochlea without appreciable degeneration of SGNs. Dexamethasone-eluting arrays caused reduced inflammatory foreign body response compared to regular implants: reduced cellular, and macrophage infiltration, and mitigated fibrotic response. Local injection of dexamethasone is partially effective for mitigation of FBR post-CI.
Conclusions: Dexamethasone-eluting electrodes elute the drug and reduce inflammatory foreign body response and electrical impedance for an extended period (112 days post-CI). Local injection of dexamethasone is only partially effective in mitigating the foreign body response suggesting a superior performance of dexamethasone eluting implant compared to the current clinical protocol.

T123. Chirp-Evoked Electrocochleography During Cochlear Implantation

Rachel Scheperle*[1], Rustin Kashani[1], Alexander Claussen[1], Bruce Gantz[1], Marlan Hansen[1]

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Category: Auditory Prostheses
Background: Intraoperative electrocochleography (ECochG) has emerged as a technique to assess peripheral auditory function during hearing preservation cochlear implant (CI) surgery. Multifrequency ECochG has been recommended as one approach to aid in the distinction between traumatic and atraumatic amplitude decrements that can occur when using the apical electrode of a CI array for recording (Saoji et al., 2019; 2023; Skarzynski et al., 2022; Walia et al., 2022). Specifically, decreased response amplitudes at multiple frequencies may be more indicative of trauma than a decrease at a single frequency. Intraoperative ECochG obtained with multifrequency stimuli, therefore, may offer surgeons better feedback during electrode array insertion and possibly improved rates of hearing preservation compared to ECochG obtained with single-frequency stimuli.

One way to evoke a multifrequency ECochG is by interleaving the presentation of multiple tonebursts. Although clicks and chirps are typically used to enhance the compound action potential, these broad-spectrum stimuli can also evoke frequency-following components of the ECochG (Skarzynski et al., 2022), which are more typically used for intraoperative CI monitoring. This report contains preliminary chirp-evoked ECochG recordings during electrode array insertion and hearing preservation outcomes. The aim is to describe insertion track patterns and to evaluate how post-operative outcomes compare to those observed from a single toneburst monitoring method.

Methods: Seven adult patients meeting FDA criteria for CI with preserved residual hearing (i.e., thresholds less than 65 dB HL at 125, 250, and 500 Hz) received intraoperative ECochG monitoring with a suprathreshold chirp stimulus during surgery. Chirps were composed of octave frequencies from 250 to 4000 Hz and were presented with alternating polarity. The apical electrode of the array was used to record the ECochG while the array was inserted with the robotic iotaSOFTTM Insertion System (iotaMotion Inc., Iowa City, IA). A frequency analysis performed on the difference waveforms provided response amplitudes at the harmonics associated with the tonal components of the stimulus. The harmonic amplitudes were monitored real time as an "insertion track" and analyzed post-hoc. Post-operative behavioral hearing thresholds were compared to pre-operative thresholds to quantify hearing preservation. Hearing preservation results from a group of 11 patients monitored with a 500-Hz toneburst will be included for comparison.
Results: Insertion track patterns evoked with chirp stimulation will be described and related to patterns observed with tonebursts. Hearing preservation outcomes for patients undergoing CI surgery with chirp ECochG monitoring will be compared to outcomes for patients monitored with a single 500-Hz toneburst.
Conclusions: The use of ECochG for intraoperative monitoring during electrode array insertion is a promising technique to identify inner ear damage during CI surgery. Improving real-time interpretation is ongoing.

T124. Cochlear Insertion of Neuronal-Based Living Electrodes

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Category: Auditory Prostheses
Background: Cochlear implants are the standard of care for addressing profound hearing loss and are the most prevalent clinically available neural implant. However, their performance is limited by the anatomy of the inner ear and current spread along the length of the cochlear duct. Biologically inspired, neuronally-based 'living electrodes' have been developed for various applications. These consist of neuronal populations cultured on biocompatible scaffolds which allow for directed and controlled axonal outgrowth. The neuronal populations and scaffold properties can be customized to the application of interest and axonal extension is achieved in vitro prior to in vivo implantation. Previous work has described successful cortical and brainstem implantation, however cochlear applications have yet to be explored. Here we present successful insertion of 'living electrode' scaffolding into a human cochlear model.
Methods: Biocompatible constructs were fabricated using 3% agarose molded with glass capillary tubes and acupuncture needles to the desired size (outer diameter: 398 μm; inner diameter: 160 μm) and cut to the desired length. Once formed, microcolumn channels were sterilized under UV light (30 min) and filled with extracellular matrix comprised of rat tail collagen I (1.0 mg/mL) and mouse laminin (1.0 mg/mL). To simulate clinical cochlear duct insertion, custom spirals were designed and 3D printed. The lumen of the artificial cochlear duct was represented by a cylindrical void with a width of 2.63 mm and length of 115.31 mm. The area simulating the round window remained open, and a small well designed to contain and aggregate of spiral ganglion neurons was located at the central portion of the distal end. Translucent material was used for fabrication to allow for visual inspection throughout the insertion.
Results: Blank constructs were inserted in a manner similar to existing clinically inserted cochlear implant electrodes via the simulated round window and monitored for insertion characteristics. Full insertions were achieved without buckling or breaking of the constructs and position was maintained after release of the proximal end of the construct. Constructs could be explanted and reimplanted without apparent damage or change to physical characteristics.
Conclusions: The work described here represents a novel advance in the efforts to improve cochlear implant performance and apply the 'living electrode' concept to the peripheral auditory system. While this work involved blank constructs, their characteristics mimicked those of commercially available traditional electrodes which are currently in clinical use. The similarity in behavior allows for direct clinical translation as they could be placed using current surgical techniques. Further work will involve insertion of constructs following neuronal culture, and axonal damage assays. Finally, the central well of the model will house a spiral ganglion aggregate and synaptic formation will be confirmed histologically and physiologically.

T125. Residual Hearing Mechanics and Fibrotic Burden in a Rodent Model of Cochlear Implantation

George Burwood*[1], Wenxuan He[1], Julia Dietlmeier[2], Teresa Wilson[1], Jordan Hill[1], Rubing Xing[1], Kevin O'Leary[2], Noel O'Connor[2], Lina Reiss[1]

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Category: Auditory Prostheses

Background: Cochlear implantation is a highly successful intervention that has restored hearing function to tens of thousands of people worldwide. The benefit of a cochlear implant can be improved further where any residual, low frequency hearing capacity is preserved and supported with a hearing aid, in the implanted ear. This strategy of electroacoustic stimulation often leads to improved speech in noise intelligibility, and musicality.

Unfortunately, the benefit of this hybrid approach is marred by the gradual worsening of residual hearing thresholds in patients following implantation. Causes such as surgical trauma, foreign body response and disruption of cochlear mechanics are implicated.

In this study, set out to test the hypothesis that basal turn fibrosis/ossification causes residual hearing loss via interference with energy propagation to the apex. We also sought to appraise the utility of optical coherence tomography imaging and vibrometry for direct assessment of residual hearing function in the apex, and fibrosis distribution in the base of the guinea pig cochlea.

Methods: We implanted guinea pigs with both active and inactive cochlear implants. Active implants were electrically stimulated for up to 8 hours a day for 8 weeks. Regular auditory brainstem response testing was conducted throughout the postoperative period. Following the postoperative period, the apical cochlear function of the animals was appraised using optical coherence tomography vibrometry. Cochleae were then harvested, fixed, decalcified and scanned using optical coherence tomography imaging to measure the amount of fibrosis/ossification present in each implanted cochlea. The cochleae were then processed for comparative histology.

Results: We present our preliminary analysis, which to our knowledge represents the first use of optical coherence tomography for imaging and vibrometry of cochlear implanted rodents. We compare these data to one another, and the audiometric data. Additionally we present the output of our attempt to train a convolutional neural network to segment fibrosis in implanted guinea pig cochleae.

Conclusions: The results show that optical coherence tomography imaging is appropriate for non-destructive estimation of cochlear fibrosis/ossification, and that cochlear implantation indeed disrupts the passive inner ear mechanics and energy propagation.

T126. Hearing Loss and Cochlear Implantation in Patients With Usher Syndrome

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Category: Auditory Prostheses

Background: Usher syndrome is a rare genetic disorder and the most common cause for combined deafblindness. Several mutations of genes involved in hair cell development and signal transduction have been identified as triggers for this disease. As these mutations are inherited in an autosomal recessive pattern, this disease could be highly suitable as a future target for gene therapy. Currently, however, treatment options for Usher syndrome are limited as only hearing aids or cochlear implants are available as remedies for hearing loss experienced by affected patients. As of today, reports on outcomes after cochlear implantation (CI) in Usher syndrome often lack high case numbers or genetic testing, which both are crucial to link treatment success with Usher subtype (USH 1-3) and clinical progression.

Methods: Data were retrospectively collected from patients with Usher syndrome as verified by genetic testing. Included parameters consisted of general demographic variables (e.g., sex, comorbidities), disease-specific information (e.g., USH subtype, previous treatments), audiological data (pure-tone and word recognition; with or without hearing aids) and – in case of CI – information on CI surgery (e.g., implant, electrode insertion depth). Subsequently, data were analyzed accordingly to investigate natural progression of hearing loss and therapeutic effect of hearing aids or cochlear implants.

Results: Overall, 31 patients (17 female) with Usher syndrome were identified and included in this study. In this patient cohort, USH type 2 was the most prevalent subtype observed (76%) and a total of 11 patients received either uni- or bilateral cochlear implants. The majority of patients without or before CI had been fitted with hearing aids. Mean (± SD) hearing function with 3-5 years of age was 62.2 ± 13.8 dB SPL and continuously deteriorated with increasing age (21-30 years: 75.7 ± 23.1 dB SPL; 31-40 years: 90.8 ± 25.5 dB SPL). Hearing aids significantly improved hearing thresholds especially at younger age but their benefit diminished with progression of disease in adulthood. On the contrary, CI significantly improved pure-tone hearing levels one year after surgery (p less than 0.0001) and patients using their implant correctly identified 57.5 ± 17.2% of monosyllabic words at 80dB.
Conclusions: In most patients with Usher syndrome, progressive hearing loss is inevitable. Even though hearing aids may alleviate this symptom in children and adolescents, their therapeutic effect gradually decreases in adults. CI constitutes a highly effective treatment option for hearing loss in Usher syndrome by restoring pure-tone hearing and positively affecting speech comprehension as well.

T127. Dexamethasone-Eluting Cochlear Implants Reduce Intracochlear Foreign Body Response Compared to Perioperative Topical Dexamethasone Treatment Following Surgery in a Murine Model

Peter Eckard*[1], Muhammad Rahman[2], Brian Mostaert[2], Bryce Hunger[2], Alexander Claussen[2], Ibrahim Razu[2], Cristina Garcia[2], Shakila Mahmuda Fatima[2], Nashwaan Khan[2], Douglas Bennion[2], Jonathon Kirk[3], Keiko Hirose[4], Marlan Hansen[2]

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Category: Auditory Prostheses
Background: Cochlear implantation (CI) invariably induces a foreign body response (FBR) that contributes to the loss of residual acoustic hearing and diminished effectiveness of the CI. Multiple animal studies have demonstrated that corticosteroid-eluting CIs can mitigate the FBR compared to a standard cochlear implant. Perioperative treatment with dexamethasone is commonly used in clinical practice to potentially reduce the FBR. However, clinical studies have demonstrated variable results in the effectiveness of perioperative dexamethasone. This study aims to explore the effectiveness of perioperative local dexamethasone in reducing the intracochlear inflammatory and foreign body response compared to implantation with a dexamethasone-eluting CI or standard CI in a murine model.
Methods: The left ears of 12-week-old CX3CR1+/GFP Thy1+/YFP C57BL6 mice were implanted using a round window approach with a dexamethasone-eluting CI or standard CI. Another group was implanted with a standard CI and treated with 10mg/kg of perioperative dexamethasone sodium phosphate injected into the middle ear. The contralateral ears were unimplanted controls. The functionality of the CIs was tested with serial measurements of electrical impedance and neural response telemetry. The implants were then stimulated between 7- and 28-days post-implantation. At 10- and 28-days post-implantation, the mice were euthanized, and the implants were explanted. The cochleae were fixed in 4% PFA and cryosectioned in 30µm mid-modiolar sections. Macrophages and neurons were intrinsically labeled. Sections were labeled with antibodies against MHCII and α-Smooth Muscle Actin (α-SMA), and nuclei were labeled with DAPI. Images were taken with confocal microscopy and analyzed using IMARIS software. The cochlear regions of the scala tympani, Rosenthal's canal, lateral wall, and modiolus were manually traced, and the volume of each area was measured. The cellular, CX3CR1+ macrophage, Thy1+ spiral ganglion neuron, and MHCII+CX3CR1+ macrophage densities were measured for all areas. The ratio of the α-SMA volume to the scala tympani volume measured to quantify the fibrotic response.
Results: Cochleae implanted with standard CIs develop a robust FBR characterized by macrophage, fibrotic tissue, and cellular infiltration. Dexamethasone-eluting CI arrays reduce the inflammatory and FBR compared to standard implants. Cochleae implanted with standard CIs and treated with perioperative topical dexamethasone partially reduce the FBR with decreased cellular infiltration, macrophage infiltration, and fibrotic response. Cochleae implanted with standard CIs treated with perioperative topical dexamethasone develop a more robust FBR with increased cellular infiltration, macrophage infiltration, and scala tympani fibrosis compared to dexamethasone-eluting CIs at 10- and 28-days post-implantation.

Conclusions: Perioperative treatment with local dexamethasone partially reduces post-implantation FBR compared to implantation with a standard CI. However, perioperative local dexamethasone treatment caused a more robust FBR than cochleae implanted with dexamethasone-eluting CIs. These results suggest dexamethasone-eluting CIs decrease the FBR and improve CI performance compared to standard clinical perioperative dexamethasone treatment in a murine model.

T128. Open Board

T129. Functional and Biological Effects of Systemic Dexamethasone After Facial Nerve Crush Injury in Mice

Seong Hoon Bae*[1], Haeng Ran Park[1], Hyunseo Lim[1], Young-Min Hyun[1]

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Category: Other, Facial nerve

Background: Corticosteroid therapy is commonly recommended for facial nerve weakness; however, its effectiveness in treating traumatic nerve injuries remains controversial. This study investigated the functional recovery and cellular effects of systemic dexamethasone administration after facial nerve injury.

Methods: Wild-type and CX3CR1(GFP/+) transgenic C57BL/6 mice were assigned to two groups by intraperitoneal injection: the phosphate-buffered saline group and the dexamethasone group. Facial nerve crush injury was induced, followed by the functional grading of recovery. Cellular effects were investigated using transmission electron microscopy, flow cytometry, immunofluorescence, and intravital imaging.

Results: The administration of systemic dexamethasone slightly delayed the functional recovery of the facial nerve compared to the PBS group; however, the morphological changes in the nerve were not significantly different between the two groups at 14 days post-injury. Macrophage infiltration into the facial nerves was significantly inhibited by systemic dexamethasone administration. However, myelin basic protein levels, which indicate the clearance of myelin debris, did not differ between the groups.

Conclusions: In summary, systemic dexamethasone successfully inhibited leukocyte infiltration; however, functional recovery was slightly delayed after crushing injury, without significant differences in morphology and myelin clearance. Clinically, these findings do not support the use of steroid pulse therapy for the treatment of traumatic facial nerve injuries.

T130. Comparative Study of Tympanoplasty Type I Using Fibrous Layer Versus Chondrogenic Layer of Tragal Cartilage

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Category: Clinical Otolaryngology and Pathology

Background: Chronic otitis media is a pathological condition characterized by the enduring presence of a perforated tympanic membrane accompanied by recurrent or persistent mucopurulent otorrhea. Tympanoplasty, a surgical procedure aimed at repairing the tympanic membrane, has utilized various grafting materials and techniques. This study's objective is to conduct a comparative analysis of the outcomes of tympanoplasty using tragal perichondrium.

Methods: This retrospective study encompassed patients who underwent Type I tympanoplasty utilizing tragal perichondrium as a graft material within the timeframe spanning from March 2016 to July 2022 at Samsung Changwon Hospital. Patients were categorized into two distinct groups: Group A, wherein the fibrous layer of the tragal perichondrium was oriented outward, and Group B, where the chondrogenic layer was facing outward. A comprehensive one-year follow-up was conducted, and an extensive comparative analysis was performed. Parameters examined included age, gender distribution, surgical site, disease progression timeline, preoperative air-bone gap (ABG), surgical duration, audiometric improvements, wound healing efficacy, and the incidence of postoperative complications.

Results: There were no significant differences between two groups in other parameters. However, the postoperative myringitis was more frequent when the chondrogenic layer of tragal perichondrium oriented outward.
Conclusions: When conducting tympanoplasty with tragal perichondrium, orienting the fibrous layer, which constitutes the outermost layer, outward can lead to a reduction in the incidence of postoperative myringitis.

T131. Association Between Fatty Liver Disease and Hearing Impairment in Korean Adults

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Category: Clinical Otolaryngology and Pathology
Background: We hypothesized that fatty liver disease (FLD) may be associated with a high prevalence of hearing loss (HL) owing to metabolic disturbance. We aimed to evaluate the association between FLD and HL using a large sample of the Korean population.
Methods: Our study used a dataset from adults who underwent voluntary routine health checkups (n = 21,316). Fatty liver index (FLI) was calculated using Bedogni's equation. Patients were divided into two groups: the non-FLD (NFLD) group (n = 18,518, FLI less than 60) and the FLD group (n = 2,798, FLI ≥ 60). The hearing thresholds were measured using an automatic audiometer. The average hearing threshold (AHT) was calculated as the pure-tone average at the four frequencies (0.5, 1, 2, and 3 kHz). HL was defined as AHT greater than 40 dB.
Results: HL was observed in 1,370 (7.4%) and 238 (8.5%) patients in the NFLD and FLD groups, respectively (P = 0.041). Compared with the NFLD group, odds ratio for HL in the FLD group was 1.16 (P = 0.040) and 1.46 (P less than 0.001) in univariate and multivariate logistic regression analyses, respectively. Linear regression analyses revealed that FLI was positively associated with AHT in both univariate and multivariate analyses. Analyses using a propensity score-matched cohort showed similar trends compared to those using the total cohort.
Conclusions: FLD or FLI was associated with poor thresholds and HL. Therefore, active monitoring for hearing impairment in patients with FLD may be helpful for early diagnosis and treatment of HL in the general population.

T132. Proteomics and Immunophenotyping of Patients With Hearing Instability Disorders

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Category: Clinical Otolaryngology and Pathology
Background: Hearing instability (HI) disorders are characterized by hearing fluctuation or sudden changes in hearing thresholds that can be associated with vertigo, poor speech recognition, endolymphatic hydrops, or a sensation of fullness in the ear. Although the immune system has been implicated in some HI disorders, the pathogenesis of hearing instability disorders is poorly understood and contributes to the challenge of diagnostic imprecision and inadequate treatment options for hearing loss. We therefore sought to investigate the molecular mechanisms of HI pathogenesis by studying the proteome and immune profile of patients with hearing instability disorders.
Methods: At the NIH Clinical Center, under a longitudinal deep phenotyping clinical protocol, patients with hearing instability disorders were recruited. The patients were grouped based on the presence of MRI proven endolymphatic hydrops (EH). Plasma and peripheral blood mononuclear cells (PBMCs) were isolated from the patient’s blood samples. Plasma samples and supernatants from the PBMC cultures were used for cytokine and proteomic analyses while PBMCs were analyzed using a 40-fluorescent marker panel full spectrum flow cytometry (FSFC).

Results: Our cytokine analyses implicated inflammation in some HI patients who exhibited an elevated level of proinflammatory cytokines. Interestingly, increased levels of Th17 cytokines were observed in some EH-HI patients, suggesting autoimmunity in these patients. Furthermore, monocytes, natural killer cells, and CD8+ T-cells profiles on FSFC were found to be significantly different between hydrops and non-hydrops patients. Finally, analysis of the proteomics data has identified a list of potential proteins that can be explored as biomarkers for HI disorders.
Conclusions: Preliminary analyses suggest that patients with HI disorders have distinct immune profile characteristics which can facilitate identification of potential diagnostic and therapeutic targets. Ongoing patient recruitment and longitudinal assessment of patients with HI disorders with multiple deep phenotyping measures including FSFC, scRNA-Seq, and proteomic profiling may build support for the involvement of the immune system in these poorly understood disorders.

T133. Preliminary Study of an Adaptive Algorithm for Efficient Estimation of Auditory Brainstem Response Threshold in Humans

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Category: Clinical Otolaryngology and Pathology
Background: The Auditory Brainstem Response (ABR) is a common tool for evaluating hearing sensitivity in animals and humans who cannot respond to behavioral exams. However, the measurements require a considerable amount of time which may waste laboratory resources and divert clinicians from other tasks. Previously, we developed an adaptive algorithm for efficient and objective ABR threshold estimation, which was validated through simulation experiments using previously collected animal ABR data. Here, we seek to evaluate the feasibility of applying this algorithm to humans
Methods: The threshold is estimated using a model trained on a small number of measured waveforms at different frequencies and levels. The algorithm begins with testing a short, pre-defined list of stimuli, which are presented and ABR waveforms recorded, followed by stimuli iteratively optimized by an algorithm based on modeling the ABR waveform as a Gaussian Process. This iterative optimization process is repeated until a termination criterion is achieved.

The adaptive algorithm was validated on a cohort of 8 young adults. First, baseline ABR data was measured using a pre-defined list of stimuli consisting of tone-bursts at 0.5, 1.0, 2.0, and 4.0 kHz ranging from 10-90 dB SPL in 10 dB increments. Next, two runs of the adaptive algorithm are performed, utilizing a subset of the stimuli list, although the model could be modified to choose stimuli at any frequency or level within the range. The adaptive algorithm is run until a fixed number (20) of stimuli have been presented. Baseline data and the two adaptive runs all occur on separate days.

Results: The adaptive algorithm is validated for test/retest reliability where the threshold estimates of the two adaptive runs are compared. The mean absolute difference is 10 dB or less for 5 out of 8 ears. Of the other ears, discrepancies between threshold estimates of the two adaptive runs vary for varied reasons. In one case, a significant neural response with a latency around 15 ms appears to have destabilized the model predicted waveforms. In another, significant differences in ABR waveforms are observed across the two sessions. The algorithm accurately captures those differences, leading to different threshold estimates. In the third ear, threshold estimates from the two adaptive runs are close for 3 out of 4 frequencies, while a large discrepancy at the 4th frequency causes a large mean absolute error.

Although the adaptive algorithm was run up to a fixed number of stimuli, the interim threshold estimates are typically within 10 dB (the minimum increment utilized in this study) of the final estimate with fewer stimuli (mean: 11.8; standard deviation: 3.5).

Conclusions: The current experiment indicates that it may be feasible to use a Bayesian adaptive algorithm for rapid ABR threshold estimation in human adults.

T134. Intractable Chronic Otitis Media as First Manifestation of ANCA Associated Vasculitis

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Category: Clinical Otolaryngology and Pathology

Background: We have seen patients who presented with chronic otitis media that was not controlled by ordinary medical treatments. We found that these patients developed otitis media as the first symptom of ANCA associated vasculitis (AAV), and had their symptoms controlled only by the use of immunosuppressant drugs. So, the aim of the present study was the investigate the clinical manifestation, diagnosis and treatment outcome of intractable chronic otitis media as first manifestation of AAV.

Methods: We retrospectively reviewed the medical record of patient with intractable chronic otitis media as first manifestation of AAV treated in the Department of Otolaryngology, Head and Neck Surgery, School of Medicine, Kyungpook National University, between 2007 and 2023. Patients who were followed up for more than 12 months were included in this study.

Results: Ten patients were enrolled in this study. They were characterized as follows: (1) first manifestation of disease is intractable chronic otitis media, which did not respond to ordinary medical treatments such as antibiotics, steroid alone and ventilation tube, followed by progressive hearing loss. Hearing outcome after treatment with a combination of corticosteroids and immunosuppressant therapy was classified CR(30%, n=3), PR(60%, n=6) and NR(10%,n=1).; (2) predominantly female (80%, n=8) and older (median age, 63 years); (3) predominantly myeloperoxidase (MPO)-ANCA positive (80%, n=8), followed by proteinase3(PR3)-ANCA positive (20%,n=2); (4) frequently observed accompanying facial palsy (60%, n=6). The average time from the onset of otitis media to the development of facial palsy is 3.7 months, and the degree of facial palsy was almost HB grade III-IV. Two patients developed bilateral facial palsy, and four patients developed unilateral facial palsy, and the side with facial palsy was consistent with the side with worse bone hearing level in all patients with bilateral otitis media. The last HB grade for all patients was either grade I or II within 1-2 months after starting treatment with a combination of corticosteroids and immunosuppressant therapy. There were no cases in which palsy relapse; (5) disease often involving lung (30%, n=3) and kidney (10%, n=1) lesion.

Conclusions: This study revealed that intractable chronic otitis media as first manifestation of AAV is a disease that initially occurs in the middle ear and subsequently spreads to other organs such as the lungs and kidneys, with eventual involvement of all body organs. Severe otolgic sequelae such as facial palsy, complete deafness can also occur. If prompt diagnosis and taking immunosuppressants, facial palsy and hearing loss can be reversed. When encountering the patients with intractable chronic otitis media, appropriate examination including ANCA titer should be performed and treated with immunosuppressants as soon as possible.

T135. From the Lab to the Clinic: Using fNIRS to Accelerate Early Intervention for Infants With Hearing Loss

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Category: Clinical Otolaryngology and Pathology

Background: Functional near infra-red spectroscopy (fNIRS) is a child-friendly light-based neuroimaging technology, that is well suited to application in audiology. The cortically derived haemodynamic responses can be evoked by acoustic or electric stimulation of the auditory system, while listening via hearing aids or with cochlear implants and uses ecologically valid speech signals. Our team has developed fNIRS tests that objectively assess sound detection and speech discrimination, addressing critical audiological questions. These include the need for hearing aids in infants with mild hearing loss or auditory neuropathy, the sufficiency of hearing aids for speech and language development, and the optimal programming of cochlear implants.

Methods: We tested infants aged 5-22 months who had varying degrees of sensorineural hearing loss (mild through to profound) or auditory neuropathy. We measured haemodynamic responses from bilateral pre-frontal and temporal regions while they slept, either with and/or without hearing aids. Our two test protocols, optimised for clinical application, measured sound detection at different levels (35-80 dBSPL) and speech

discrimination of different consonant-vowel tokens (e.g., “ba” vs “bee”). The fNIRS responses were analysed using our proprietary EarGenie® automated detection algorithm and by studying the epoched waveforms.

Results: The results from these case studies illustrate how fNIRS testing can contribute valuable information to key audiological decisions along the care pathway. It supported the provision of hearing aids for infants with mild hearing loss and auditory neuropathy, as their aided responses were larger and showed more normal morphology compared to their unaided responses. It also revealed the impact of noise in an infant with profound unilateral hearing loss, affecting both response amplitude and morphology. For infants with moderate to profound sensorineural hearing loss, the results confirmed that hearing aids enabled detection of conversational speech and discrimination of speech sounds. In one case, an infant with severe/profound hearing loss who was a regular hearing aid user, showed aided responses with normal morphology and amplitude that more than doubled over a period of 4 months.

Conclusions: fNIRS is a promising brain imaging technology ready for clinical translation. Our research has demonstrated how our tests provide additional, objective information to support evidence-based decisions in audiological care. These cases highlight the value of objective test information in helping families understand their baby's hearing abilities and participate in clinical decision-making. We are currently conducting a clinical trial using our prototype device, EarGenie® Minimum Viable Product (MVP), to further demonstrate the value of fNIRS testing in clinical decision-making.

T136. Risk of Otologic Symptom Onset Following mRNA COVID-19 Vaccination: An Institutional Cohort Study With High-Dimensionality Propensity Score Matching

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Category: Clinical Otolaryngology and Pathology

Background: The primary objective of this study was to evaluate for the onset of otologic symptoms in older individuals who received mRNA vaccination against SARS-CoV-2 relative to a control group who received influenza vaccination.

Methods: We conducted a population-based retrospective cohort study using electronic health records collected during routine care at Stanford Health Care (SHC) on adult patients aged 50 and older with no prior otologic diagnoses. The intervention group included patients who received an mRNA COVID vaccine between December 2020 and January 2022 (n=30,434). The non-overlapping control group included patients who received an influenza vaccine at Stanford Health Care in the pre-pandemic years of 2016-2019 (n=42,859). The outcome measures were defined as the first mention of ICD-10 codes for hearing loss, sudden hearing loss, tinnitus, dizziness, vertigo, aural fullness, and otalgia in the medical record in the six months following vaccine administration for the two groups. A comparison group of patients was chosen from the control group based high-dimensionality propensity score (hdPS) matching using age, sex, Charlson Comorbidity Score, diagnostic codes, procedure codes, medication codes and number of encounters.

Results: The risks of sudden hearing loss (OR 2.5, 95% CI 0.4-26.3) and aural fullness (OR 2.2, 95% CI 1.3-3.7) were higher in the COVID vaccination cohort, and these effects persisted when a history of COVID-19 infection was used as an exclusion factor in a sensitivity analysis (OR 3.0, 95% CI 0.5-30.4; OR 1.9, 95% CI 1.1-3.3). There were no differences in the risks of hearing loss (OR 1.0, 95% CI 0.8-1.2), tinnitus (OR 1.1, 95% CI 0.8-1.5), and otalgia (OR 0.9, 95% CI 0.6-1.4) between the two cohorts. There were reduced risks of dizziness (OR 0.8, 95% CI 0.7-1.0) and vertigo (OR 0.8, 95% CI 0.7-0.9) in the mRNA cohort as compared to the influenza vaccination cohort.

Conclusions: In our single institution study among adults aged 50 and over, currently available mRNA vaccines may be associated with higher risks of sudden hearing loss and aural fullness, and lower risks of dizziness and vertigo compared to the influence vaccine. The small effect sizes and imprecision of the estimates do not allow for definitive conclusions. Further studies are required to verify these findings and elucidate the mechanism behind these possible associations.

T137. Effect of Radiation on Viabilities of Schwann and Squamous Cell Carcinoma Cell Lines

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Category: Other, Cancer Biology

Background: Broadly defined, perineural invasion (PNI) is the extension of cancer into or around nerves. When present in head and neck squamous cell carcinoma (HNSCC), PNI is a poor prognostic factor with worse disease-free survival and higher rates of metastasis and recurrence. In addition, PNI can be associated with significant cranial nerve morbidities which, depending on the site of the primary tumor, can lead to dysphagia, loss of taste, facial paralysis, paresthesia, and pain. Though the clinical implications of PNI-positive disease have been well-documented, the molecular mechanisms underlying PNI remain largely unknown. Recent investigations suggest PNI is a dynamic process involving reciprocal signaling between cancer and nervous system cells and is mediated by secreted molecules within the tumor-nerve microenvironment. One treatment that has been shown to clinically improve outcomes for PNI-positive disease is adjuvant radiation therapy. However, the impact of radiation on the tumor-nerve microenvironment is understudied. In our study, we aim to analyze and compare the secretome of irradiated and non-irradiated cancer and Schwann cells.

Methods: Normal Schwann cell lines (HS11 and FHSC), a primary human keratinocyte cell line (HeKa), and an oral tongue SCC cell line (SCC9) were cultured in 6-well plates with a cell seeding density of 100,000 cells per well. Cells were irradiated on day 3 with one fraction of radiation at 0, 2, or 8 Gray. Culture media was harvested on post-radiation day 2 and 3. Subsequently, cytokine arrays were conducted for each experimental condition. Arrays were imaged on a chemiluminescence imager and analyzed using ImageJ software with microarray plug-in.

Results: HS11, FHSC, SCC9, and HeKa cells secreted several cytokines into the tumor condition media. Compared to HeKa cells, SCC9 cells expressed more IL-6, IL-8, RANTES, VEGF, IP-10, and MIP-3-α and expressed less osteoprotegerin, TIMP-1, TIMP-2, MCP-1, MCP-2, MCP-3, and angiogenin. HS11 and FHSC cells expressed high levels of IL-6, IL-8, and MCP-1. When HS11 cells were exposed to higher levels of radiation, IL-6 and MCP-1 expression increased while IL-8 expression decreased. We also observed cytokine changes with radiation using SCC9 and HeKa cells.

Conclusions: Radiation modulates the secretion of several cytokines by normal Schwann cells and SCC9 cells. The changes observed in the secretome may provide insight into the biology of PNI and how adjuvant radiation may halt PNI progression. Ultimately, understanding the molecular crosstalk between cancer and supporting nerve cells will further elucidate the mechanisms underlying PNI and identify novel therapies to suppress PNI.

T138. Effectiveness of Cartilage Conduction Hearing Aids in Patients With Unilateral Hearing Loss

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Category: Clinical Otolaryngology and Pathology

Background: In 2004, Hosoi et al. discovered that applying vibrations containing sound information to the aural cartilage produces a sound that is as clear as that of air or bone conduction 1）. This phenomenon was termed cartilage conduction (CC). CC was subsequently established as the third auditory pathway, following air and bone conductions. A new type of hearing aid using CC (cartilage-conduction hearing aid [CC-HA]) has been developed in Japan (HB-A2CC, RION, Tokyo, Japan) and has been available in clinical practice since 2017.

Methods: Our department measured and evaluated the effectiveness of hearing aids during the fitting of CC-HA and after purchase by measuring the aided sound field thresholds and assessing subjective hearing using a questionnaire.

The department has extensive experience fitting CC-HAs on people with unilateral hearing loss (UHL). This study investigated the effectiveness of CC-HA in patients with unilateral hearing loss. Cartilage conduction hearing aids were fitted in patients between November 2017 and March 2021, using pure tone average (PTA), wearing threshold, and subjective hearing assessment with the Spatial Hearing Questionnaire (SHQ).

Results: The subjects were 17 patients with UHL for whom CC-HA was fitted. The median age was 31 (6–78) years, and the most underlying diseases were aural atresia and ear canal stenosis. The PTA was 76.5 ± 15.1dB and 15.0 ± 11.7dB for air and bone conduction hearing, respectively, in the affected ear. The aided sound field threshold was 49.0 ± 15.0dB, and the mean functional gain was 23.0 ± 7.9dB. The SHQ total score improved from 48.8 ± 9.5 before to 68.1 ± 18.1 after hearing aid use.
Conclusions: Wearing a CC-HA on the affected side of the UHL may improve quality of life by allowing sound information to be received from the affected side, reducing disorientation and difficulty hearing in noise.
1） Hosoi, H. Approach in the Use of Cartilage Conduction Speaker. Japanese Patent 4541111, 17 November 2004.

T139. Sensorineural Hearing Loss as a Complication of Type-2 Diabetes Mellitus: Evidence of Several Cellular and Neural Impairments

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Category: Other, Hearing Loss in Metabolic Disorders: Evaluation in Diabetic Model
Background: While retinopathy, nephropathy, and peripheral neuropathy are well-established complications of type 2 diabetes, sensorineural hearing loss is increasingly recognized as a comorbidity of this metabolic disease. Several causes have been suggested, including cochlear microangiopathy, chronic systemic inflammation, or auditory peripheral neuropathy (synaptopathy), which can lead to early-onset hearing loss. No extensive research has been conducted on preclinical models of type 2 diabetes and a better understanding of the establishment of sensorineural hearing loss (SNHL) would be beneficial for the diabetic population. Our study aims to better characterize the development of SNHL concomitantly with diabetes biomarkers in a diabetic mouse model.
Methods: Genetically modified mice with mutations in the leptin receptor gene (BKS(D)-Leprdb/JOrlRj)2 were monitored from 5 to 13 weeks of age to assess the parameters of their diabetes (blood glucose, glycosylated hemoglobin, biochemical analyses) and their hearing, using auditory evoked potentials and otoacoustic emissions. Terminal histological analyses of the cochlea were performed to visualize hair cells, supporting cells, and synaptic connections with auditory nerve fibers.
Results: In this model, Leprdb/Leprdb mice exhibited a phenotype of obesity and hyperglycemia, with fasting blood glucose levels of ~4 g/L and HbA1c at 5.3%, compared to 2 g/L and 4.2%, respectively, in heterozygous control mice. Blood analyses revealed a significant increase in albumin and monocyte-to-lymphocyte and granulocyte-to-lymphocyte ratios, indicating renal and systemic inflammatory involvement. Diabetic mice displayed early-onset hearing loss characterized by a significant increase of auditory brainstem response (ABR) thresholds and a significant decrease of distortion product otoacoustic emission (DPOAE) amplitudes compared to the Leprdb/+ control mice. These data correlated with morphological and histological changes in hair cells and associated synapses, as well as supporting cell loss at the base of the cochlea.
Conclusions: Here, we present data from a murine model illustrating the detrimental consequences of type 2 diabetes on hearing. This translational preclinical model may be useful for evaluating the efficacy of drug candidates for the preservation and restoration of hearing in type 2 diabetic patients.

T140. Hearing Screening in Private Practice Family Medicine

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Category: Clinical Otolaryngology and Pathology
Background: Hearing loss is a common deficit that remains underdiagnosed. To address this issue, automatic self-hearing tests have been developed. These tools are based on pure tone detection and speech in noise evaluation. The aim of the present study was to evaluate the feasibility of hearing screening of the patients consulting in private practice medicine. More precisely, we evaluated i) the time needed for the medical doctor

to realize the protocol, ii) the pertinence or not to perform both pure tone and speech in noise test, iii) the number of hearing-impaired patients detected and those who consulted an ENT specialist for hearing aids rehabilitation.
Methods: Data were collected in three medical care centers from May to November 2022 using the Sonup application (Sonup company, France, Montpellier). Hearing tests (SoTone and SoNoise) were implemented on Android OS tablet (Galaxy Tab A7) and circumaural headphones (Orosound Tilde Pro) were connected to the tablet via Bluetooth. SoTone is a self-test based on pure tone stimulus, designed to detect hearing losses superior to 20 dB HL (disabling hearing losses as defined by the World Health Organization, 2021). SoNoise is a speech-in-noise test designed to automatically determine the signal to noise ratio at which a participant recognize 50 percent of words in a noisy environment. After a brief explanation of the tests by the medical doctor, the patients were invited to probe their hearing by themself. Patients with suspected hearing impairment were encouraged to consult an ENT doctor. The question whether the patient effectively consult for hearing rehabilitation was evaluated by phoning ~1 to 3 months after the recommendation.
Results: Among the 521 eligible patients, 235 were able to perform the tests. The average time to complete both tests with the explanations was 6 minutes and 14 seconds. The SoTone classified 3 time more hearing-impaired patients (23%) than SoNoise (7%). Consequently, most of the patients who refused ENT consultation for hearing aid prescription were classified as positive with SoTone only, probably because SoTone generated much more false-positive than SoNoise. Finally, among the 235 patients tested, 13 patients (5%) presented a hearing impairment confirmed by the ENT doctor with a classical audiometry. Among them, 8 were fitted with hearing aids afterwards.
Conclusions: Based on our results, the use of SoNoise to detect hearing loss is more accurate and specific than SoTone. Using only SoNoise will be easier, less time consuming for the general practitioner. Hearing screening via applications on tablet may help a large population of general practitioners to screen hearing impaired patients and improve the diagnosis of hearing disorders.

T141. Characterizing Sudden Sensorineural Hearing Loss Outcomes With Associated Socioeconomic Status and Comorbidities Across the SARS-CoV-2 Pandemic

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Category: Clinical Otolaryngology and Pathology
Background: Sudden sensorineural hearing loss (SSHL) is commonly defined as an abrupt loss of hearing in one or both ears due to dysfunction of the inner ear organs. Though the underlying pathophysiology of SSHL is poorly understood, increased incidence rates may be associated with circulating infections. Previous research has suggested temporal patterns and seasonality consistent with viral illnesses, though the unprecedented magnitude and duration of the SARS-CoV-2 pandemic necessitates further investigation. Additionally, a better understanding of underlying factors predictive of SSHL is required, including investigation into social determinants of health (e.g., socioeconomic status and other comorbidities that may predispose individuals to developing SSHL or influence treatment outcomes). Improved understanding of potential risk factors may aid in the development of prophylactic interventions and optimized treatment modalities to minimize incidence as well as lead to better outcomes following SSHL.
Methods: Through a retrospective chart review (#20230698), patients (n=230) with SSHL who underwent intratympanic dexamethasone injections (ITI) were identified at the University of Miami Miller School of Medicine Ear Institute during discrete pre (before pandemic), during (peak restrictions), and post-COVID-19 (following significant decrease in restrictions) timepoints. Each chart was reviewed for demographic information, including postal code, comorbidities (e.g., cardiovascular disease), pertinent social history (e.g., history of smoking), treatment (e.g., intratympanic injection), as well as audiometric outcomes (e.g., change in speech (SRT)/word recognition thresholds (WRS). Postal codes were cross-referenced with National Census data to gather median household income, employment rates, and health insurance coverage. Linear and logistic regression models were generated to examine relationships between demographic factors and comorbidities to hearing outcomes following ITI. Responder status was classified as a change in a decrease in SRT $\geq$10 and/or 15% improvement in WRS.
Results: Overall, 112 males (48.7%) and 118 females (51.3%) with a mean age of 52 ($\pm$12.7) years old were included in this analysis. Following both simple linear regression and logistic regression, we observed no

significant relationship between median household income or percentage of individuals without health insurance to SRT change or responder status following ITI. Logistic regression models between independent comorbidities and responsiveness to steroid treatment yielded mostly insignificant results, with no predictive relationship between endocrine and other diseases at any time point assessed. However, in the Pre-COVID group, otologic disease significantly predicted non-responder status (estimate = -1.61; p = 0.05). Interestingly, in the During COVID sub-cohort, we found that cardiovascular disease predicted positive responder status (estimate = 0.40; p = 0.05). This was also observed in the post-COVID group (estimate = 2.51; p = 0.01).
Conclusions: Social determinants of health and comorbidities, including otologic and cardiovascular diseases, may predict outcomes related to SNHL. More work is needed to understand the role of these factors in the clinical course and recovery of SNHL.

T142. Assessing the Impact of the COVID-19 Pandemic on Risk Factors for Sudden Sensorineural Hearing Loss

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Category: Clinical Otolaryngology and Pathology
Background: Sudden sensorineural hearing loss (SSNHL) refers to a sudden loss of hearing either all at once or over the span of a few days, due to damage to the inner ear, vestibulocochlear nerve, or central hearing processing centers. Most cases are unilateral, with up to 90% of cases being idiopathic, though potential causes include viral infection, autoimmune disease, or circulatory abnormalities. In this work, we sought to characterize changes in the rate of SSNHL at three discrete time points (Pre-, During, and Post-COVID-19 pandemic) and investigate how demographics may predict hearing recovery following intratympanic steroid injections.
Methods: For this retrospective chart review (#20230698), n=249 charts of patients who visited the University of Miami Health System between November 2018 – December 2019 (prior to the COVID-19 pandemic), April 2020 – August 2021 (during COVID-19 restrictions), and February 2022 – February 2023 (following significant relaxation of COVID-19 restrictions) who were at least 18 years old, diagnosed with SSNHL and who received intratympanic steroid injections, had no history of vestibular schwannoma, and who did not receive chemotherapy within 4 weeks of the start of their hearing loss were examined. Data obtained included age, ethnicity, sex, and pre- and post-intratympanic injection audiograms. Logistic regressions were generated to examine relationships between demographics (e.g., age, sex, ethnicity) at the three different time points, with responder status being a primary outcome measure. Responders were defined as patients who had at least a -10 decibel (dB) recovery in speech reception threshold (SRT) following intratympanic steroid injections into the affected ear.
Results: Across all time points examined, our retrospective patient population had a mean age of 51.7 ± 9.3 years and was 51.3% female (48.7% male). Nearly 50% were Hispanic or Latino (44.3% non-Hispanic or Latino; 7% unknown ethnicity), and 84.3% identified as White. Following logistic regression, we observed that younger age was a significant predictor of being a responder following intratympanic injection (β = -0.09, p = .002) prior to the COVID-19 pandemic. Interestingly, we also investigated age vs. post-injection responder status for both During COVID-19 and Post-COVID-19 time points, but the predictor of age was no longer significant at either time point (β = 0.03, p = 0.12 and β = 0.04, p = 0.34, respectively). Across sex, race, and ethnicity, we observed no significant relationships with responder status following intratympanic steroid injection among any time point examined.
Conclusions: Through our retrospective study, we found that younger patients with SSNHL were more likely to benefit from intratympanic steroid injections compared to older patients with SSNHL, though no other predictors were found to influence hearing recovery, including sex, race, or ethnicity.

T143. Auditory and Balance Manifestations in Fabry Disease Patients: A Comprehensive Evaluation in Taiwan

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Category: Clinical Otolaryngology and Pathology

Background: Fabry disease (FD) is a genetic disorder known for its systemic accumulation of globotriaosylceramide (Gb3), leading to multi-organ pathology. While previous literature has underscored its systemic involvement, such as cardiovascular or neurologic implications, the potential auditory and vestibular manifestations impacting patients' quality of life in Taiwan remain under-explored. This study aimed to understand the prevalence and severity of auditory, tinnitus, and balance function abnormalities in FD patients.

Methods: A cross-sectional study was conducted at Taipei Veterans General Hospital from January 2022 to December 2022. We enrolled 100 FD patients aged 19-85; 20 had classical FD, and 80 were non-classical FD. Otologic assessments included pure tone audiometry and balance function tests.

Results: Of the 100 FD patients evaluated, nearly four-fifths (79%) were confirmed to have hearing loss. Asymmetrical hearing loss was observed in 36% of these patients, while 46% exhibited high-frequency hearing loss. Balance function abnormalities were evident in 79 patients, representing 80.6% of the cohort. Moreover, the prevalence of tinnitus was identified in almost 60% of the subjects. Most hearing challenges encountered were centered on high-frequency deficits.

Conclusions: This study significantly underscores the need for incorporating audiological and vestibular evaluations in the multidisciplinary assessment of FD patients in Taiwan. It was discerned that FD is substantially associated with specific auditory manifestations, including hearing loss, sudden deafness, and tinnitus. Healthcare providers managing FD patients should remain vigilant about these potential otologic manifestations, and routine otologic consultations are imperative. Future pathologic research is crucial to delve deeper into the mechanisms underlying these otologic comorbidities in FD.

T144. Therapeutic Effect of Intraperitoneal Dexamethasone on Noise-Induced Permanent Threshold Shift in Mice Model

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Category: Hearing Loss: Consequences and Adaptation

Background: The study of acquired hearing loss focused on industrial noise in the past but now also considers various social noises, environmental noises, toxic deafness due to drug misuse, and aging. Due to the increasing size of the aged population, advances in science and technology, and the development of industrialization, the prevalence of noise-induced hearing loss is on the rise.

As hair cells in mammals do not regenerate once damaged and hearing loss is not easily restored, a steroid drug called dexamethasone is widely used for treating noise-induced hearing loss. Dexamethasone has potent anti-inflammatory and immune response-modulating effects and can be clinically administered in various ways, mainly systemic steroid therapy and intratympanic steroid injection. However, the effects of dexamethasone on hair cells and hearing recovery are not fully understood.

Methods: Animals: CBA/Jackson mice are widely used general purpose strain is the only CBA substrain that carries the Pde6brd1 mutation. Research includes immunology and inflammation, metabolism, hearing and cochlear function, infectious disease, and fetal development. (KOATECH, Pyeongtaek, South Korea)

Auditory brainstem response (ABR) measurement: Mouse auditory function was assessed by measuring ABR with an ABR workstation-System 3 (Tucker Davis Technology, Alachua, FL, USA), as previously described. Auditory function was measured with click stimuli and tone burst sound frequencies of 8, 16, and 32 kHz, and acoustic thresholds of sound pressure level (SPL) were determined using BioSigRP software (Tucker Davis Technology, Alachua, FL, USA).

Cochlear whole mount: The normal saline solution and dexamethasone were injected and anesthetized 21 days later and perfusion was performed at 1xPBS. The temporal bone was removed and the inner ear was extracted and fixed at 4% PFA. After washing with 1xPBS, desalination was performed at 10% EDTA for 48 hours.

Results: The experimental groups were 3 mg/kg of dexamethasone (3 mpk) and 10 mg/kg of dexamethasone (10 mpk), and the control group was a saline-administered group. The results showed that compared to the control group, the hearing threshold value was recovered by 10 dB SPL compared to the saline group from the 14th day in the 3 mpk group. In the 10 mpk group, thresholds were recovered from the 7th day compared to the saline group. This difference was similar at 4 kHz, and in the case of the 10 mpk group, the threshold was recovered by 20 dB SPL compared to the saline group. The study also confirmed the restoration of nerve cell activity and showed a recovery effect of about 20 μV in the amplitude value change in the 10 mpk group.
Conclusions: The study suggests that dexamethasone has a therapeutic effect for noise-induced hearing loss by increasing the activity of nerve cells and showing a recovery effect from hair cells damaged by noise.

T145. Clinical Characteristics and Hearing Impairment in 42 Patients With Mitochondrial Disease

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Category: Hearing Loss: Consequences and Adaptation
Background: Mitochondrial diseases vary in symptoms, organs affected, age of onset, and inheritance. MELAS syndrome (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes) presents severe stroke-like episodes. MIDD (maternally inherited diabetes and deafness）, with diabetes and hearing loss, emerges in the thirties. Hearing loss (HL) and short stature are common symptoms. On the other hand, patients with like m.1555A greater than G mutation, manifest only with HL. In this report, we investigated the clinical features, and hearing in 42 consecutive patients with mitochondrial DNA mutation.
Methods: The subjects were 42 patients from 29 unrelated families with confirmed mitochondrial DNA pathogenic variants. A retrospective review was conducted on genetic testing, family history, degree, type and configuration of hearing loss, complications, and BMI (Body Mass Index).
Results: m.3243A greater than G mutation was identified in 23 families, 36 patients, m.1555A greater than G was in four patients, m.7445A greater than G was in two patients. Median age of the patients was 42 (5–78) years old. There was no obvious family history in five patients.
In patients with m.3243A greater than G mutation, flat-type HL was most common, and observed in 16 patients, followed by 10 patients with gently sloping-type HL, and 5 patients with other types HL. Degrees of HL were variable in each patient, but late-onset HL was common in m.3243A greater than G mutation.

In patients with m.3243A greater than G mutation, patients with multiple complications tended to have BMI less than 18.5, and more severe HL, compared to patients with HL and/or diabetes alone. Variations in phenotype were observed even within the same family. In patients with m.1555A greater than G mutation, three patients showed steeply sloping-type HL, and one patient showed deaf-type HL. All of them wear hearing aids. Two patients with severe HL received cochlear implantation after considering the results of genetic testing.

Two patients with m.7445A greater than G mutation showed progressive and severe steeply sloping-type HL. Patient with m.7445A greater than G mutation was associated with palmoplantar keratosis, which was difficult to diagnose without genetic testing. Patients with m.1555A greater than G mutation and m.7445A greater than G mutation had severe progressive congenital HL.

Conclusions: In conclusion, early diagnosis, prevention, and comprehensive examinations are crucial in the management of HL in the patients with mitochondrial disease. Understanding the clinical features of mitochondrial disease will enable accurate diagnosis and lead to the individualized optimal management.

T146. Noise Exposure Profiles of 100 College Students Who Participate in Musical Activities

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Category: Hearing Loss: Consequences and Adaptation

Background: Musicians are routinely exposed to sound levels that place them at risk for noise-induced hearing loss. Although the dangers of noise exposure from music are well-known, less is known about which specific types and combinations of activities contribute to this loss. Our past work combining body-worn noise dosimeters with daily journals shows that college musicians experience higher noise levels than non-musicians in both musical and non-musical environments. That earlier work focused predominantly on college musicians in marching band. We now expand this line of investigation to a larger dataset that includes college musicians with different music histories.
Methods: One hundred collegiate musicians completed 1 week of personal noise dosimetry. We intentionally recruited a diverse group of musicians, including majors and non-majors, across all prominent Western instrument families and genres and different ensemble types (e.g., marching band, orchestra, symphony). In addition to noise dosimetry, participants kept daily journals to record their activities and completed a comprehensive questionnaire on their musical experience and practice, including their instrument(s), ensemble(s), and whether they were majoring in music.
Results: By using personal noise dosimetry and extensive profiles of musicianship, we show (i) a wide range of daily noise exposure dose, spanning 2 to 1,648% of the recommended daily noise dose (using National Institute for Occupational Safety and Health criteria) stemming from both musical and non-musical activities; (ii) that majors and non-majors experience similar daily noise doses on average once accounting for differences in total years of musical training; (ii) that noise dose scales as a function of the size of the ensemble and the number of ensembles (iii) that brass and wind players tend to experience higher doses than string players; but that (iv) even within these musician subpopulations there is tremendous variation in average daily noise doses.
Conclusions: College musicians vary widely in their noise exposure profiles, but their day-to-day noise exposure still puts them at significant hearing health risk. Our results confirm that musicians' noise exposure stems from both musical and non-musical activities and identifies attributes of musicianship correlated to daily noise exposure. Finally, our results reinforce the need to develop scalable yet individualizable assays of noise exposure and hearing health risk.

T147. Exploring Level Effects on Auditory Enhancement: Implications for Hearing Loss

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Category: Hearing Loss: Consequences and Adaptation
Background: Auditory enhancement describes a form of auditory context effect, in which the perception of a target tone within a complex of simultaneous tones is enhanced by the presentation of the masker components alone as a precursor. This effect is thought to represent the auditory system's capacity to adapt in challenging acoustic environments and maintain perceptual constancy. Young, normal-hearing listeners demonstrate as much as 20 dB enhancement of the target; however, for reasons currently unknown, enhancement seems to decrease at higher overall sound levels. The aim of this study is to systematically examine the effects of masker level on enhancement, and to study any potential interaction with spectral resolution.
Methods: In a single interval paradigm, young, normal-hearing participants (N=30) were asked to determine whether the pitch of a 100-ms probe tone matched that of a preceding target embedded in a masker complex. Masker stimuli were 100-ms inharmonic five-tone complexes, symmetrically centered around 1414 Hz on a logarithmic scale. The masker and target were followed by the probe tone, either with (ENH condition) or without (MSK condition) a 300-ms precursor that was identical in spectral content to the masker. All components in the complex and precursor were spaced apart by either 0.3 or 0.6 octaves, and the masking tones were presented at a fixed sound level of 45, 57.5, or 70 dB SPL per component. Target tone thresholds were measured adaptively, with the amount of enhancement quantified as the difference in threshold between the MSK and ENH conditions for each level and component spacing.
Results: With the 0.3-octave component spacing, results replicated previous findings of a large effect (15-20 dB) of enhancement at the 45-dB per component masker level, as well as greatly reduced effects at the 70-dB masker level. Enhancement at 57.5-dB SPL was intermediate. With the 0.6-octave spacing, more enhancement was produced by the moderate 57.5 dB masker level relative to the other level conditions, with the 70-dB condition still producing the least enhancement.

Conclusions: The results demonstrate a large effect of level on enhancement, as well as an interaction between level and spectral component spacing. These findings may help to shed light on the reduced enhancement found in listeners with hearing loss at the high levels needed to maintain audibility. [Work supported by NIH grant R01 DC012262.]

T148. Functional Hearing Difficulties in Veterans: Interactive Contributions of Traumatic Brain Injury, Age, and Cognitive Ability

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Category: Hearing Loss: Consequences and Adaptation

Background: Many blast-exposed Veterans have normal audiometric thresholds but difficulty processing suprathreshold sounds, e.g., speech in background noise (SIN). These functional hearing difficulties (FHDs) likely reflect changes in the central auditory system or at the interface between peripheral and central systems.

Like older listeners, blast-exposed Veterans show deficits in temporal processing and neural encoding of suprathreshold sounds, suggesting that aging and blast exposure affect similar mechanisms. Moreover, SIN performance is modulated by higher-level systems that are vulnerable to aging and blast exposure.

Here, subjective/objective FHDs are measured in young-to-middle aged Veterans with reported blast exposure and other mild traumatic brain injuries (TBIs). We hypothesize: (i) greater cumulative TBI (cTBI) is associated with greater FHD regardless of age, cognitive ability, or hearing status; and (ii) effects of cTBI on FHD will interact with age and cognitive ability.

Methods: Veterans (n=71) aged 25-60 with overseas deployment and normal/near-normal hearing completed SIN tests in several types of background noise, as well as a large cognitive battery. Subjective FHD was assessed via the Hearing Handicap Inventory for Adults (HHIA). Lifetime cTBI was measured via the Quantification of Cumulative Blast Exposure (QCuBE) interview. Regression analyses were performed with composite SIN score, HHIA score, and the average standard score of SIN and HHIA as the dependent variables. Covariates included age, high-frequency (HF) audiometric thresholds, PTSD symptom severity, cognitive ability, and cTBI.

Results: Lifetime cTBI was significantly associated with poorer SIN scores, greater subjective hearing handicap, and greater composite FHD. To test whether cTBI moderated the effects of other covariates, we performed a second regression on the composite FHD metric where cTBI was allowed to interact with each covariate. Significant positive interactions were observed between cTBI and age, PTSD severity, and cognitive ability, and a negative interaction was observed between cTBI and HF audiometric thresholds. That is, in subjects with greater cTBI, FHD was best explained by age, PTSD, and cognition. For subjects with little or no cTBI, FHD was best explained by audiometric thresholds.

Conclusions: Results reinforce that cTBI interacts with higher-level mechanisms to produce FHD. Interactions between cTBI and age may reflect the common effects of these variables on central auditory mechanisms. Interactions of cTBI, PTSD, and cognition may indicate these variables jointly limit the potential to utilize latent neural resources to support complex auditory tasks like SIN. When cTBI is low, FHD is generally low and variance is best explained by audiometric thresholds, suggesting the relative dependence of complex auditory processing on peripheral vs. central mechanisms depends on cTBI.

T149. Open Board

T150. Neural Signatures of Tinnitus in Subjects With Normal Hearing

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Category: Tinnitus

Background: Tinnitus, reduced sound-level tolerance, and difficulties hearing in noisy environments are the most common complaints associated with sensorineural hearing loss in adult populations. This study aims to clarify if cochlear neural degeneration estimated in a large pool of participants with normal audiograms is associated with self-report of tinnitus using a test battery probing the different stages of the auditory processing from hair cell responses to the auditory reflexes of the brainstem.

Methods: 294 native speakers of English, 18 – 72 yrs old, with normal audiometric thresholds, normal middle-ear function and no history of somatic tinnitus were enrolled. 3 groups were defined based on self-report: 1) those who never experienced tinnitus or occasionally heard phantom sounds that emerged and resolved within minutes (control group), 2) those who experienced at least one episode of temporary/intermittent tinnitus of less than six months duration, or 3) those who reported a continuous tinnitus percept for more than 6 months. Hearing sensitivity was measured behaviorally at standard and extended high frequencies (9-16 kHz). Neural responses were assessed using ABRs obtained in response to 100 µs-clicks delivered at either 125 or 110 dB pSPL in alternating polarity at a rate of 9.1 or 40.1 Hz, in the presence or absence of a 90-msec forward masker (8-16 kHz). ABR waveforms were processed through two bandpass filters to separate the contributions of auditory-nerve spikes from other generators ([3.3-470 Hz] vs. [470-3000 Hz]). To assess the middle-ear muscle reflex, changes in ear-canal sound pressure to a probe stimulus (95 dB pSPL click) were evoked by a 500-msec ipsilateral broadband noise elicitor raised in 5 dB steps from 40 to 95 dB SPL. The medial olivocochlear reflex was measured as the average difference between the 1-2.8 kHz TEOAE spectral band obtained with or without a 60 dB SPL contralateral broadband noise elicitor.

Results: Self-report of chronic tinnitus was significantly associated with 1) reduced cochlear nerve responses, 2) weaker middle-ear muscle reflexes, 3) stronger medial olivocochlear efferent reflexes and 4) hyperactivity in the central auditory pathways, even when differences in sex and thresholds at standard frequencies or EHFs were accounted for.

Conclusions: These results support the model of tinnitus generation whereby decreased neural activity from a damaged cochlea can elicit hyperactivity from decreased inhibition in the central nervous system.

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T151. Early Markers of Hidden Hearing Loss in Patients With Acute and Chronic Tinnitus

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Category: Tinnitus

Background: The occurrence of tinnitus after noise exposure remains unpredictable, both in individuals with and without elevated hearing thresholds. Therefore, it has been suggested that hidden hearing loss could be a trigger for tinnitus, leading to central amplification through the neural auditory pathway. Cochlear synaptopathy (CS) has been hypothesized as a possible underlying mechanism of hidden hearing loss. This phenomenon, which affects the synapses between inner hair cells and afferent nerve fibers, has already been observed in aging or after extensive noise exposure. Previous research has shown mixed results regarding CS in individuals with tinnitus. In an earlier study, we found no differences in electrophysiological markers of CS between individuals with and without tinnitus, after compensating for age and hearing thresholds. In the current study, we aim to investigate the effects of tinnitus duration on early markers of hidden hearing loss by comparing patients with acute and chronic tinnitus.

Methods: A comprehensive audiological testing protocol was conducted in three groups with young normal hearing subjects (18-32 years): (I) individuals with chronic tinnitus (greater than 1 year; N=15), (II) individuals with acute tinnitus (less than 3 months; N=10) and (III) a control group without tinnitus (N=15). Four subjects with acute tinnitus were re-measured after 6 months (chronic stage). Peripheral hearing was assessed by tonal audiometry and otoacoustic emissions (DPOAE), neural processing by auditory brainstem responses (ABR) and cochlear synaptopathy by envelope following responses (EFR) and filtered speech (in noise) intelligibility tests. Questionnaires (Tinnitus Sample Case History Questionnaire, Tinnitus Functional

Index, Hyperacusis Impact Questionnaire and Inventory of Hyperacusis Symptoms) were used to assess tinnitus distress and hyperacusis complaints.
Results: In the statistical analyses performed on the subjects tested so far (N=19), we observed decreased ABR wave-I amplitudes in the acute group, while wave-V amplitudes were significantly increased. This results in decreased wave I/V ratios, which were used in other studies to quantify central gain. However, outer-hair-cell damage seems to be the underlying cause of our decreased wave I ratios, as the acute group showed significantly lower DPOAE amplitudes than the chronic and the control group. No significant differences were observed in DPOAE, ABR and EFR results between the chronic and control group. We are currently measuring additional test subjects and analyzing additional (longitudinal) data to investigate whether these observations remain.
Conclusions: Firstly, our initial results highlight the importance of considering tinnitus duration and DPOAE measurements when interpreting EEG measurements in patients with tinnitus. Secondly, this data may provide further insights into the role of cochlear synaptopathy and outer hair cell damage in the pathogenesis of tinnitus and the central gain effect.

T152. Optimisation of Operant Silent Gap-In-Noise Detection in Humans to Avoid Ceiling Effect by Reducing Predictability and Saliency of the Silent Gap

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Category: Tinnitus
Background: In the auditory domain, temporal resolution is defined as the ability to respond to rapid changes in the envelope of a sound over time and is essential for accurate speech perception. Silent gap-in-noise detection tests assess temporal resolution that can be impaired by aging, neurolological dysfunction, inattention, or fatigue. Whether temporal resolution is impaired in tinnitus and whether silent gap-in-noise detection tests are useful for identifying the condition is still debated. We have revisited these questions by assessing the performance of human participants in operant silent gap-in-noise detection.
Methods: This project was approved by the Medical Sciences Interdivisional Research Ethics Committee at the University of Oxford (CUREC R57971). Participants were forty-seven healthy young adults with normal hearing, separated into three gender-balanced groups. The first group (n=18) was allocated to examine the effect of the position and the salience of the gap on the operant silent gap behaviour. The other remaining two groups, a tinnitus group (n=12) and a matched-control group (n=16), were used in a case control observational study.
Results: For each group, an equally distributed number of gap-containing and no gap stimuli were randomly presented in a two-alternative forced choice paradigm (stimulus duration 400 ms at 75 dB SPL). Broadband white noise and one octave-narrowband white noise (NBN) centred at 1, 2, 4, 8 and 16 kHz were used. To reduce its predictability, the position of the silent gap was randomly varied within the stimulus (100, 200, 300ms).
In the first group, the depth was modulated at 100%, 80% and 60% intensity attenuation. Detection thresholds increased when the gap was less predictable, with hit rates higher when the salient gap was in the middle of the stimulus (gaps greater than 3 ms, mixed-effects ANOVA, $F(2,635)=0.47$, $p=0.01$). Similarly, thresholds increased and the slopes of the psychometric function decreased when salience decreased, being significantly lower at low modulation values for all NBN except 8kHz (e.g. 4 kHz NBN, threshold increased from 5.23 ± 0.05 ms to 8.92 ± 0.04, $F(2,5)=183.55$, p less than 0.001).

In the case control observational study, the position of silent gaps in each stimulus was randomly varied and 80% modulation depth was used for all trials. The average gap detection threshold was lower overall for control than tinnitus participants at all tested frequencies (2-way ANOVA, $F(1,24)=12.3$, p less than 0.01). Post hoc analysis revealed this difference was significant at 2 and 8kHz NBN (Student's t-test, p less than 0.05).

Conclusions: Operant silent gap-in-noise detection is impaired in tinnitus when reducing the predictability and saliency of the silent gap to avoid the ceiling effect. The paradigm can distinguish tinnitus and control

groups suggesting that temporal resolution is impaired in tinnitus. However, it is unable to objectively identify tinnitus at the individual level in young adults with normal hearing.

T153. Supra-Threshold Hearing Characteristics in Chronic Tinnitus Patients

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Category: Tinnitus

Background: Hearing loss is the most common comorbidity of tinnitus and approx. 80-90% of people with tinnitus have a measurable hearing loss. Furthermore, many tinnitus patients report difficulties with various hearing abilities such as speech perception in noise. However, the majority of previous studies that investigated these difficulties were mainly based on self-reported challenges. Hearing loss does not only lead to a reduction in audibility but can also cause other hearing deficits, such as impaired binaural, spectral or temporal processing abilities. Systematic and well controlled studies comparing these so-called supra-threshold hearing abilities between tinnitus patients and controls are necessary to further increase the understanding of how tinnitus affects the hearing. The first purpose of the study is to investigate if the supra-threshold hearing abilities differ between a tinnitus group and a matched control group. Furthermore, the tinnitus population is very heterogeneous also with regards to hearing abilities and identifying subgroups with common hearing characteristics may help personalize interventions and thereby reduce outcome variability. Therefore, the second purpose of the study is to subgroup the tinnitus participants based on their supra-threshold hearing abilities.

Methods: In this study, the supra-threshold hearing abilities of tinnitus participants were measured with a test-battery consisting of five psychophysical measurements in addition to standard audiometry. Moreover, the speech perception, the loudness perception, the spectro-temporal modulation sensitivity, the temporal fine structure processing and the binaural pitch detection were measured. Furthermore, the tinnitus distress was evaluated with the Tinnitus Functional Index, while the tinnitus loudness and pitch were measured with psychoacoustic tests. The supra-threshold hearing abilities of the tinnitus group were compared to a matched control group without tinnitus. A cluster analysis was used to identify potential tinnitus subgroups.

Results: The preliminary results showed that both the measured and self-reported speech perception were worse in the tinnitus group compared to the matched control group. Furthermore, the tinnitus group were more sensitive to loud sounds, which was found both in the self-reported hyperacusis evaluation and in the categorical loudness measurement. However, no differences were found between the groups when comparing the binaural processing abilities.

Conclusions: The preliminary results suggest that there are large individual differences in the supra-threshold measurements in the tinnitus group which can be utilized to subgroup the participants based on their hearing abilities. Further investigation of the subgroups is needed to evaluate the possible clinical applications.

T154. Binaural Temporal Fine Structure Sensitivity Development in Children With Developmental Dyslexia

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Category: Binaural Hearing and Sound Localization

Background: Speech-in-noise perception is known to mature over the first 10 – 12 years of life. In this age range, children with language and/or reading difficulties have been reported to experience poor speech-in-noise perception compared with controls (Ziegler et al., 2009; Calcus et al., 2018). However, the underlying aetiology for this finding is debated. Binaural Temporal Fine Structure sensitivity (bTFSs) is known to be beneficial for attending to sound sources in challenging environments. For young normal-hearing adults (YHNA), the upper frequency limit of bTFSs is known to be around 1400 Hz. Recent research found the upper frequency limit of bTFSs to be significantly lower (worse) for typically-developing children than for YHNA, with age being a significant predictor of the upper limit (Flanagan et al., 2021). If bTFSs contributes to impaired speech-in-noise perception in dyslexia (DYS), poorer bTFSs would be expected in DYS. In contrast

to this, the Temporal Sampling (TS) theory of developmental dyslexia (Goswami, 2011) predicts that the perception of bTFS of speech may be preserved in children with dyslexia. By TS theory, reduced sensitivity to low-frequency envelope modulations is the core auditory impairment regarding DYS.
Methods: Binaural TFS sensitivity was measured here utilising the Temporal Fine Structure-Adaptive Frequency (TFS-AF) test (Füllgrabe et al., 2017) with 88 children aged 7-9.5 years (30 age-matched [CA], 20 male and 58 DYS, 31 male). Using an adaptive 2-up-1-down paradigm, the highest frequency at which interaural phase differences (IPD) of 30 and 180 degrees could be distinguished from an IPD of 0 degrees was assessed. The starting frequency was 200 Hz. Frequency increased by fixed factors until 8 reversals, or 40 trials had occurred. Frequency thresholds were calculated from the geometric mean of the last 4 reversals.
Results: A Linear Mixed Effects Model was fitted to the log-transformed data with independent variables group (CA, DYS) and Phase (180 degrees, 30 degrees). A Satterthwaite's ANOVA revealed no effect of group ($F[1,44] = 0.18$, $p = .68$), a significant effect of phase, with 30 degrees lower than 180 degrees ($F[1,44] = 214.83$, p less than .001), and no phase by group interaction ($F[1,44] = 0.04$, $p = .84$). The upper frequency limit of bTFSs in DYS compared to YNHA was significantly lower (p less than .001) for both levels of phase difference tested (30 and 180 degrees).
Conclusions: These results suggest that development of bTFSs is similar for DYS and CA children. Hence, the developmental pattern of bTFSs found by Flanagan et al., (2021) was supported. A smaller frequency range of bTFSs may limit the benefit gained from spectral release from masking (Swaminathan et al., 2016) contributing to the known speech-in-noise deficit found in children compared with adults. However, bTFS sensitivity was not found to be more impaired in DYS, supporting TS theory.

T155. Effects of Contextual Cues on Speech Recognition and Spatial Release From Masking in Children With Typical Hearing Using Vocoded Speech

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Category: Binaural Hearing and Sound Localization
Background: Children with cochlear implants (CIs) show significant variability in speech recognition due to many factors that influence outcomes, including cognitive abilities, neural integrity and auditory experience. In order to better understand how these children function in realistic listening situations, this study investigated recognition of sentences that are either low- or high-context, with the first step being to study typically-hearing children who listened to speech that was degraded using vocoding. The multi-channel vocoder was used here to simulate aspects of CI processing, in typical hearing children, where some of the sources of variability are more controlled. One critical aspect of this study is also pre-testing exposure to vocoded speech. Previous work has shown that several hours of experience listening to vocoders promotes better speech recognition, thus providing a better assay for simulating CI listening in individuals with typical hearing. In the current study, we tested typically-hearing children with unprocessed and vocoded speech, with the incorporation of vocoder exposure prior to testing on experimental conditions. Speech recognition was measured for unprocessed and vocoded speech in quiet and in noise, using sentences with low- or high-context, to evaluate spatial release from masking (SRM). In addition, cognitive aspects of executive function were measured.
Methods: Children with typical hearing ages 9 to 15 years participated. Sentence materials were either semantically coherent or anomalous to elicit low- vs high-context listening conditions. Each participant participated in an exposure session with vocoded IEEE sentences prior to testing in experimental conditions. Testing was conducted in quiet, and at 8 dB SNR for co-located and spatially separated conditions. Cognitive testing consisted of digit span forward, backward and flanker inhibitory control tests to measure executive functioning skills including attention, working memory and attention inhibition.
Results: Preliminary data indicate that speech understanding improves for vocoded IEEE sentences during the vocoder exposure session, in both quiet and co-located conditions. During the experimental conditions, scores in quiet were higher overall for coherent than anomalous sentences irrespective of whether the speech was unprocessed or vocoded. In the presence of maskers, effects of sentence material and vocoding were observed. Moreover, results may reveal correlations between measures of attention and anomalous unprocessed sentences. Effects of listening conditions on SRM will be evaluated.
Conclusions: Preliminary findings suggest that children may rely on contextual cues for extracting speech information from sentences, whether unprocessed or spectrally degraded using vocoding, in quiet or in the

presence of interfering maskers. Further, there may be an association between performance on the speech recognition measures and attention.

T156. Head-Related Transfer Functions of Rabbits Within the Front Horizontal Plane

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Category: Binaural Hearing and Sound Localization

Background: The head-related transfer function (HRTF) is the direction-dependent acoustic filtering by the head that occurs between a source signal in free-field space and the signal at the tympanic membrane. HRTFs contain information on sound source location via interaural differences of their magnitude or phase spectra and via the shapes of their magnitude spectra.

Methods: The present study characterized HRTFs for source locations in the front horizontal plane for nine rabbits, which are a species commonly used in studies of the central auditory system.

Results: HRTF magnitude spectra shared several features across individuals, including a broad spectral peak at 2.6 kHz that increased gain by 12 to 23 dB depending on source azimuth; and a notch at 7.6 kHz and peak at 9.8 kHz visible for most azimuths. Overall, frequencies above 4 kHz were amplified for sources ipsilateral to the ear and progressively attenuated for frontal and contralateral azimuths. The slope of the magnitude spectrum between 3 and 5 kHz was found to be an unambiguous monaural cue for source azimuths ipsilateral to the ear. Average interaural level difference (ILD) between 5 and 16 kHz varied monotonically with azimuth over ±31 dB despite a relatively small head size. Interaural time differences (ITDs) at 0.5 kHz and 1.5 kHz also varied monotonically with azimuth over ±358 μs and ±260 μs, respectively. Remeasurement of HRTFs after pinna removal revealed that the large pinnae of rabbits were responsible for all spectral peaks and notches in magnitude spectra and were the main contribution to high-frequency ILDs, whereas the rest of the head was the main contribution to ITDs and low-frequency ILDs. Lastly, inter-individual differences in magnitude spectra were found to be small enough that deviations of individual HRTFs from an average HRTF were comparable in size to measurement error.

Conclusions: The average rabbit HRTF may be acceptable for use in neural or behavioral studies of rabbits implementing virtual acoustic space when measurement of individualized HRTFs is not possible.

T157. Investigating Sound-Localization in Head-Fixed Mice Using Two-Alternative Forced Choice Behavior

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Category: Binaural Hearing and Sound Localization

Background: Humans and animals can re-learn to localize sounds following monaural hearing loss, even when binaural cues are absent. It is assumed that the re-learning relies on context-dependent plasticity mechanisms that ‘re-calibrate’ the representation of auditory space in sound localization circuits. However, the exact processes underlying this learning-dependent plasticity remain unknown. This gap in knowledge is mainly derived from methodological difficulties in chronically recording the same neurons over extended periods and multiple acoustical conditions. To overcome this drawback of standard physiological recordings, high-resolution Ca2+ imaging could be used to track neuronal activity over multiple weeks. Using this approach however requires behavioral setups and paradigms for head fixed animals.

Methods: Here we present a reward-based two-alternative forced choice behavioral paradigm for the investigation of sound localization while probing central circuits of spatial hearing in head-fixed and water-deprived mice. We developed a setup to present broadband noise stimuli from 15 distinct spatial positions within the horizontal frontal field by moving a speaker around the animals’ head using a servo motor. Two capacity-sensing lick ports were placed in front of the animal, which had to discriminate right from left sound presentations by licking either the right or the left lick port. Licking the correct port on each trial was rewarded with a droplet of sugar water; incorrect responses were punished with a time-out of 5 seconds.

Results: Training was performed at two positions (-65°/ 65°), for which 50 % of all mice reached a performance above chance level after 1-3 weeks. Following initial learning, performance was stable when varying the presentation angle (-85°, -65° / 85°, 65°) and robust to level roving. As a next step, psychometric functions will be derived by recording the response to the full range of servo positions (-85°, -65°, -45°, -30°, -15°, -10°, -5°, 0° 10°, 15°, 30°, 45°, 65°, 85°). Trials on the midline (0°) will serve for the determination of side biases.
Conclusions: Our paradigm provides an approach for the determination of murine sound localization behavior, and our setup can be used for the investigation of neuronal space representation within the central auditory pathway using high resolution Ca2+ imaging. Thus, this setup-paradigm combination enables the long-term quantification of auditory space representation in the same neurons while mice engage in a spatial hearing task.

T158. Open Board

T159. Human Sensitivity to Interaural Time and Level Differences Conveyed via Bilateral Bone Conduction

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Category: Binaural Hearing and Sound Localization
Background: Bone conduction (BC) defines a mode of hearing different from the conventional air conduction (AC) pathway. The auditory contributions of BC under typical listening conditions are minimal. However, purpose-built transducers, including BC hearing aids and implants, can provide effective BC stimulation and have become essential for the diagnosis and treatment of hearing loss. Despite the prevalence of BC technology, fundamental aspects of BC hearing remain poorly understood. For example, it is clear that a single BC transducer stimulates both cochleae (i.e., transcranial attenuation is minimal compared to AC), but factors affecting intracranial interaural disparities, or psychophysical sensitivity thereto, have proven difficult to measure and predict. A growing body of evidence suggests that spatial hearing, including sound source localization, is improved during the use of bilateral versus unilateral BC devices, but the basis of such improvement and thus barriers to further improvement remain unclear. Here we sought to quantify sensitivity to binaural cues for sound source localization, interaural time and level differences (ITD and ILD), conveyed via bilaterally worn BC transducers.
Methods: Two BC transducers (RadioEar B81) were integrated into an adjustable elastic headband using custom in-line adapters. Headbands were fitted onto normal-hearing subjects and static force was adjusted to ~3 N (±0.5 N) at each transducer using integrated force sensors. Stimuli of varied frequency, bandwidth, and/or duration were presented with varied ITD or ILD cues at a nominal level of 50 dB HL. Stimuli were presented, in separate conditions, via BC transducers and via standard AC insert earphones (Etymotic ER-2). Subjects were asked to indicate the perceived intracranial position of presented stimuli using a touch-sensitive display. Simultaneously, probe microphones were used to measure the signal in each ear canal.
Results: For AC presentation, variation of ITD or ILD yielded systematic and reproducible lateralization of all presented stimuli. For BC presentation, variation of ILD yielded systematic but compressed lateralization of a 1/3-octave 4000 Hz noise stimulus, consistent with partial preservation of presented ILDs at high frequencies due to modest transcranial attenuation. In contrast, variation of ITD cues resulted in variable and non-monotonic lateralization of a 1/3-octave 500 Hz noise stimulus. Ear canal recordings suggested that ITD-magnitude-dependent interference between ipsilateral and contralateral signals resulted in the introduction of spurious ILD cues, consistent with a simple model. Performance improved with increased stimulus bandwidth (1-octave 500 Hz stimulus) or decreased duration (1-octave 500 Hz Gaussian pulse) – conditions expected to augment the perceptual salience of presented ITD cues.
Conclusions: Data from the present study, in tandem with limited published BC localization data, suggest that for BC hearing, high-frequency ILD may be a relatively better spatial cue than low-frequency ITD – the opposite of AC hearing. Potential pathways for improved BC spatial hearing outcomes are considered.

T160. Fine-Structure Interaural-Time-Difference Sensitivity as a Function of Frequency and Level

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Category: Binaural Hearing and Sound Localization

Background: Low-frequency temporal-fine-structure interaural time differences (ITDs) are potent and heavily weighted sound-localization cues. Pure-tone fine-structure ITD discrimination sensitivity improves up to ~700 Hz. The best ITD sensitivity occurs between 500 and 1000 Hz, which is also coincides with the "ITD frequency dominant region". Above the dominant region, ITD discrimination sensitivity worsens until ~1400 Hz at which point fine-structure ITDs become undetectable. While high-resolution measurements of ITD sensitivity as a function of frequency have been made near 1400 Hz, such high-resolution measurements in the 500-1000 Hz dominant region have not yet been made; the size of the dominant region and specific best frequency can only be approximated from the current data. Therefore, we performed high-resolution fine-structure ITD sensitivity measurements as a function of frequency.

Methods: ITD discrimination sensitivity was measured in normal-hearing listeners. Measurements were made with pure tones between 300 and 1500 Hz with 100-Hz resolution and at a range of levels (10, 30, 50, and 70 dB HL).

Results: We hypothesize that there will be (1) best ITD sensitivity near 700 Hz, (2) better ITD sensitivity with increasing level, and (3) a frequency x level interaction, such that the sharpest ITD sensitivity curve is observed at the lowest level.

Conclusions: These results will help understand the size and location ITD frequency dominant region and will inform binaural models.

T161. Effects of Spatial Attention on Localization of Moving Sounds

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Category: Binaural Hearing and Sound Localization

Background: The offset location of a moving sound is often perceived ahead of its true location; an illusion termed "representational momentum". Under some conditions, the offset of moving sounds can be perceived behind the true location, which we term "representational lag". Our previous results found these perceptual displacements to be related to sound duration: 2 sec durations had forward displacement (representational momentum), while a 1 sec duration had backward displacement (representational lag). We hypothesized that the sudden onset of a moving sound generates an attentional orienting response, which delays the generation of forward displacements and is proportionally greater for brief sounds. This study compared offset localization of sounds moving from lateral to medial locations as a function of whether spatial attention was focused at the onset location (valid trials), the side opposite of the onset (invalid trials), or divided between left and right sides.

Methods: Young adult participants with normal hearing (N=29) heard virtual sounds with an abrupt onset in the lateral left or right hemispace (white noise, 5 ms rise/decay, 70 dB SPL). The sound smoothly moved towards the midline (20° or 40°/sec), had a random offset at one of 3 locations (0° midline, ±4°). Participants made left/right judgments of offset location relative to midline. Offsets at 0° were analyzed, and the % of left/right judgments that were ahead of motion direction (forward displacements) were quantified. Participants attended to the left or right side in focused attention blocks (counterbalanced order). Onsets were mostly on the attended side (75%, valid trials), with 25% on the unattended side (invalid trials). There was also a divided attention condition, where onsets were equally likely on the left/right sides. Factors included trial type (valid, invalid, divided), stimulus duration (1s, 2s), and movement direction (clockwise, counterclockwise).

Results: Results showed a main effect of trial type ($p = .002$), with progressively more forward displacement for invalid less than divided less than valid trials. Representation momentum was evident for valid trials, representational lag was present for invalid trials. Additionally, there was the expected main effect of duration, with more forward displacement for 2 sec vs. 1 sec sound (p less than .001), and direction (displacement for CW greater than CCW; $p = .001$).

Conclusions: These findings show that attention influences spatial localization of moving sounds, with progressively more forward displacement as attention is more strongly focused on where a moving sound first appears. Trial type did not interact with stimulus duration, which does not support the hypothesis that orienting responses promoted representational lag more in the shorter-duration sounds.

T162. Expression of BDNF and its Associated Receptors in the Fetal Development of the Human Inner Ear

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Category: Development: Human Subjects

Background: For mammals the nerve-growth factor (NGF), brain-derived-neurotrophic factor (BDNF), neurotrophin-3 (NT-3), neurotrophin-4 (NT-4) and their corresponding receptors, the tropomyosin-related kinase (Trk A, B, C) and p75 are known. Neurotrophins play a role in survival of the afferent sensory neurons, innervation and sprouting. These proteins and receptors are synthesized and expressed in hair follicles, Schwann cells, fibroblasts and in injured nerve tissue. A lack or loss in synthesis and secretion leads to incomplete development and impaired regeneration of neural tissue. In the inner ear, neurotrophins are important in the development of the auditory system especially for axon guidance and spiral ganglion survival. Previous studies showed that neurotrophins are expressed in supporting cells, hair cells and in the spiral ganglion of adult inner ears. A loss of neurotrophins in the inner ear resulted in hearing impairment and/or deafness.

Methods: BDNF and its associated factors are analysed with fetal human inner ear samples between GW 12 to 19. We determine the protein expression level with immunostaining, RNA expression with In-situ hybridisation and RNAseq for pathway/gene ontology analyses. Semiquantitative analysis was performed with a dedicated imaging system (TissueFAXS plus).

Results: We investigate the expression of BDNF, Trk and p75 in the development of human inner ear and correlate with time points of hair cell formation and Organ of Corti maturation. Our semiquantitative comparison of BDNF transcripts and RNAseq data among gestational weeks (GW) 12-19 exposes a spatio-temporal gradient of BDNF and associated receptors depicts differences to animal data.

Conclusions: Results enhance our understanding Neurotrophins in the normal development of the human inner ear and set important normative date to detects aberrations in pathological situations

T163. Neural Signatures of Stream Segregation: From Childhood to Adulthood

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Category: Development: Human Subjects

Background: From classrooms to playgrounds, children's communication occurs in noisy environments. Despite the peripheral auditory system reaching maturity approximately six months after birth, children have difficulties in perceiving speech in noise.

Auditory segregation is a fundamental mechanism of auditory scene analysis, involving the organization of similar sound waves into a coherent stream, while distinguishing dissimilar acoustic components and attributing them to distinct sources. This process is closely associated with speech perception in noisy environments. Two Event-Related Potential (ERP) components have been identified as associated to auditory segregation: the Object-Relative Negativity (ORN) and the P400.

Methods: The current study aims to investigate the development of the relationship between auditory segregation and speech perception in noisy environments, along with the maturation of the neurocorrelates associated with auditory segregation. Participants aged 8 to 27 (children n =17, adolescents n=13 and adults n= 16) performed in auditory segregation (stochastic figure-ground task) and speech perception in noise tasks.

Results: Behavioral results indicate an improvement in auditory segregation mechanisms and speech intelligibility in noise.
Furthermore, our neurophysiological findings show a reduction in both the ORN and the P400 amplitude from childhood to adulthood.

Conclusions: The results demonstrate that auditory segregation is a mechanism that matures during adolescence. This mechanism underlies speech perception in noise, which may explain the late development of speech perception in noise.

T164. Developmental Effects of Concurrent Auditory and Vestibular Impairments on Working Memory, Language, and Academic Abilities in Children With Bilateral Cochlear Implants

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Category: Development: Human Subjects
Background: This study investigates the developmental consequences of vestibular impairments on working memory, language proficiency, and academic abilities in children with bilateral cochlear implants (BCIs).

Balance deficits, attributed to vestibular impairments, are prevalent in children with hearing loss. Impaired spatial perception, working memory, academics, and learning have been shown in children with hearing loss (McSweeny et al., 2021) and impaired working memory has been shown in young adults with both hearing and vestibular dysfunction (Benjamin et al., 2023). However, the potential role of the vestibular impairment above and beyond the hearing loss are not clear.

The hypothesis of the study is that deficits in working memory, language skills, and academic performance in children with bilateral cochlear implants are further exacerbated in the children with concurrent vestibular impairments.

Methods: The study included 53 children (25 female, 28 male) divided into three groups: 1) typically developing (n=15, average(SD) age = 12.75(2.38), range: 8.11-15.91 years); 2) sensorineural hearing loss (n=13, average(SD) age = 11.42(2.34) years, range: 6.95-15.84 years); and 3) concurrent hearing loss and vestibular impairment (n=24, average(SD) age = 10.46(3.74), range: 4.65-17.85 years). Working memory was tested by measuring span recalls in the Dot Matrix, Corsi Block, and Digit Span tests. Academic skills were tested using the Weschler Individual Achievement Test 3rd Edition (WIAT) and language was measured with the Clinical Evaluation of Language Fundamentals 5th Edition (CELF). Data analyses utilized mixed model regressions, accounting for group, age, sex as fixed effects. Post-hoc analyses were conducted on significant findings using estimated marginal means.
Results: Development in all tests increased with age as expected (academics (F(1)=35.27, p less than 0.01); working memory (F(1)=25.57, p less than 0.01); language (F(1)=13.33, p less than 0.01)). Children with bilateral cochlear implants exhibited poorer language skills (F(2)=7.85, p less than 0.01) when compared to typically developing peers. Concurrent vestibular impairment may have additional adverse effects on academics (t(2)=2.21, p=0.08) and language (t(45)=2.56, p less than 0.04).
Conclusions: Early findings of this study suggest developmental effects of hearing loss, which may be further intensified by the presence of concurrent vestibular impairments in academic and language. Non-significant findings will be addressed relative to previous work. Overall, these findings may underscore the importance of early intervention and tailored support to address the unique challenges faced by children with dual sensory impairments.

T165. Development of Factors Underlying Spectral Resolution in Normal Hearing Infants

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Category: Development: Human Subjects

Background: Acoustic spectral resolution (SR), the ability to perceive intensity variation across frequency, is essential for speech perception and predicts clinical benefit in pre- and post-lingually deaf cochlear implant (CI) users. A clinical SR tool for infants could improve optimal audiologic and habilitative care earlier than currently possible. SR depends on two factors: frequency resolution (FR), the ability to perceive intensity peaks in the presence of adjacent peaks and spectral modulation sensitivity (SMS), the ability to perceive peak/trough intensity differences. We have used a spectral ripple discrimination to derive the spectral modulation transfer function, where slope defines FR and intercept defines SMS (Supin et al., 1999), from two thresholds. Previous works suggest that FR is mature, but SMS immature, in normal hearing (NH) 6-month-olds. Here, we include younger 3-month old infants based on evidence that frequency tuning is not yet mature at this age (Spetner, Werner-Olsho, 1990). The hypothesis was that both FR and SMS would improve with age but that FR would be immature only at 3-months whereas SMS would be immature in 3- and 6-months olds.
Methods: Participants included 13 3-month olds, 15 6-month olds, and 16 adults without hearing loss, risk factors, and able to pass bilateral OAE screening. Stimuli were 1-second pure-tone-complex carriers with spectrotemporally-modulated envelopes. Temporal rate was fixed at 5 Hz and spectral peak density varied from 1 to 20 "ripples" per octave (RPO). The listeners' task was to respond behaviorally to "target" trials with spectral density less than 20 RPO and not to respond to "no-target" trials with spectral density = 20 RPO. A single-interval forced choice observer based procedure was used to estimate thresholds adaptively (Noble et al., 2022). For the SMS threshold, modulation depth was varied at fixed target density = 1 RPO. Then, RPO was varied at fixed target depth (2 X SMS) to determine FR. Thresholds were based on the 70% correct point of the psychometric function.
Results: An additional 25 infants started, but didn't complete testing due to inability to pass response criteria (18) or inability to complete both thresholds (7) corresponding to 47% attrition. Mean SMS improved with age and t-tests showed significant differences between all three age-groups (p's less than 0.01). Similarly, mean FR improved with age but t-tests showed differences only between 3-month and older groups (p's less than 0.0001) while 6-month and adult means were not different ($p = 0.4$).
Conclusions: The findings support the hypothesis and are consistent with previous studies showing maturation of FR between 3- and 6-months of age. An analogous study is being conducted in CI infants between 3 and 6 months of activation and at later school ages to examine FR as a potential marker of device efficacy in implanted infants.

T166. A Matched Comparison Across Different Sensory Pairs of Cross-Modal Temporal Integration in Balance Maintenance

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Category: Multisensory Processing/Interactions
Background: Various sensory information involved in balance maintenance is integrated together with different timing characteristics when they are processed from peripheral to central stages. There are two important parameters for analyzing multisensory temporal integration in humans: the point of subjective simultaneity (PSS) and the temporal binding window (TBW). The aim of this study was to explore the relationship of PSS and TBW within and between individuals for the different sensory pairings and to relate these measures to the relative timing perceived by reaction time (RT) differences in unimodal sensory stimulation.
Methods: Twenty-five healthy adult subjects (12 males, 13 females, aged 21-60 years) participated in the temporal measurements of unimodal and cross-modal sensory perceptions. We conducted a simultaneity judgment test by pairing two out of four stimuli, which included auditory, visual, vibrotactile, and galvanic vestibular stimulation (GVS), each lasting for 1.5 seconds. The subject's responses were fitted to asymmetric Gaussian functions to estimate TBW and PSS. We analyzed the correlation between TBW ranges and PSS values obtained from the six different combinations of tests, and their results were compared with the unimodal RT differences. An asymmetry index (AI) was also calculated to quantify the degree of perceptual asymmetry involved in multisensory temporal integration.
Results: Among the TBW of the six sensory cross-modal stimulus pairs, the TBW of the three combinations that included GVS (987-1,227 msec) exhibited a wider range than those without GVS (481-516 msec).

However, in the case of PSS, it showed relatively similar values regardless of the inclusion of GVS (16-346 msec). The results show that the correlation between the TBW measurements in the three combinations involving GVS (visual-GVS, vibrotactile-GVS, and auditory-GVS) showed a significant positive correlation, while there was no correlation among the combinations in terms of PSS. Further, no correlation between either TBW or PSS and the RT differences was observed. The degree of the temporal binding asymmetricity (i.e., AI magnitudes) ranged from 8% to 92%.
Conclusions: The findings in this study suggest that the strong connections between TBW widths in the balance-related multisensory combinations (visual-GVS, vibrotactile-GVS, and auditory-GVS) show that how people combine information from different senses varies from person to person but is closely connected within each sensory system in balance maintenance. On the other hand, the lack of connections between PSSs in the various sensory pairings suggests that this measure is more flexible and depends on the changing patterns of sensory information in the world, rather than being strongly influenced by individual factors. In addition, the results of this study demonstrate that temporal judgment processing in multisensory events might have a different mechanism than that in unisensory events, and there is evidence for the existence of perceptual asymmetricity in multisensory temporal integration processing.

T167. Different Cortical Activities Associated With Susceptibilities in Audiovisual Integration in Normal Hearing Subjects

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Category: Multisensory Processing/Interactions
Background: Audiovisual integration combining auditory and visual speech cues helps normal hearing to listen to speech sounds in noise and patients with hearing loss to discriminate speech. The capacity of audiovisual integration is influenced by the severity of hearing loss and mother languages, which varies widely between individuals. A combination of /ba/ sound and an incongruent lip movement often leads to illusory auditory percepts, called the McGurk effect. Susceptibility to the McGurk effects might be associated with audiovisual integration capacity. The present study, recruiting normal-hearing adults, aims to reveal event-related cortical activities associated with McGurk effect susceptibility 180-300 trials were conducted for each stimulus.
Methods: The silent videos of lip movements uttering /ba/, /da/, and /ga/ and 66 ms /ba/ sounds were used as V-only and A-only stimuli. We also created three audiovisual (AV) stimuli by combining the silent videos and the /ba/ sound (a congruent and two incongruent stimuli). The onset of lip movement and auditory sound was -380 ms and 0 ms in each AV stimulus. Twenty-two normal-hearing Japanese adults were recruited and divided into responder and non-responder groups; the responder and non-responder groups experienced audiovisual illusion in ≥50% and less than 50% of trials, respectively. Each participant randomly underwent AV, V-only, and A-only conditions, and cortical activities were recorded using a 32-channel EEG system. The recorded data were high-pass (1Hz), low-pass (40Hz), and notch (multiple number of 60Hz) filtered and average-referenced.
Results: Twelve and ten participants were included in the responder and non-responder groups, respectively. Within-group analysis for the responder group revealed significant differences between the congruent and incongruent AV conditions at parieto-occipital electrodes around 175ms ($p=0.02$) and between the congruent and incongruent V-only conditions at occipital electrodes around 180 ms (p less than 0.0001). In contrast, the non-responder group showed no significant difference between these conditions. Between-group analyses demonstrated that the congruent AV stimuli induced different responses restricted in the vertex (Cz, $p=0.03$) around 170 ms. In contrast, the incongruent AV /da/ and /ga/ stimuli led to different cortical activities in the parieto-occipital regions ($p=0.04$), in addition to the vertex ($p=0.03$). Interestingly, no significant between-group difference was detected in V-only conditions, but the A-only stimuli induced significantly larger responses in the non-responder group than the responder group around 175 ms at the vertex and parieto-occipital regions (Cz: p less than 0.0001, Oz: $p=0.002$), which is similar to those observed in the incongruent AV condition.
Conclusions: The results of within-group analyses suggest that different cortical activities at the parieto-occipital regions are associated with susceptibility to audiovisual integration. The similar pattern of between-group differences at the parieto-occipital regions among the AV and A-only conditions implies that in subjects

with a high capacity for audiovisual integration, speech sounds may activate cortical areas associated with the audiovisual integration, even without visual stimuli.

T168. Parameters of Paired Stimulation Change Coding Properties in the Auditory Cortex of Guinea Pigs

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Category: Multisensory Processing/Interactions

Background: Paired stimulation, which consists of neural stimulation using two modalities (e.g., acoustic and electric stimulation), is a method of neuromodulation that induces plasticity in the cortex of the brain. Specifically, paired stimulation has been shown to change the responses of neurons in the primary auditory cortex to noise and tones. Paired stimulation has been shown to be effective in treating tinnitus and may be effective in treating other hearing disorders, such as hyperacusis and hearing loss. Many variables—such as the amplitude, frequency, and duration of both acoustic and electric stimulation—can affect the type and extent of plasticity induced by paired stimulation. This study aims to characterize the effects of the frequency and time of paired stimulation, consisting of acoustic stimulation and electric stimulation of the pinna, on the threshold and spectral coding properties of neurons in the primary auditory cortex of guinea pigs.

Methods: Paired stimulation was delivered to ketamine-anesthetized guinea pigs up to 5000 times at 2 Hz. Acoustic stimulation, ranging from 1-16 kHz and 0-70 dB SPL, was delivered through a speaker to the left ear. Electric stimulation, with a ±150 ms onset time relative to acoustic stimulation, was delivered using subdermal electrodes to the left and right ear. The threshold and spectral properties of neurons in the right primary auditory cortex before and after paired stimulation were collected using a 4-shank, 32-site electrode array with sites centered at layer IV. Tuning curves were collected and analyzed for sensitivity, specificity, and selectivity and compared before and after paired stimulation using spike counts, thresholds, Q10 values, and best frequencies.

Results: Acoustic-driven activation thresholds did not significantly change. After paired stimulation, Q10 values increased, and best frequencies shifted towards the frequency of the acoustic stimulation during paired stimulation. Not all neurons or recording sites shifted significantly in coding properties; further experiments are needed to better apply the parameters of paired stimulation to change the responses of neurons in the primary auditory cortex more effectively and differentially.

Conclusions: Changes in the spectral and threshold properties of neurons in primary auditory cortex are consistent with previous studies. However, the magnitude of the changes was less than magnitudes observed in previous studies. The use of anesthesia and the relatively noninvasive quality of the paired stimulation may decrease the plasticity induced by paired stimulation: previous studies directly stimulated neural pathways with invasive implants. Further studies are needed to characterize effects of additional parameters and their interactions on the plasticity induced by paired stimulation. Algorithms, such as Bayesian optimization, are promising to explore the complex parameter space of paired stimulation more efficiently. These studies can lead to the design of a noninvasive, accessible, and personalized device that uses paired stimulation to treat various hearing disorders.

T169. Open Board

T170. Associations of Distortion- and Reflection-Based Otoacoustic Emissions With Metabolic and Sensory Components of Age-Related Hearing Loss

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Category: Otoacoustic Emissions

Background: Age-related hearing loss is challenging to study, because of varying genetic risks and environmental exposures that contribute to changes in cochlear function across the lifespan. Cochlear pathologies including stria vascularis degeneration and outer hair cell damage are each hypothesized to affect

active cochlear amplification, with differential effects across frequencies or at higher frequencies, respectively. Otoacoustic emission (OAE) measures are used to assess the energy added to the traveling wave in cochlear amplification, which weaken as pure-tone thresholds increase. Given this relationship, our goal was to characterize the extent to which different OAEs provide sensitivity to age-related hearing loss components. Thus, the current study compared intercepts and slopes for distortion-based and reflection-based OAEs and their associations with metabolic and sensory estimates.
Methods: Two independently collected, retrospective datasets were examined: Medical University of South Carolina (N=539; 59% female; 19-89+ years of age; 26% non-White) and Boys Town National Research Hospital (N=429; 55% female; 18-86 years of age; 10% non-White). Each dataset included demographics, self-reported noise history, distortion-product otoacoustic emissions (DPOAE), and cochlear reflectance (CR) responses. Estimates of metabolic and sensory components of age-related hearing loss were calculated from audiograms, then linear mixed model regression analyses were performed to characterize the extent to which the component estimates were associated with intercepts and slopes for DPOAE and CR responses fitted across frequencies. We predicted associations of component estimates with both DPOAE and CR responses, with lower intercepts related to higher metabolic estimates and steeper, more negative slopes related to higher sensory estimates.
Results: As predicted, metabolic estimates significantly increased with participant age (t greater than 8.29, p less than 0.001) and were significantly associated with lower fitted intercepts (CR and DPOAE: t greater than 5.29, p less than 0.001). Sensory estimates also significantly increased with participant age, with larger age-related differences for males than females (age-sex interactions: t greater than 3.12, p less than 0.01), and with positive noise history (t greater than 2.65, p less than 0.01). Sensory estimates were significantly associated with steeper, more negative slopes for DPOAE (t greater than 3.11, p less than 0.01) but not CR (p greater than 0.19). For participants with both measures collected, DPOAE intercepts explained unique variance in metabolic estimates when modelled with CR intercepts.
Conclusions: Both the DPOAE and CR responses were related to component estimates of age-related hearing loss. DPOAE and CR intercepts were each significantly associated with metabolic estimates, and DPOAE slopes were significantly associated with sensory estimates while the CR slopes were not. These findings suggest that distortion-based and reflection-based otoacoustic measures differ in their sensitivity to sensory losses. Determining cochlear pathologies that may be associated with hearing loss for individual older adults could be important for supporting targeted therapeutics, and the current findings suggest that distortion-based measures may provide more sensitivity than reflection-based measures.

T171. Temporal Correlations of Medial Olivocochlear Reflex With Auditory Brainstem and Cortical Responses

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Category: Otoacoustic Emissions
Background: Medial olivocochlear (MOC) fibers are efferent projections that emerge from the superior olivary complex 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 has been termed the medial olivocochlear reflex (MOCR). We have previously reported that MOCR is enhanced by anticipation based on the regularity of preceding sound sequence. Since there are efferent projections from the auditory cortex to the MOC via brainstem nuclei, it is necessary to comprehensively measure the activity throughout the auditory pathway to identify the neural basis for the predictive control of MOCRs. Here we simultaneously measured MOCR, auditory brainstem response (ABR) and cortical auditory evoked potentials (CAEP) and examined those temporal correlations.
Methods: Click sequences with varying inter click intervals (ICIs) based on maximum length sequences (MLS) were used for the MOCR, ABR and CAEP elicitation. The minimum ICIs of MLS-clicks was 4 ms. In the regular condition, interstimulus interval among the MLS-clicks was fixed at 500 ms. In the irregular condition, the onset of the MLS-clicks was randomly delayed or front loaded by 200 ms compared to the regular condition. The peak equivalent sound pressure level (peSPL) and the duration of the MLS-clicks were set to 60 dB and 508 ms, respectively. The temporal fluctuations of the MOCR, ABR and CAEP were monitored during eight-minute measurement sessions. MOCR was assessed noninvasively using otoacoustic

emissions (OAEs) evoked by clicks presented right after the MLS-click offset. ISIs of the clicks were 25 ms and presented at 60 dB peSPL. OAE level differences between the adjacent irregular and regular condition (ΔMOCR_reg) can be considered as MOCR changes associated with the timing predictability of the MLS-clicks. The ABR induced by MLS-clicks was measured. To recover the ABR waveform, the measured waveforms were separated by minimum ICIs and convolved with the corresponding MLS. CAEPs synchronized with the MLS-click onset were assessed by the potential difference between the peaks of N1 and P2.

Results: The time variation of ΔMOCR_reg was negatively correlated with that of N1-P2 amplitude, i.e., the rhythm-based enhancement of MOCR is associated with reduced CAEP. On the other hand, no correlation was identified between the time variation of ΔMOCR_reg and that of ABR amplitude.

Conclusions: Costa-Faidella et al. (2011) reported that temporal regularity enhances repetition suppression (RS), the attenuation of neural responses to repeated stimulation, and proposed RS as a potential mechanism underlying regularity encoding. Given the reduced CAEP reflects enhanced RS, the association between the rhythm-based enhancement of MOCR and reduced CAEP implicates that MOCR reflects regularity encoding at cortical levels. By contrast, brainstem activities are less likely to be involved with the predictive control of MOCR.

T172. Temperature Dependence of Spontaneous Otoacoustic Emissions From Lizards With Free-Standing Hair Cell Bundles

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Category: Otoacoustic Emissions

Background: Spontaneous otoacoustic emissions (SOAEs) are sensitive to physiological changes. In ectothermic vertebrates such as lizards, SOAE peak frequencies increase with body temperature. However, the cause of these frequency shifts with temperature is unknown. Although models suggest that cooperativity between hair cells is a prerequisite for SOAE generation, it is unclear how temperature changes affect generation mechanisms and inter-cellular coupling. We investigated the temperature dependence of SOAE spectra and temporal statistics of the peaks in the green anole lizard (Anolis carolinensis). The anole ear produces robust emissions despite its comparatively simple basilar papilla (only ~150 hair cells). Notably, SOAEs are most prominent between 1–5 kHz, corresponding to the basilar papilla region in which hair cell bundles are free-standing; that is, the bundles lack an overlying tectorium.

Methods: SOAE waveforms were recorded from anoles in "cold" (body temperature less than 23.5°C) and "warm" (greater than 24.0°C) conditions. Body temperature was manipulated by increasing the set point of a heating pad in 1–2°C steps (maximum temperature ≥33.0°C) and allowing at least ten minutes for acclimation to the temperature change. For some subjects, SOAEs were measured at each temperature. SOAEs were analyzed in the spectral domain to evaluate structure and peak frequency shifts. The temporal statistics of the peaks were determined from the analytic signals of spectral peaks isolated by filtering.

Results: SOAE peak frequencies increased with temperature, but most peaks maintained their bandwidth relative to the peak frequency. Generally, an SOAE's spectral structure was stable across temperatures, with "warm" spectra resembling frequency-scaled versions of "cold" spectra. There were minor changes in some spectra, like a single peak splitting into two or two peaks merging. These results are generally consistent with previous studies examining other lizard species (e.g., Manley and Köppl 1994; Manley 2006). However, we found that the temporal statistics of the peaks could change with temperature, even in cases in which only the peak's frequency changed. Preliminary observations indicate that as body temperature increased, some peaks' temporal amplitude distributions transitioned from bimodal to unimodal. The data also allowed us to examine how interpeak correlations in amplitude and frequency fluctuations are affected by temperature.

Conclusions: Our results align with previous work in lizards demonstrating the preservation of SOAE spectral structure with increasing body temperature. However, the temporal statistics of the peaks qualitatively changed as temperature increased. These results provide foundational information on the origins of emission generation. To explain the changes in the temporal statistics of the peaks, we propose that temperature may affect homeostasis and act as an in-vivo "bifurcation parameter" for a fully intact ear.

T173. Mechanisms of Frequency Modulation Perception Across the Adult Lifespan

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Category: Psychoacoustics

Background: Humans exhibit fine-grained frequency modulation (FM) perception, especially at the slow rates (fm less than ~5-10 Hz) and low carrier frequencies (fc less than ~4-5 kHz) that are important for speech and music. This slow-rate advantage is present for FM, but not amplitude modulation (AM), and has been classically thought to be driven by precise neural phase locking to temporal fine structure (time coding). At faster rates and higher carriers at all rates, FM is thought to be represented by fluctuations in average neural firing rate, once FM has been converted to AM via cochlear filtering (place coding). However, recent evidence suggests a unitary central place code may account for all FM perception. Age has been found to selectively impair FM, but not AM, detection at both slow and fast rates. Our hypothesis was that the pattern of FM detection thresholds previously ascribed to neural phase-locking to TFS, including poorer slow-rate FM detection with age, can instead be explained by the properties of out-of-phase envelope processing, based on a place code.

Methods: This hypothesis was tested by assessing sensitivity to AM with pure-tone dyads, where two carriers were centered around 1 kHz and separated in frequency by 2/3 octave. The slow-rate AM (fm = 2 Hz) was either incoherent (i.e., out of phase), to simulate the out-of-phase AM produced by FM (simulated FM), or coherent (i.e., in phase), as in traditional AM. The depth of the AM on each carrier was set to equal sensitivity (d' = 1) for each listener, and sensitivity to simultaneous AM on both carriers was assessed using a two-interval constant stimulus method. Sensitivity for pure-tone FM and AM detection was measured within the same participants (fc = 1 kHz; fm = 2 Hz) using an adaptive method.

Results: Preliminary results from 33 participants varying in age (18 – 76 years) show that FM but not AM perception worsens with age, and that FM and AM perception are highly correlated, consistent with previous research. Consistent with place theory and with prior research, the slow-rate advantage in FM perception was present for detection of out-of-phase AM dyads, even though there is no viable timing information to perform the task. However, variability in out-of-phase AM sensitivity was not related to age or FM sensitivity, either before or after controlling for sensitivity to in-phase AM.

Conclusions: These results could suggest that poor FM perception with age is not due to sensitivity to out-of-phase AM processing. An explanation for the effects of age on both slow- and fast-rate FM but not AM, despite the strong multicollinearity between FM and AM, remains elusive. [Supported by NIH grant R21 DC019409.]

T174. Simulating Psychophysical Modulation Masking Experiments in an Artificial Neural Network Trained for Sound Classification

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Category: Psychoacoustics

Background: Neurons in the auditory system exhibit selectivity to modulation frequency and thus can be viewed as modulation filters. Consistently, psychophysical studies indicate the presence of modulation filters by modulation masking experiments: the detection of the "signal" sinusoidal modulation in the presence of a "masker" modulation is improved with increasing differences between the two modulation frequencies (Houtgast, 1989, JASA; Ewert and Dau, 2000, JASA). Our computational studies have suggested that an artificial neural network (NN) trained for sound classification contains units with neuron-like modulation selectivity (Koumura et al., 2019, J Neurosci), and exhibits human-like psychophysical modulation sensitivity (Koumura et al., 2023, J Neurosci). These studies suggest that the trained NN can be a computational model of auditory modulation processing. The present study tries to test this notion further by simulating modulation masking experiments in the NN model.

Methods: The NNs were the same as in our previous study (Koumura et al., 2023). The model takes a 0.2 sound waveform as an input, processes it with 13 convolutional layers, and outputs an estimated sound category. Models were trained for the classification of either everyday sounds or phonemes in speech sounds.

The parameters in the trained models were frozen. To measure their masked threshold patterns (MTPs), we simulated the modulation masking experiment (Ewert and Dau, 2000). The simulation protocol was the same as our previous computational study (Koumura et al., 2023) except that the measured sensitivity was of discriminating the masked signal from the masker-only stimulus. The stimulus parameters were the same as in the previous psychoacoustic study (Ewert and Dau, 2000): signal modulation frequencies were 4, 16, 64, and 256 Hz; the masker modulation was the narrow-band noise with a depth of -10 dB and a width of a half octave of the signal; the carrier was white noise.
Results: MTPs in the conditions of 4- and 16-Hz signal modulation frequencies had their peaks at the signal modulation frequencies, indicating the existence of modulation frequency selectivity in those modulation frequency regions. MTPs of the higher modulation frequencies did not have notable peaks. For those conditions with peaked MTPs, we tried to calculate Q values using the widths at peak -3dB. Although most of the conditions resulted in too "shallow" MTPs to calculate the widths, some MTPs deeper than 3dB showed Q values comparable to humans.
Conclusions: We simulated psychophysical masked modulation detection experiments in NNs trained for sound classification. Mostly, the resulting MTPs were shallower than those in humans, suggesting less selectivity on the modulation domain in the models. The conditions in which -3dB widths could be calculated showed somewhat comparable selectivity with humans.

T175. Optimization Under Ecological Realism Reproduces Signatures of Human Speech Perception

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Category: Psychoacoustics
Background: Machine learning has enabled new models of sensory systems via optimization of artificial neural networks. Such models replicate aspects of human perception and brain responses but also show discrepancies with humans, and it remains unclear how to remedy them.
Methods: We analyzed a model of the auditory system obtained by optimizing a neural network to recognize words from simulated cochlear responses to sound, previously shown to reproduce aspects of auditory brain responses and behavior.
Results: The model exhibited signatures of cortical responses to speech and human-like recognition across some speech manipulations (e.g., inharmonicity, local time-reversal). However, it diverged from humans on some ecological manipulations not encountered during training (e.g., reverberation). A new model, optimized in more ecological conditions, produced human-like behavior across all conditions.
Conclusions: The results suggest that optimization under ecological realism yields more accurate models of perception, here yielding a model accounting for many characteristics of speech perception.

T176. The Role of Perceptual Grouping in Auditory Enhancement Effect

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Category: Psychoacoustics
Background: One of the manifestations of an "auditory-enhancement effect" is a significant reduction of masking of a tone (target) by a set of surrounding tones (masker) when the masker-target complex follows its own copy (a precursor), but with the target component removed. The amount of auditory enhancement is much larger (in excess of 20-25 dB) when the frequency range of the precursor and masker components is roved between the target and comparison observation intervals compared with that observed without a frequency rove (6-10 dB). Two possible reasons for this large effect of rove are: 1) the rove limits the influence of longer-term effects between intervals and trials of an adaptive tracking procedure; and 2) roving increases the effects of informational masking, by increasing uncertainty surrounding the frequency and pitch of the target component. The first aim of the current study was to distinguish between these two hypotheses by investigating the effects of an additional manipulation, amplitude modulation (AM), which should help

segregate the target from the masker components. The second aim was to test for effects of age on overall enhancement, on the assumption that age may affect inhibitory processes thought to underlie enhancement.
Methods: We used two durations for the seven-tone masker-target combination, 440 ms and 100 ms, to manipulate the effectiveness of imposing 40-Hz AM on the target in segregating it from the masker. The frequencies of the precursor and the masker-target components were roved between the observation intervals over a one-octave range. Enhancement was calculated from the difference between the masked thresholds observed for a precursor with and without the target component. Younger (18-30 yrs) and older (55-70 yrs) listeners with normal hearing took part.
Results: For both groups of listeners, the auditory enhancement was greatly reduced for the AM target when the masker-target duration was 440 ms, and was comparable with that reported in studies that did not use a frequency rove. However, for the 100-ms masker enhancement was not affected by AM and remained large. Enhancement was on average greater for the younger than the older group for the shorter but not the longer AM target.
Conclusions: The results are inconsistent with the idea that once the effect of obligatory grouping of target and masker component is eliminated, loss of neural inhibition in older adults is associated with reduced auditory enhancement. [Supported by NIH grant R01DC012262.]

T177. Mid-Level Auditory Computations Predict Human Speech Recognition in Natural Environmental Noise

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Category: Speech Perception
Background: Recognizing speech in noisy environments, such as on a busy street or restaurant, is an essential listening task. While both temporal and spectral auditory cues contribute to listening in noise, current cognitive models are unable to account for differences in real-world hearing sensitivity across backgrounds. Here we develop and optimize a biologically inspired auditory model that is able to predict speech recognition sensitivity in real-world environmental noises.
Methods: Here we assess how the spectrum and modulation statistics of natural sounds mask the recognition of spoken digits (0 to 9). We enrolled participants (N=27) in a psychoacoustic study where digits were presented in various natural background sounds (e.g., water, construction noise, speaker babble; tested for signal to noise ratios of SNR=-18 to 0 dB) and their perturbed variants. Background sounds were perturbed by either phase randomization (PR), which whitens the modulation statistics and preserves the original sound spectra; or spectrum equalization (SE), which whitens the sound spectrum but preserves the modulation statistics.

We next developed a physiologically inspired model of the auditory system to predict perceptual trends across backgrounds, perturbations, and SNRs. Sounds were decomposed through a cochlear filter bank (cochlear stage) and a subsequent set of spectrotemporal receptive fields that model modulation selectivity in auditory midbrain (mid-level stage). Spectrum and modulation based summary statistics were then derived for both the foreground and background sounds. Logistic regression was performed on these features (summary statistics) to estimate perceptual transfer functions and predict human accuracy. The logistic model was optimized using a cross-validated Bayesian approach which maximizes the a posteriori probability.

Results: Summary statistics from the mid-level model accurately predict single trial perceptual judgments, accounting for ~ 92% of the response variance across backgrounds and noise levels. By comparison, a reduced model consisting of a only a cochlear stage (which only contains spectrum statistics) predicted ~67% of the perceptual variance. Furthermore, perceptual transfer functions in the model space identify specific natural sound features and the foreground / background interference that impact recognition. The mid-level model transfer functions demonstrate that slow modulations in background sounds (1-6 Hz) are a critical source of interference that competes with comparable fluctuations in speech. The interference is also frequency specific: the transfer functions indicate maximal background interference at ~1 kHz and a reduction of interference and possible enhancement for frequencies greater than 1KHz.

Conclusions: The results suggest that speech recognition in natural backgrounds involves interference of multiple low-dimensional cues that are well described by an interpretable auditory model. The model identifies the specific spectral and/or modulation cues that contribute to listening in noise and their complex interference pattern. The model also allows for estimating perceptual transfer functions which have potential implications for hearing loss intervention and hearing diagnostics.

T178. Auditory Processing: Effects of Silent Intervals on the Extraction of Human Frequency-Following Responses Using Non-Negative Matrix Factorization

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Category: Speech Perception

Background: Source-Separation Non-Negative Matrix Factorization (SSNMF) is a mathematical algorithm recently developed to extract scalp-recorded frequency-following responses (FFRs) from noise. Despite its initial success, the effects of silent intervals on algorithm performance remain undetermined. Our purpose in this study was to determine the effects of silent intervals on the extraction of FFRs, which are electrophysiological responses that are commonly used to evaluate auditory processing and neuroplasticity in the human brain.

Methods: We used an English vowel /i/ with a rising frequency contour to evoke FFRs in 23 normal-hearing adults. The stimulus had a duration of 150 ms, while the silent interval between the onset of one stimulus and the offset of the next one was also 150 ms. We computed FFR Enhancement and Noise Residue to estimate algorithm performance, while silent intervals were either included (i.e., the WithSI condition) or excluded (i.e., the WithoutSI condition) in our analysis.

Results: The FFR Enhancements and Noise Residues obtained in the WithoutSI condition were significantly better (p less than 0.05) than those obtained in the WithSI condition. On average, the exclusion of silent intervals produced a 11.78% increment in FFR Enhancement and a 20.69% decrement in Noise Residue.

Conclusions: These results not only quantify the effects of silent intervals on the extraction of human FFRs, but also provide recommendations for designing and improving the SSNMF algorithm in future research.

T179. Individualized White Matter Connectivity of the Articulatory Pathway: An Ultra-High Field Study

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Category: Speech Perception

Background: The ultra-high field 7T MRI has revolutionized the visualization of white matter tracts involved in neurocognitive functions. The present study aimed to investigate the characteristics of the articulatory motor pathway during speech production and its potential common path with speech perception, as it plays a crucial role in the accurate and efficient execution of spoken language. To achieve this, ultra-high field diffusion-weighted imaging was utilized to visualize the white matter tracts of the articulatory motor pathway in participants performing a basic speech production task.

Methods: Twenty healthy right-handed adults (12 females, 19–48 years, average 30 years) were studied. 7 T MRI data were recorded using a whole-body scanner (MAGNETOM Terra, Siemens, Erlangen, Germany) with a custom-built 63-channel receive array coil. fMRI blood oxygen level-dependent (BOLD) signal data were obtained by using a simultaneous multi-slice (SMS) echo planar imaging (EPI) acquisition. In a speech-sound production task, subjects were asked to produce a silent lip-round vowel /u/ in response to the visual

cue ‘‘U’’ or to purse their lips in response to seeing the cue “P”. Diffusion data were obtained using a 2D pulsed gradient spin-echo sequence. Axial diffusion-weighted images (DWIs) were acquired with a b-value of 1000 s/mm2 along 68 non-collinear directions at 1 mm isotropic resolution. We used the data from the speech production task to identify the relevant individual ROIs for white matter tractography. We calculated contrasts using a general linear model (GLM) for /u/-sound vs. baseline, lip purse vs. baseline, and /u/-sound vs. lip purse (“U”-“P”). The GLM regressors defined onsets and offsets of /u/ or lip purse trials. Diffusion MRI tractography was performed using the TrackVis software (www.trackvis.org).
Results: To identify the articulatory motor pathways, we used deterministic fiber tracking and demonstrated that the motor areas of the dominant hemisphere associated with speech production exhibit evidence of the path with the structural connection between the primary motor cortex and posterior temporoparietal receptive language areas, which is referred to share the expected path with the arcuate fasciculus (AF). The white matter fiber tractography in the left motor area revealed significant connectivity patterns across the language areas.
Conclusions: This study provides valuable insights into the macrostructural basis of speech production and highlights the critical role of white matter tracts in facilitating communication between various regions of the brain. With continued advancements in imaging techniques and hardware, we can continue to push the boundaries of our understanding of the brain and its intricate networks. Our results provide new insights into the neural mechanisms underlying speech production and the intricate connections within the language areas of the brain. These findings may have implications for the development of novel therapies aimed at treating speech and language disorders.

T180. Semantic and Acoustic Ambiguity: An fMRI Investigation of Speech Perception Under Challenging Conditions

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Category: Speech Perception
Background: Processing speech relies on a network of interconnected frontal and temporal regions known as the core speech network. The ventral anterior temporal lobe (ATL), a key region for semantic cognition, is often not shown to be active in fMRI studies of speech perception. This is partly due to significant image distortions and signal dropout within this region. Several techniques have been proposed to overcome this issue, yet they have seldom been utilized in auditory research. As such, understanding of the contribution of this ATL region is limited.
When faced with challenges such as background noise or semantic ambiguity, we see activation extend beyond core speech areas into the multiple demand network – a domain-general network recruited for various cognitive tasks. Different types of challenges present different obstacles to overcome. For example, acoustic ambiguity from background noise renders the identity of word forms uncertain. Similar-sounding words cannot be distinguished based on sound alone, and the listener must use context to determine which phonological form is correct. Alternatively, words like ‘bark’ have multiple meanings, and the listener must use the context to determine which meaning is correct. fMRI studies have revealed different patterns of brain activation associated with these challenges. Acoustic challenges activate more cingulo-opercular regions, while semantic challenges activate regions more associated with language.

The present study employed ATL-optimised fMRI to investigate 1) how acoustic and semantic ambiguity challenges interact in the brain and 2) how the ATL is involved in overcoming these challenges.

Methods: We collected fMRI from 30 participants’ brains while they listened to naturalistic speech. The stimuli comprised 112 sentences, half containing homophones (creating semantic ambiguity). Half of these sentences were presented clearly, and half with 12-talker babble at an SNR of +4 dB (to create acoustic ambiguity). After each sentence, participants were asked whether they understood the speech. Sentence trials were intermixed with trials of silence and signal-correlated noise (SCN).
Results: We found that the left ventral ATL was active during all speech conditions relative to SCN; however, we found no differences between different speech conditions. Acoustic ambiguity increased activation in bilateral insula, anterior cingulate, and inferior frontal gyrus. Semantic ambiguity led to increased activation

in left inferior frontal gyrus. We await results from psychophysiological interaction analyses investigating how functional connectivity changes across speech conditions.
Conclusions: The present study extends our knowledge of how the brain processes speech, especially in difficult listening situations. Results for semantic and acoustic ambiguity were consistent with previous findings. The ATL activation we found is consistent with its role as a semantic hub and suggests a non-selective involvement in semantic processing of naturalistic speech. That is, the left ATL appears to support processing of semantically rich auditory stimuli, regardless of listening challenges.

T181. A Concussion Signature Without the Need of a Baseline: Comparison to Healthy Athlete Norms

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Category: Speech Perception
Background: Concussion disrupts the frequency following response (FFR) in male athletes when comparing post-concussion data with baseline data from the same individual. This study explored the feasibility of using normative data from a large cohort of Division I student-athletes to obviate the need for individual baseline FFR assessments.
Methods: We tested the difference in the encoding of F0, F1, and HF between three groups of Division I collegiate male student athletes. The first group - Healthy - included data from student athletes with no history of concussion, the second was a cohort of acutely concussed male athletes, and the third was the second groups' pre-concussion baseline. We hypothesized the absence of difference between Healthy and Baseline across the three frequency ranges and the presence of differences between both Healthy and Baseline with respect to Acute. To test our hypothesis, we ran t-tests between groups.
Results: Results confirmed our hypothesis revealing no differences between Healthy and Baseline across the three frequency ranges and the presence of differences respectively between Healthy and Acute and Baseline and Acute. This evidence supports the feasibility of using normative data as a reliable comparison with data post-concussion obviating the need of an individual's baseline assessment.
Conclusions: This study confirms the power of the FFR as a biologic measure of the impact of concussion on our auditory brain. Ongoing analyses are exploring the same question in Female student-athletes and examining the role of sub-concussion, considering the level of contact in a given sport.

Supported by NIH R01NS102500

T182. Open Board

T183. Can Narrower Frequency Allocation Improve Speech Perception in Korean Cochlear Implant Users?

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Category: Speech Perception
Background: Cochlear implant (CI) mapping is post-operative adjustment process of the CI device and it should be precisely implemented according to individual's condition and needs. Frequency allocation is an important parameter of CI mapping and is known to affect the speech perception of CI. In previous studies, restricting the upper end frequency is known to improve speech perception, but few studies have been conducted on this. Therefore, in this study, the authors conducted a study on the relationship between frequency allocation and speech perception.
Methods: We prospectively conducted a study on 10 Korean cochlear implant users. All participants were post-lingual hearing-impaired CI users who were implanted with the Cochlear Nucleus. All participants were assigned a frequency of 188 Hz to 7938 Hz, and a speech perception test was performed 2 weeks later. After

that, a frequency allocation of 188 Hz to 5938 Hz was set, and a speech perception test was performed after 2 weeks, and compared with the previous results.
Results: In this study, the narrower frequency allocation of 188 Hz to 5938 Hz showed statistically significant improvement in monosyllable speech perception tests compared to the frequency allocation of 188 Hz to 7938 Hz (88.3 ± 8.7 vs 79.4 ± 8.0, P=0.03). There was no significant difference in the CAP score, Ling 6 sound test, vower, consonant, disyllable, and sentence test.
Conclusions: This study estimated that narrower frequency allocation improves monosyllable speech perception in Korean CI users. CI mapping should be precisely implemented according to individual's condition and needs, and there are various factors and parameters that need adjustment. Among them, the authors think that when it is necessary to improve the speech perception of Korean CI users, setting a narrower frequency allocation could be considered.

T184. A Comparison of Pupillometric, Cortical-Alpha, and Self-Report-Based Measures of Listening Effort During Speech-in-Speech Recognition Task

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Category: Speech Perception
Background: Speech perception plays a key role in human life, but commonly deteriorates with ageing and hearing loss, particularly in noisy backgrounds. A growing body of work has recognized that these populations rely on increased use of cognitive resources, referred to as listening effort (LE), to compensate for the reduced fidelity of peripheral neural acoustic representations.

Although existing research on LE has utilized a wide range of biophysiological and behavioral measures, it is unclear whether these measures reflect the use of the same cognitive processes. Several studies have employed multiple simultaneous LE measures with correlational analyses to assess the extent to which these measures reflect common underlying neural mechanisms (e.g., McMahon et al., 2016; Miles et al., 2017, Strand et al., 2018; Alhanbali et al., 2019). Generally, these studies have failed to find significant correlations between most measures of LE, suggesting that they largely reflect distinct cognitive mechanisms. However, the lack of significant correlations may have been driven by small sample sizes and/or the use of target and distractor stimuli lacking semantic meaning (e.g., sequences of digits with speech-shaped noise), with the latter limiting the engagement of cognitive processes that are involved in real-world speech perception.

Methods: In an ongoing study, we address these limitations by collecting data from a large sample (anticipated n = 80) of participants spanning 18-70 years in a challenging dual-talker speech perception paradigm (i.e., with meaningful target and distractor speech). While participants perform the speech task, we simultaneously acquire three commonly used measures of LE: Pupil dilation, cortical alpha-band power (ABP) measured via electroencephalography (EEG), and self-reported effort. The stimuli are meaningful sentences embedded in spatially co-located distractor speech sampled from a narrative audiobook. To vary the task difficulty, the target-to-masker ratio is randomly selected for each trial from a range between -10 to 5 dB SNR. Following each sentence (trial) and a subsequent 3-sec retention interval, participants are prompted to repeat the target sentence, and then rate the subjective LE they exerted in the trial on a 7-point scale.
Results: The EEG and pupillometric data are analyzed to extract trial-by-trial ABP and baseline-corrected peak pupil dilation, respectively, for use in subsequent pairwise correlational analyses with the self-report data. Additionally, we will explore the effects of SNR, participant age, and measures of cognitive performance (working memory capacity, attentional inhibition, fluid intelligence) on these measures of LE and on speech-perception performance.
Conclusions: The results will provide insights about the extent to which popular measures of LE rely on common underlying cognitive mechanisms and help inform our understanding of how they are affected in healthy ageing. [Supported by NIH grant R21DC020788]

T185. Estimation of Speech Recognition Threshold in Noise From High-Frequency Pure Tone Threshold in Normal-Hearing

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Category: Speech Perception

Background: People with hearing loss are known to have more difficulty hearing speech in noise than people with normal hearing. Clarification of the relationship between speech recognition threshold in noise ability and the pure tone threshold may facilitate improvements in speech recognition in noise ability through the appropriate fitting or compensation related frequency region of hearing aids or cochlear implants. In this study, we clarified this relationship through comparison of the scores of the Japanese Hearing in Noise Test (J-HINT), a threshold test for speech recognition threshold in noise ability, with pure tone thresholds.

Methods: Seventy subjects with normal hearing aged 30 to 69 years old (mean age of 52 years) participated in the present study. The J-HINT was conducted in three conditions: Noise Front (NF), Noise Right (NR), and Noise Left (NL), where the noise signal was presented from the subject's front (0°), right (+90°), left (-90°), respectively. The speech signal was always presented from the frontal loudspeaker. The Noise composite score (Ncs) was calculated by the equation Ncs = (NF score×2+NR score + NL score)/4. The correlation coefficients between the J-HINT scores and pure tone thresholds (0.125, 0.25, 0.5, 1, 2, 4, 8 kHz) on the good ear side were calculated to evaluate their relationship. Multiple regression analysis was further conducted to obtain an equation to estimate the J-HINT score from pure tone hearing threshold on the good ear side.

Results: The correlation coefficients between pure tone threshold and J-HINT score showed a tendency to increase as the frequency increased. Multiple regression analysis showed that the multiple correlation coefficients were 0.62 (p less than 0.01) for NF score and 0.70 (p less than 0.01) for Ncs, with 57-63% of subjects within 1.0 dB of the residuals of the estimated equations for NF and Ncs, and 79-81% within 1.5 dB.

Conclusions: The results of this study suggest that there is a relationship between speech recognition threshold in noise ability and pure tone threshold at high frequency. It may be important to compensate for hearing threshold at high frequency range when fitting of hearing aids or cochlear implants to improve speech recognition in noise ability. Furthermore, these results showed that use of the obtained multiple regression equation may be used to estimate speech recognition threshold in noise ability from the pure tone thresholds.

T186. Relationships Between Spectral Cues and Consonant Perception Errors in Pediatric Cochlear Implant Users

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Category: Speech Perception

Background: Even though cochlear implants (CIs) improve communication and assist in overcoming many educational challenges for pediatric users, performance outcomes are highly variable. Speech requires that listeners perceive changes in sound intensity over time (temporal cues) and across frequency (spectral cues). Spectral resolution, an important predictor of speech perception, is an ability to perceive differences in intensity across the frequency spectrum in a complex sound. It relies independently on frequency resolution (FR) and spectral modulation sensitivity (SMS). In children with CIs, speech perception is also influenced by natural developmental constraints, amount and quality of acoustic information accessible through the CIs, as well as duration of device use. For production, studies demonstrate NH children can produce some consonants by age 3 and all English consonants by age 7; stop consonants are acquired earlier than fricatives (Dodd et al., 2003). We anticipate that children with CIs will also follow this pattern in the perception domain. We hypothesize that younger children will make more errors and fewer errors will be evident in stops compared to fricatives. In addition, we predict that better spectral resolution will be associated with fewer errors for fricative and stop consonants.

Methods: The study included 15 children (5-16 years old) implanted prior to age two. Listeners were tested using one CI, in their better ear if bilaterally implanted, in soundfield using their clinical processor. Spectral ripple discrimination was measured using a 3-afc 2-down 1-up adaptive procedure to determine the highest ripple density distinguishable from a highly-rippled reference (Noble et al., 2023). For each listener, thresholds were obtained at modulation depths 3, 7, 10, and 15 dB, in random order, and data were fit to a spectral

modulation transfer function (SMTF) where intercept reflects SMS and slope reflects FR (Horn et al, 2017; Noble et al., 2023). Consonant perception stimuli included 12 specific target consonants (6 fricatives and 6 stops), spoken by a single male talker in /a/-consonant-/a/ format. Each consonant was presented 3 times, in random order, and scored as percent correct. For analysis, error patterns were compared between two age subgroups: younger (ages 5-8, N=7) and older (ages 9-16, N=9).
Results: The number of errors revealed a negative association with age, suggesting improved performance with increasing age. However, this association was only evident for stops, not for fricatives. In addition, better spectral resolution was correlated with fewer errors in stop consonants but did not show the same association with fricatives.
Conclusions: The different patterns in development of stop and fricative consonant perception suggest different mechanisms are involved beyond spectral auditory acuity. In future research, we plan to expand the sample size and introduce measures of temporal resolution to further investigate the sources of performance variability among CI users.

T187. Cochlear, Neural, Cognitive, and Linguistic Contributions to Speech-In-Noise Perception in Middle-Aged Adults

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Category: Speech Perception
Background: Perception of speech-in-noise (SPIN) is influenced by a variety of factors including Cochlear, Neural, Cognitive, and Linguistic mechanisms. However, we are still uncertain about the independent contributions of each of these measures to SPIN. In this study, we employed a host of behavioral and electrophysiological metrics to explore the independent contributions of each of these factors towards SPIN.
Methods: We recruited middle-aged Monolingual English speakers with hearing acuities ranging from normal to moderate sensorineural hearing loss. All participants underwent a total of 10 tests corresponding to the different factors – Cochlear (Standard and Extended High Frequency audiometry, Distortion Product Oto-acoustic Emissions), Neural (click-evoked Auditory Brainstem responses at 80 dB SPL), Cognitive (NIH toolbox and Woodcock-Johnson tests), Linguistic (Test of Word Reading Efficiency and Test of Silent Word Reading Fluency). Each of these tests provided multiple metrics associated with different inter-related mechanisms. SPIN was estimated using QuickSIN and Words-in-noise (WIN) tests. Principal Component Analysis (PCA) and Linear Regression analysis provided the inter-relations between each of these factors as well as the contributions of the different factors towards SPIN.
Results: PCA analyses showed both QuickSIN and WIN were loaded onto the factor containing measures of peripheral auditory acuity (hearing thresholds). Linear Regression done separately for QuickSIN ($R2 = 0.930$) and WIN ($R2 = 0.864$) revealed that QuickSIN was primarily predicted by the TOSWRF (linguistic) whereas WIN was predicted by the peripheral auditory measures (cochlear). Neural and cognitive metrics were not a factor for either SPIN test.
Conclusions: Our study shows that the inclusion of multiple dimensions successfully explains the overall SPIN difficulties in middle-aged adults. The amount of linguistic information in the test material to measure SPIN (QuickSIN vs. WIN) likely modulates the overall weight placed on each factor.

T188. Phase Coding in Phoneme Processing Slows With Age

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Category: Speech Perception

Background: The comprehension of phonemes is a fundamental component of speech processing. EEG amplitude in response to phonemes has previously been identified as an indicator of speech comprehension performance in hearing-aid users. Altered neuro-electric responses to phonemes may also occur in presbyacusis, even when only a mild or no hearing threshold elevation can be observed. If speech comprehension thresholds, even after correcting for pure-tone thresholds, reflect a clinically relevant deficit, we propose that speech EEG provides a biomarker for consequences of cochlear synaptopathy on central neural processing.

Methods: To test this possibility, we collected pure-tone and speech audiometric data (PTA4, PTA-EHF and the German word matrix test OLSA) and EEG during passive listening to six different phoneme stimuli (/y/,/di/,/bi/,/o/,/du/,/bu/) in 80 subjects aged 18 to 75. The phoneme stimuli contained either one (/o/,/du/,/bu/) or two (/y/,/di/,/bi/) formants below the human phase-locking limit (~1500 Hz) and thus have different amounts of information available for temporal fine structure coding.

Results: We confirm that phoneme-evoked EEG amplitude (PEA) correlates with speech comprehension. Specifically, PEA decreased with elevated thresholds for speech and pure tones as well as with increasing age. Furthermore, we found that the temporal delay of phoneme-evoked EEG responses (PED) increased with hearing thresholds and age. However, PED and PEA are non-redundant neural parameters of phoneme processing. Pure-tone thresholds expressed as PTA4 (0.5-4 kHz) correlated best with PEA, while hearing loss in extended high-frequencies (EHF: 11.3-16 kHz) affected PED more than PTA4 did. More importantly, increased PED correlated with speech comprehension deficits beyond what can be explained by pure-tone threshold elevation.

Conclusions: The absolute size of PED and its correlation with age, the lack of lateralization of PEA in combination with the fundamental frequency of the phoneme stimuli suggest a predominantly thalamic generator of phoneme-evoked EEG responses. When analyzing subjects who over- or under-performed their expected OLSA threshold, based on a multivariable regression between OLSA and all pure-tone thresholds (PTA4 and EHF), we discovered that proficient listeners could compensate for elevated pure-tone thresholds at the expense of decreased temporal processing speed, whereas poor performers could not. We therefore conclude that a proper understanding of PED can lead to the development of a new objective marker for speech comprehension.

T189. Decoding Speech From Cochlear Implant Users' EEG

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Category: Speech Perception

Background: In the typical auditory system, different speech syllables evoke different temporal patterns of cortical activity, which makes it possible to decode speech from EEG signals. How accurately speech is decoded from EEG predicts the listeners' speech intelligibility. We ask if such speech decoding is possible using cochlear implant (CI) users' EEG, and whether individual differences in the decoding accuracy predict the CI listeners' speech intelligibility. Thus, our goal in the present study was to use a large cohort of CI users to examine item-level differences in auditory evoked responses to a large battery of phonetically balanced test items. This approach can reveal the relationship between the neural pattern similarities and phonetic categories, and whether such similarities can predict speech intelligibility.

Methods: One hundred twenty adult CI users were asked to identify isolated monosyllabic words. A total of 30 item sets were used. Within each item set were four words that were only differentiated by their initial consonant. Each of the four words served as a target once in each of two noise conditions while the other three

words in the set were the foils for that trial. Simultaneously, 64-channel EEG measured cortical responses to the speech. Grand average evoked potentials were obtained for each word to examine dissimilarities in neural encoding across various phonetic categories as well as similarities across items within the same category. Finally, similarities between the evoked potentials for a target word and its three foils were used to predict the accuracy of recognizing the word.
Results: The mean speech recognition performance across all the items was reasonable (mean: 70.9%) with the hardest individual items at closer to chance level (29.9%) while easiest items were close to ceiling (98.0%). The morphology of evoked potentials for each item tends to be similar within each phonetic category. Stop consonants all appear to have similar morphologies to one another with slight differences in timing or amplitude. Similarly, nasals, glides, and liquids all have similar morphologies to one another. In contrast, the morphology of fricatives varies widely from one another. Comparing the evoked response to a target fricative to its three competitors showed a moderate correlation between speech recognition performance and the disparity between the evoked potentials.
Conclusions: Even among a large cohort of CI users with various devices and configurations, we were able to find clusters of similarity in the speech-evoked neural signals that obey phonemic categories in the language. Furthermore, evoked responses to fricatives (which had the least similar morphologies to one another) predicted the perceived difficulty of each item. The modeling of neural response patterns to various speech content derived from this study could lead to the use of EEG to help with the tuning of CIs.

T190. Non-Native and Noise-Masked Speech Rely on Different Auditory Processes in NH and SNHL Middle-Aged Adults

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Category: Speech Perception
Background: Communication rarely takes place under pristine listening conditions. Often, distortion of the talker's message occurs before it even reaches the listener's ears. Speech in noise (SIN) and non-native accents are two common types of distortion that can make recognition challenging for everyone, but exceedingly so for individuals with hearing loss. Moreover, what is known about how hearing loss impacts recognition of distorted speech comes from studies in older adults, where interactions such as cognitive decline can complicate the interpretation. What happens during middle-age, an age span that represents the majority of the workforce and a period when speech-recognition difficulties emerge, is unknown.
Methods: We tested middle-aged monolingual adults between the ages of 30-60 years on a test of accented-speech and speech-in-noise recognition, standard and extended high-frequency audiograms, and the frequency-following response to the speech sound 'd'. The FFR to a single complex sound (e.g., a speech syllable), contains multiple subcomponents that can be quantified independently, therefore, we predict that the fundamental frequency (F0), a component that underlies pitch perception and is important for grouping auditory objects and following a talker's voice in noise, would support speech-in-noise recognition. In contrast, harmonics are especially important for language skills, such as phoneme discrimination. Non-native phoneme pronunciation differs from native pronunciation in large part on the basis of harmonic content and so we predicted that formant-encoding strength would support accented speech recognition. We also predicted that hearing acuity would play a role in both, especially for the hearing loss group.
Results: Consistent with our predictions, we found that F0 encoding aligned with SIN abilities while formant encoding aligned with accented-speech recognition. Moreover, we found that adults with mild sensorineural hearing loss (SNHL) rely more strongly on these auditory mechanisms to understand distorted speech.
Conclusions: Importantly, the majority of these individuals show only a very mild SNHL, with levels that fall just outside of normal limits, suggesting that even subtle differences in hearing acuity alter how people comprehend distorted speech. Together, these findings highlight how listeners engage different mechanisms to understand speech in noise and accented speech, and the exacerbation subtle hearing deficits have on these processes.

T191. Porosity and Bone Density Distribution in the Human Ossicular Chain: A Synchrotron-Based Microtomography Study

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Category: Middle and External Ear

Background: The auditory ossicles amplify and transmit sound from the environment to the inner ear. The distribution of bone mineral density is crucial for the proper functioning of sound transmission as the ossicles are suspended in an air-filled chamber. However, little is known about the distribution of bone mineral density along the human ossicular chain and within individual ossicles.

Methods: We investigated bone density distribution in three fresh-frozen human specimens using synchrotron-based phase-contrast microtomography images. Color-coded 3D volume renderings were analyzed with the help of medical image analysis software. In addition, we analyzed the volume and porosity of the ossicles.

Results: The porosity for the auditory ossicles lies between 1.38 and 4.59%. We observed a high inter-individual variability. The average volume for the mallei is 13.85mm3 ± 2.15, for the incudes 17.62mm3 ± 4.05, and 1.24mm3 ± 0.29 for the stapedes. Although the bone density distribution varied considerably, all three samples showed a low-density area surrounding nutritional cavities and a relatively high bone mineral density for the stapes footplate. We could also see a correlation between low bone mineral density and the degree of porosity.

Conclusions: Our study has shown that the overall mineralization of the ossicles is higher than that of the cortical and trabecular bone. Regarding bone density, we identified a pattern for all three samples, showing a low-density area surrounding the nutritional cavities. Further, we observed a relatively high density of the stapes footplate, which is also evident in all samples and may indicate its importance in transmitting sound waves to the inner ear.

T192. Human Middle-Ear Forward and Reverse Transfer Functions: A Finite-Element Modeling Study

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Category: Middle and External Ear

Background: Sounds entered in the ear canal (EC) traverse the middle ear (ME) in the "forward" direction to reach the cochlea. Complex electro-mechanical processes within the cochlea, in turn, generate faint sounds that propagate through cochlear fluids and traverse backwards through the ME in the "reverse" direction toward EC, where these sounds are measured as otoacoustic emissions (OAEs). Much remains to be understood about how OAEs are generated and how they are shaped when traveling through the ME and measured in the EC. In a recent article, we could map the stimulus-frequency OAE to the ME round-trip transfer function in a mouse model by removing the reflections at the EC-entrance and stapes–cochlea boundaries. In this study we quantify the conventional and modified ME transfer functions in a human model.

Methods: Using image registration methods, we coupled human ME and cochlear models, each separately verified in the previous studies. The viscosity effect of cochlear fluid on its impedance was studied with mesh convergence analysis with at least three computational nodes within the viscous boundary layers at each frequency. Impedances and reflections at the EC and cochlea were calculated alongside the conventional ME forward and reverse transfer functions. The effect of the ME properties (e.g., the stapes annular ligament stiffness) were studied in a series of sensitivity analyses to quantify the variations of the transfer functions.

Results: Mesh convergence study indicated that the cochlear input impedance is not sensitive to fluid viscosity; two order magnitudes variation of the viscosity coefficient causes less than 50% impedance change at the frequency range of 20 Hz to 20 kHz. The viscosity, however, has significant effect on the traveling wave of the basilar membrane. The spectra of modified ME forward and reverse gains are smoother than the conventional gains, as the standing wave effects are mostly eliminated by removing the reflections at the EC-entrance and stapes–cochlea boundaries.

Conclusions: The viscosity of the cochlear fluid has negligible effects on the cochlear impedance, but it affects the traveling wave of the basilar membrane and thus, the motion of the organ of Corti is significantly influenced. Limiting the viscosity to the boundary layers of the basilar membrane reduces the computational

time significantly. By removing reflections at the EC-entrance and stapes footplate–cochlear-fluid interfaces, we are able to quantitatively map OAE changes to changes in the modified round-trip ME gain due to variations in the ME parameters. Understanding how OAEs are shaped by the ME enables us to interpret OAEs in terms of the status and pathologies of both cochlea and the ME.
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T193. Shelf-Life Studies on a Single Application Otitis Externa Drug Delivery System

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Category: Middle and External Ear
Background: Otitis externa, or outer ear infection, is a pathological condition that impacts both humans and animals. The recommended approach for treating otitis externa involves the application of ear drops. Nonetheless, the need for frequent administrations and prolonged treatment duration have been linked to low patient adherence and heightened risk of complications. In order to enhance ease of use and enhance patient outcomes, our group has developed a tetraethyl orthosilicate-based thixogel that serves as a one-time treatment/drug delivery option for otitis externa. We also assessed the drug delivery system formulation repeatability and reproducibility, material sources and tolerances, release of a variety of model drugs, and impact of application-specific physiological factors such as local pH or enzymatic activity on drug release. In this study we present our findings on the shelf life of the thixotropic drug delivery systems.
Methods: Thixogels were prepared by the hydrolysis of tetraethyl orthosilicate (hTEOS) and subsequent combination with aqueous hyaluronan (HA) at different concentrations, followed by the adjustment of the solution's pH to ~7.65. Accelerated aging studies were performed based on the 10-degree-rule methodology (Q10). Real time shelf-life determinations were additionally performed for all formulations. At the end of the shelf-life study, samples were assessed for visual appearance, rheological properties, drug release, and dry substance content.
Results: The shelf-life of the evaluated thixogels was found to be cold-chain independent and ranged from 4 months to 8.5-month real time, depending on the amount of HA in the formulation. We also found the HA component of the thixogel was the main determinant of shelf-life. Various product formulations were then explored to extend the product shelf-life
Conclusions: Our findings suggest that thixogels are cold-chain independent with their shelf-life significantly impacted by the HA component of the formulation. Application specific optimization of HA parameters such as molecular weight and/or concentration can clearly be exploited to adequately tailor the final product's shelf life.

T194. Auditory Steady-State Response Elicited by Rising-Frequency Silent Interval Embedded Within Broadband Noise

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Category: Other, Auditory Temporal Processing
Background: Auditory temporal resolution plays an important role in speech perception. Gap detection threshold (GDT) is one of the proposed indices to determine auditory temporal resolution. We propose an objective GDT measurement method using auditory steady-state response (ASSR) elicited by silent intervals at a rate of 40 Hz or 80 Hz embedded within broadband noise (this ASSR is termed "GapASSR") [Kadowaki et al., 2022; Kadowaki et al., 2023]. The amplitude of ASSR can be increased by compensating for the cochlear delay characteristics, i.e., by using a rising-frequency chirp signal [Elberling et al., 2007]. Therefore, the amplitude of GapASSR can be improved using a stimulus that has a rising-frequency silent interval, i.e., the location of the interval differs slightly with each frequency based on the cochlear delay characteristics. Cochlear delay characteristics have been studied based on auditory brainstem response (ABR) elicited by the

click-on high-pass masking condition [Don et al., 2005], tone-burst-evoked ABR [Neely et al., 1988], stimulus-frequency otoacoustic emissions [Shera et al., 2005], and the linear cochlear model [de Boar, 1980]. However, delay characteristics have not been evaluated based on responses elicited by the silence interval. Therefore, delay characteristics and their effects on GapASSR were evaluated.
Methods: GapASSRs under three stimulus conditions were measured for normal hearing participants: (A) when compensating for the cochlear delay characteristic measured for each participant; (B) when compensating for the cochlear delay formulated by Neely et al.; and (C) when not compensating for the cochlear delay, i.e., the timing of the silent interval insertion was constant regardless of the frequency. The delay characteristic in condition A was obtained by approximating the delay of the ASSR elicited by a silent interval embedded within the narrowband noise by the equation tg = k f^(-d), where f denotes the frequency, tg denotes the delay, and k and d denote constants estimated by approximation. In addition, experiments were conducted on patients with suspected auditory processing disorders under conditions B and C.
Results: The results showed that the per-individual estimated cochlear delay was longer than those of the Neely et al. data, that the phase synchrony and amplitude of GapASSR were significantly larger under compensating conditions (conditions A and B) than under non-compensating conditions (condition C), and that the phase synchrony and amplitude of the ASSR were maximized when compensating for the estimated cochlear delay for each individual (condition A).
Conclusions: It is desirable to measure the auditory ability index immediately, especially when using an electroencephalogram to examine neonates in a clinical setting. The results of this study show that it is expected that the measurement time can be shortened by compensating for the cochlear delay compared to the conventional GapASSR.

T195. Cerebellar Activity for Compensatory Saccade During the Head Impulse Test in the Vestibular Impaired Monkey

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Category: Vestibular: Basic Research and Clinical
Background: The vestibulo-ocular reflex (VOR) plays a critical role in gaze stabilization. Patients with vestibular loss cannot maintain their gaze on the 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 the 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 reduced by the saccade. Also, vision is greatly reduced during the saccade so there is little perceived retinal slip. Thus, learning to generate covert saccades is important for improving the patient's quality of life. Despite this, little is known about the neural basis of these movements.
Methods: We examined saccade behavior during HIT in a vestibular impaired monkey. We also recorded unit activity from the flocculus (a cerebellar cortical region that encodes vestibular signals) during HIT. In both experiments, we also recorded visually-guided saccades and catch-up saccades during sinusoidal chair movement (i.e., well-studied saccade types) to compare covert and overt saccades with them.
Results: Similar to human patients, vestibular impaired monkeys made covert saccades and overt saccades during HIT. Overt saccades showed a typical main sequence similar to visually-guided saccades; i.e., a strong correlation between saccade peak velocity and saccade amplitude. However, covert saccades did not show the same correlation. We also found that flocculus neurons exhibited a burst and pause for covert saccades. The flocculus neurons did not exhibit the burst, but rather only paused for overt, visually-guided, and catch-up saccades.
Conclusions: The results of these experiments suggest that the mechanisms to induce covert saccades are fundamentally different from those of overt and visually-guided saccades. Flocculus neurons may be involved in driving covert saccades. We speculate that the burst-pause activity of the flocculus neurons might induce rebound excitation of vestibular nucleus neurons, which, in turn, may induce a burst in motoneurons to evoke covert saccades.

T196. Photodynamic Therapy-Induced Precise Attenuation of Targeted Semicircular Canals for Treating Intractable Vertigo

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Category: Vestibular: Basic Research and Clinical

Background: Vertigo is a common symptom of various diseases, affecting a large number of people worldwide. Intractable vertigo can significantly handicap patients in daily activities. Current treatments for intractable peripheral vertigo can be invasive and associated with complications such as hearing loss. Photodynamic therapy (PDT) is a non-invasive therapeutic approach due to its excellent specificity and minimal invasion of non-targeted tissues. Inspired by its space and time precision, we developed a PDT-based approach for treating intractable peripheral vertigo in the mouse model, in order to avoid hearing loss.

Methods: Our PDT used photosensitizer chlorin e6 (Ce6) packed in nanoparticles (NPs) with PLGA as the shell coated with polyethylene glycol (PEG). A small hole was opened on the horizontal semicircular canal (SCC), about 2 mm from the cupula. Photosensitizer was then injected into the SCC via the hole. Thirty minutes later, red light (650 nm) was applied via an optical fiber to induce lesion on the cupula. Before and seven days after surgery, vestibular function of the animal was evaluated with angular vestibulo-ocular reflex (aVOR) and off-vertical-axis rotation (OVAR) for the SCC and otolith organ, respectively. Hearing test were performed at the same time with auditory brainstem response (ABR).

Results: 1) A high dose (10-minute illumination) of PDT attenuated the function of both the semicircular canal and otolith organ and damaged their hair cells. 2) A lower dose (2-minute illumination) limits the damage within the semicircular canal with less intense. 3) The PDT effect can be achieved with red light illumination through bony wall of the SCC. 4) PDT exerted no effect on hearing function or cochlear hair-cell viability in all experiments.

Conclusions: Our PDT-based approach for attenuating the function of the semicircular canals shows promise as a less-invasive and targeted therapeutic option for treating vertigo. One significant advantage is the ability to precisely control the level of attenuation and the affected area by adjusting the duration of light exposure. It can potentially be minimally invasive if the photosensitizer can be delivered into the SCC less invasively.

T197. Effects of Galvanic Vestibular Stimulation on Vestibular System in Hypergravity-Induced Motion Sickness Animal Model

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Category: Vestibular: Basic Research and Clinical

Background: Galvanic vestibular stimulation sends electrical currents to the vestibular end organs, resulting in changes of discharge in the vestibular afferents. Galvanic vestibular stimulation plays an important role in understanding sensory signal processing in the vestibular system under normal and pathological conditions. It is known that GVS could be a relevant tool to assess the neuronal computations of vestibular system. Despite the previous various studies of GVS for the assessment or treatment of vestibular disorders, the exact mechanism for this non- invasive electric stimulation remains unclear. To investigate the effects of galvanic vestibular stimulation on vestibular system under the altered gravity condition. We assessed how the GVS modulate the response of altered gravity in the vestibular system.

Methods: For hypergravity stimulation, we use our gravity simulator, Inha G-simulator, which is a centrifuge device for animals. With 4G hypergravity stimulation was exposed for 1week with SD male rats (aged 7~8weeks, weighing 250-300g). The vestibular function with animal rotator was assessed after the hypergravity stimulation (DAY0, DAY 3, DAY7). Animals were divided into two groups according to the presence of GVS stimulation. In the GVS group, stimulation was applied for 30 minutes daily for 5 days. The western blotting and immunohistochemistry analysis to quantify the protein expression of serotonin and histamine receptors in vestibular nuclei.

Results: After 4G stimulation, hypergravity group showed decreased vestibulo ocular reflex (VOR) responses compared to the control group. VOR responses were significantly impaired in the non- GVS group. GVS stimulation significantly improved VOR gain compared to the non-GVS group. The expression of 5-HT1B, 5-HT2A receptors were increased significantly compared to the control group in the vestibular nuclei after hypergravity stimulation. The expression of 5-HT1B and 5-HT2A was significantly decreased in GVS group compared to non-GVS group. The expression of histamine receptor (H1 and H2) were increased significantly after hypergravity loading. And they showed changes between the groups. GVS stimulation effectively decreased the expression of H1 and H2 in the vesibular nuclei.
Conclusions: By observing the data, we can induce the results that hypergravity stimulation causes remarkable changes in VOR gain. GVS intervention induced the modulation of histamine and serotonin receptors expression in the vestibular nuclei.

T198. Assessment of Vertical Semicircular Canal Function: The Case for Computerized Rotational Head Impulse Testing

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Category: Vestibular: Basic Research and Clinical
Background: Clinical assessment of the vertical semicircular canals (SCC) is limited, as there is currently no single ideal test that can reliably and accurately assess their function.
The video head impulse test (vHIT) is a non-invasive and widely available test that can be used to assess the function of all six semicircular canals. Unfortunately, this test is difficult to practically administer for inexperienced practitioners, is subject to significant inter-provider variability, and may be difficult to perform in young children or patients with limited mobility. Recently, our group developed a novel, computerized rotational head impulse test (crHIT) testing paradigm to assess the function of the vertical SCC via delivery of brief, whole-body impulsive rotations comparable to the angular motion used for traditional vHIT. The purpose of this work was to compare vertical crHIT and vHIT measures among healthy subjects to characterize the viability of these tools for assessment of the vertical canals.

Methods: Sixteen male and 23 females (n=39) with a mean age of 25.7 (±5.6) years with no history of vestibular disorders or traumatic brain injury were enrolled following written informed consent (#20190034). Each participant underwent 1) vHIT testing of the horizontal and vertical canals (ICS Impulse Otometrics) and 2) oculomotor, horizontal/vertical crHIT testing (Neuro-Otologic Testing Center (Neurolign USA, Inc.). Eye position and velocity were measured using a binocular infrared video-oculography system recording at a frame rate of 250 fps (res: less than 0.1 deg) with sensors in the pitch (x), roll (y), and yaw (z) planes. Paired t-tests and Bland-Altman plots were performed.
Results: We observed that crHIT gain (1.03±0.04) was significantly higher than vHIT gain (0.93±0.10) in the left lateral SCC (p less than 0.01), though there was no significant difference between crHIT gain (1.02±0.06) and the vHIT gain (1.01±0.09) In right lateral SCC (p=0.67). Interestingly, the mean gain in the vertical SCCs was greater for crHIT than vHIT in all canals tested. The left anterior and right posterior (LARP) SCC gain of crHIT (LA: 0.98±0.08; RP: 1.02±0.09) was significantly higher (p less than 0.01) than the gain of vHIT (LA: 0.76±0.11; RP: 0.83±0.15). This was also observed when comparing crHIT gain values in the right anterior (1.02±0.06) and left posterior (0.97±0.10) (RALP) canals when compared to vHIT (RA: 0.71±0.17; LP: 0.81±0.16) (p less than 0.01). The gain of crHIT (0.97±0.10) was significantly higher than the gain of vHIT (0.81±0.16) in left posterior SCC (p less than 0.01). Bland-Altman plots indicated quantitative agreement only in the lateral SCC.
Conclusions: crHIT can reliably evaluate SCC function in normal subjects. Our lab is actively exploring this technique on series of individuals with defined vestibular pathologies.

T199. Sex Differences in Vestibular Function in Mice

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Category: Vestibular: Basic Research and Clinical
Background: Vestibular dysfunction in humans have been documented to occur more frequently in women. The mechanism for differences in incidences of vestibulopathies between sexes is not well documented. As mice are popular research models for vestibular function and dysfunction, it is important to understand sex differences in mice models to further develop our understanding of health, disease, and treatment in humans. This study seeks to understand the role of sex in vestibular function and the influence of biological sex on the effects of experimental testing of vestibular function in mice. To determine if outcomes vary between sexes which could influence the applicability of studies following only one sex.
Methods: Manuscripts were identified in OVID Medline using MeSH and keywords for the terms: mice, mouse, male, female, sex, vestibular, function, and tests. Manuscripts were included if they were original research, written in English, and used mice. The content of each manuscript was screened for sex of the mice, vestibular function testing, and discussion of sex-based results.
Results: Most studies found in the search used one sex to evaluate the variables studied to limit the confounding variable of sex. 12 studies evaluated vestibular function of males and females separately. Of these, five found no differences between the sexes in vestibular function. The seven where differences were found, showed generally worse vestibular metrics for females than males offering various explanations for these differences as applicable to the individual study.
Conclusions: These findings prompt the need to further explore the applicability of studies following only one sex. This is especially important when these findings are applied to humans in which the potentially significant differences between sexes in studies could change medical care of patients. While sex can be a confounding variable, it should also be analyzed as an important factor when formulating experiments or interventions and studying how they can be applied to medical practice.

T200. Noise Exposure Alters Head Stability and Orientation With Respect to Gravity in Rats

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Category: Vestibular: Basic Research and Clinical
Background: Exposure to intense noise damages both the cochlea and the vestibular end organs. Our previous report showed reduced vestibular short-latency evoked potentials (VsEP) and reduced number of calretinin-positive (CR+) calyces in the saccule following noise exposure (Stewart et al. 2020). Here, we examined the effect of noise on the animal's head stability and orientation.
Methods: We measured the angular velocity and linear acceleration of a rat's head motion using a motion sensor while the animal was in the dark with its body restrained but its head free to move. The measurements were made before and after a 4-hour, 120 dB noise exposure. The VsEP was also measured at baseline, 1 week, and 4 weeks after the noise exposure. The number of calretinin positive (CR+) calyces were counted in the saccule and utricle 5 weeks following the noise exposure.
Results: The VsEP N1 and P2 peak latencies were significantly delayed (p less than 0.01, t-test) and the N2P3 amplitude was significantly reduced following the noise exposure (p less than 0.001). The number of CR+ calyces in both saccule and utricle were also significantly reduced by ~25 % (p less than 0.001). The size of the reduction in the N2P3 amplitude was significantly correlated to the number of CR+ calyces in the utricle ($r = 0.78$, $p = 0.0027$, Pearson's correlation test), but not the saccule. Five out of 17 animals showed a significant change in the median pitch orientation of the head, with 4 animals going downward and 1 upward, following the noise exposure. The size of change in the pitch orientation was significantly correlated to the number of CR+ calyces in the saccule ($r = -0.67$, $p = 0.0033$), but not in the utricle. The animals with a larger delay in the VsEP N1 peak latency and/or a smaller number of CR+ calyces in the utricle following the exposure showed a significant decrease in the average speed of y-axis rotational head motion (4 out of 17 animals), while those with a smaller change showed a significant increase (5 animals). In addition, the animals with a larger noise-induced change held their head motionless for a longer time following the noise exposure.
Conclusions: Noise exposure was inherently destructive to the animal's head stability, manifested as an increase in average head motion in mildly affected animals. However, the animals with largest effect of noise

on the VsEP and fewer CR+ calyces actually exhibited reduced durations and speed of their head motion, possibly as a behavioral adaptation. The noise exposure also significantly altered the pitch of the head orientation in animals who had the fewest countable CR+ calyces in the saccule.

T201. Measuring the Effect of Self-Reported History of Noise Exposure and Noise-Induced Hearing Deficits on Verticality Perception

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Category: Vestibular: Basic Research and Clinical

Background: Noise exposure is prevalent worldwide and associated with deleterious effects including hearing loss and possible vestibular dysfunction. Previous work in mammals shows acute and chronic noise exposure can cause vestibular organ damage and sensorimotor deficits in behaviors reliant on vestibular information (e.g., postural control). A major limitation of analogous work in human populations is estimating a given participant's lifetime noise exposure, as estimation relies upon participants' recall about past noisy activities. In the current study, we examine the predictive value of using participants' recall and measurements of hearing loss for noise-induced changes in verticality perception, which reflect changes in otolith function.

Methods: We assessed lifetime noise exposure and hearing in 33 participants (15 female, mean age = 45.5 years) using the Noise Exposure Structured Interview (NESI) and pure-tone audiometry respectively. We use auditory thresholds in the 4kHz band as a proxy for noise-induced hearing damage, as an audiometric notch at this frequency is associated with noise-induced hearing loss. We assessed participants' perception of vertical using the subjective visual vertical (SVV) task while the head was upright or tilted 20° relative to the body, and the rod-and-frame task (RFT) with the frame tilted 20° relative to vertical. As expected, head tilt and frame presence resulted in systematic bias in participant's perception of vertical. In addition to biases in each condition, we calculate change in bias due to head tilt or frame presence and evaluate the extent to which NESI scores and 4kHz thresholds predict these changes alongside observed error.

We build two sets of multiple linear regression models with several predictors: age, height, functional balance (timed up-and-go test), and either lifetime noise exposure (log-transformed NESI scores; NESI models) or hearing thresholds in the 4kHz band (difference between ears/hearing in worse ear at 4KHz; audiometric models). We use the model sets to fit five separate outcome variables: absolute and directional SVV error, absolute RFT error, and changes in bias due to head tilt or frame presence. We perform stepwise reduction in each model by removing parameters and minimizing the Akaike Information Criterion (AIC) of each candidate model until reaching the model with best AIC.

Results: We show that both NESI scores as well as audiometry at the 4kHz band are broadly predictive of participant error in each task. NESI and audiometric models are significantly predictive of directional SVV error, absolute RFT error, and frame presence-induced bias change. Additionally, the audiometric model significantly predicts absolute SVV error, while the NESI model significantly predicts head tilt-induced bias change.

Conclusions: Taken together, these results suggest that audiometry and participant interview can provide complementary assessments of lifetime noise exposure that can be predictive of vestibular function and verticality perception.

T202. Mechanical Stimulation-Induced ATP Secretion Through Gap Junction Hemichannels and Vesicular Release in the Vestibular System

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Category: Vestibular: Basic Research and Clinical

Background: It was reported that ATP is released from gap junction hemichannels of supporting cells and stria marginal cells to endolymphatic space in the cochlear and mediates cochlear functions through various purinergic receptors. Similarly, ATP is thought to exist in vestibular organs to modulate the function of balance

system; however, direct functional evidence for the ATP release in the vestibular labyrinth is lacking. We tried to investigate the functional evidence and location of ATP release in vestibular organs.
Methods: The membranous vestibular labyrinths of C57BL/6 mice were harvested, and the utricle, saccule, ampulla, and common crus were separated by microdissection. To measure the ATP secretion rate per area of each tissue, the surface area of each tissue was calculated by the geometric method using a confocal microscope. Each tissue was immersed in physiologic saline and rotational mechanical stimulation (250 rpm) was applied with a three-dimensional multi-shaker. The amount of ATP secretion was measured by a bioluminescent-based method using an EDTA-containing luciferin-luciferase assay kit. In selected samples, bafilomycin A1 (100nM), carbenoxolone (CBX, 10nM), and clodronate (100nM) were applied to inhibit ATP secretion by vestibular dark cells, connexin hemichannels, and VNUT-mediated ATP release. ATP-enriched vesicles were stained with quinacrine for detecting ATP reservoir. We investigated the distribution of connexin 26 (CX26) in mouse vestibular dark cell areas by immunohistochemical staining.
Results: The ATP secretion per surface was significantly increased during the stimulation (0.11 ± 0.03 nM/mm2, 0.06 ± 0.02 nM/mm2, and 0.05 ± 0.01 nM/mm2 in utricle with ampulla, saccule, and common crus, respectively). The amount of ATP secretion in utricle with ampulla was significantly higher than common crus (p less than 0.05). ATP secretion was partially or completely blocked by bafilomycin A1 and was almost completely blocked by CBX and clodronate in all tissues. Expression of the ATP containing vesicle was detected in the dark cell area in the utricle/ampulla, roof epithelium of ampulla and saccule, utricular macula, ampullary crest, and common crus. CX26 was identified to be distributed at the apical surface of hair cells and supporting cells of utricle, ampulla, and saccule, dark cells of utricle and ampulla, roof epithelium of saccule and ampulla, and common crus. VNUT expression was also detected throughout the epithelial cells of each tissue except vestibular transitional cells.
Conclusions: The results suggested that mechanical stimulation induces ATP secretion in the various epithelial cells of vestibular organ through gap junction hemichannel and dark cells and the secretion was mediated by VNUT.

T203. Enhancement of Vestibular Compensation by Retigabine

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Category: Vestibular: Basic Research and Clinical
Background: Acute unilateral vestibulopathy induces vertigo, spontaneous nystagmus, and postural instability. So far, there is no treatment method to recover the vestibular function, instead, visual and postural stability accomplished by central vestibular compensation. Vestibular compensation can be stimulated by vestibular rehabilitation exercises, but there is no definitive therapeutic agent that can stimulate vestibular compensation as well as recovery of vestibular function. KCNQ4 is distributed over vestibular calyx at type 1 hair cells and vestibular nerve. The role of KCNQ4 is not fully revealed in the vestibular system. In this study, we tried to investigate the role of KCNQ4 stimulator (retigabine) in acute unilateral vestibulopathy mouse model.
Methods: Unilateral labyrinthectomy was performed in 8-week-old C57BL/6 mouse. Retigaine (10 μg/g) or DMSO was injected i.p. in the mice immediately after the labyrinthectomy. The mean slow phase velocity of spontaneous nystagmus and vestibulo-ocular reflex in slow harmonic acceleration and step velocity test was measured before labyrinthectomy, 1 hour, 12 hour, 48 hour, and 7 days after labyrninthectomy. The same experiment was performed in Kcnq4p.W277S/p.W277S mice to confirm the effect of retigabine in the wild type mice. To investigate the effect of retigabine on vestibular compensation, immunohistochemistry for c-Fos and KCNQ4 in vestibular nucleus was performed.
Results: After the unilateral labyrinthectomy, spontaneous nystagmus was developed to the contralateral side of the labyrnithectomy in all mice. The mean slow phase velocity of spontaneous nystagmus was more significantly reduced in the retigabine injected mice than DMSO injected mice at 48 hours (0.60 vs. 0.24 deg/sec) and 7 days (0.25 vs. 0.09 deg/sec) after the labyrinthectomy. In sinusoidal harmonic acceleration test, gain value was significantly increased in the retigabine injected mice than DMSO injected mice at 7 days in 1.28Hz (0.13 vs. 0.20). In contrast, retigabine showed no effect on labyrinthectomized Kcnq4p.W277S/p.W277S mice. In immunohistochemistry, c-Fos protein expression was increased in the vestibular nucleus of retigabine injected mice when compared to that of DMSO injected mice.

Conclusions: Based on the observation, KCNQ4 stimulator was likely to enhance vestibular compensation after acute vestibular injury. The role of other KCNQ channel in the vestibular compensation should be evaluated in the future because retigabine can stimulate other KCNQ channels.

T204. A Novel Deep Learning Approach Enable to Evaluate Sensorimotor System During Quiet Standing in Patients With Peripheral Vestibular Disorders

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Category: Vestibular: Basic Research and Clinical

Background: Maintaining a successfully balanced posture without dizziness is achieved by harmonious coordination and integration of visual, vestibular, and somatosensory systems. Sensory organization test (SOT) in computerized dynamic posturography is clinically the most widely used balance assessment test. In this study, we proposed a novel deep learning architecture using the frequency of center of pressure (COP) signals while in standing posture to estimate the equilibrium score, a sensory system contribution variable in SOT protocol.

Methods: This study analyzed the SOT data of 100 patients with Meniere's disease, 100 patients with vestibular neuritis, and 100 normal controls. The COP signals preprocessed by using low-pass filter in SOT condition 1 were converted into frequency domains through Short Time Fourier Transform (STFT). These frequency domain data in SOT condition 1 and the equilibrium score (ES) of condition 2 to 6 were trained using convolution neural network (CNN) architecture. The training and testing data ratio for the CNN model was set 80% and 20%, respectively. The predicted results of this approach were verified by comparing them to the measured results.

Results: The ES value for patients with vestibular neuritis was approximately 7 points smaller than that for the normal group under condition 4 (p less than 0.01). In addition, ES values at conditions 5 and 6 for the normal group were about 12-13% higher than those of Meniere's disease and vestibular neuritis groups (p less than 0.05), although their differences between the two patient groups were not statistically significant (p greater than 0.2). The measured ES of overall data was shown to be 96.0 (5.7), 83.4 (11.4), 63.4 (14.3), and 94.2 (7.9) for the somatosensory system, visual system, vestibular system, and visual preference, respectively. The error of each sensory system performance using the estimated ES value from the deep learning model was shown to be around 1% on average without showing statistically significant difference between the predicted and measured results for each sensory system (p greater than 0.05).

Conclusions: It is estimated that the sensorimotor system can be measured just by measuring the COP signals of the quiet standing posture through the deep learning architecture applied in this study. Through this, the examination time can be shortened and the posturography device can be miniaturized, which is expected to contribute to the widespread dissemination of the balance assessment test.

T205. KCNQ4 Dysfunction Causes Dizziness and Imbalance After Excessive Rotational and Gravitational Stimulation due to Vestibular Hair Cell Loss

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Category: Vestibular: Basic Research and Clinical

Background: KCNQ4 is a voltage-gated K+ channel distributed over the inner surface of calyx and heminode of the vestibular nerve distributed over type 1 vestibular hair cells of the inner ear. However, little is known about the vestibular phenotypes caused by KCNQ4 dysfunction or the specific role of KCNQ4 in the vestibular organs.

Methods: To investigate the role of KCNQ4 in the vestibular organ, 6-g hypergravity stimulation for 24 hours, which represents excessive mechanical stimulation of the sensory epithelium, was applied to p.W277S Kcnq4

transgenic mice. We measured the vestibule-ocular reflex from the mouse after the stimulation and the hair cell damage by immunofluorescent staining. Retigabine was injected to the wild type mouse to confirm the role of KCNQ4 in the vestibular system against hypergravity stimulation. We also investigated the human vestibular phenotype using clinical vestibular function test in the patients with KCNQ4 mutation.
Results: Vestibular function decrease was more severe in the Kcnq4p.W277S/p.W277S mice than in the Kcnq4+/+ and Kcnq4+/p.W277S mice after the stimulation. The vestibular function loss was resulted from the loss of type 1 vestibular hair cells. Retigabine, a KCNQ activator, prevented hypergravity-induced vestibular dysfunction and hair cell loss. Patients with KCNQ4 mutations also showed abnormal clinical vestibular function tests.
Conclusions: These findings suggest that KCNQ4 plays an essential role in type 1 vestibular hair cells preserving vestibular function against excessive mechanical stimulation.

T206. Development of Real-Time 3D Video-Oculography Using High Quality Infrared Video Frenzel and Galvanic Evoked Vestibulo-Ocular Response

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Category: Vestibular: Basic Research and Clinical
Background: It is essential to use an infrared CCD camera in clinical examination of the vestibular system. Devices are currently available that can quite accurately record human eye movements, based on the principle of video-oculography (VOG). We devised an original VOG (HI-VOG) system using a commercialized infrared CCD camera, a personal computer and public domain software program (ImageJ) for data analysis. We revised the VOG and image filing system for real-time 3D analysis of nystagmus, and developed high quality video Frenzel (yVOG-Glass).
Galvanic vestibular stimulation (GVS) activates the vestibular system. Galvanic body sway test provides important information for differential diagnosis between inner ear and retro-labyrinth disorders of the vestibular system. On the other hand, interpretation of nystagmus is difficult, because precise measurement of the eye movement by electronystagmography is not feasible.

Methods: The video image from a Frenzel with high quality image camera was captured at 60 frames per second at a resolution of 640*480 pixels. For real-time analysis of the horizontal and vertical components, the X-Y center of the pupil was calculated. For real-time analysis of torsional components, the whole iris pattern was overlaid with the same area of the next iris pattern, and the angle at which both iris patterns showed the greatest match was calculated. Galvanic evoked vestibulo-ocular response was monitored.
Results: Accurate measurements of horizontal, vertical and torsional eye movement were taken while recording the video image in real-time. For quantitative analysis, the slow phase velocity of each occurrence of nystagmus and the average value of the slow phase velocity were analyzed automatically. it was possible to carry out accurate recording and evaluation of nystagmus.
Conclusions: Using the yVOG-Glass system, it was possible to perform real-time quantitative 3D analysis of nystagmus from video images recorded with high quality video Frenzel. Recent technological developments, including the use of improved VOG, might lead to the rediscovery of GVS.

T207. Effects of Fluoxetine on Ionic Currents in Vestibular Calyces

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Category: Vestibular: Basic Research and Clinical
Background: Recent studies suggest a role for selective serotonin reuptake inhibitors (SSRIs) such as Fluoxetine (Prozac®) in the treatment of dizziness and inner ear vestibular dysfunction. However, the potential mechanism of action remains unclear. Fluoxetine is reported to block certain types of K+ channel in a variety of cell types. Here, we investigated the direct actions of Fluoxetine on membrane currents in hair cells and

calyx afferents of the peripheral vestibular system. In addition, we explored differences in K+ currents in peripheral zone (PZ) and central zone (CZ) calyces and their response to Fluoxetine application.
Methods: Cristae were extracted from Mongolian gerbils at postnatal days 20-30. Hair cells and afferents were isolated or studied in thin slices of the crista. Hair cells and calyces were visually identified under the microscope and membrane seals obtained with patch electrodes at their basal regions. Fluoxetine was prepared in extracellular L-15 medium to a final concentration of 10, 50 or 100 μM and perfused onto cells in the recording chamber.
Results: Calyx terminals expressed a variety of ionic conductances as demonstrated previously. We confirmed that when cells in different regions of crista slices were compared, the outward K+ currents showed significantly greater inactivation in PZ calyces compared to CZ calyces. Fluoxetine (100 μM) completely blocked the transient Na+ current in calyx terminals and strongly reduced the outward K+ current (n=12, 62% mean current blocked). Fluoxetine reduced on average 40% of outward K+ current in PZ calyces, (n=6) and 56% of outward current in CZ calyces (n=4). In PZ calyx terminals, fluoxetine blocked a non-inactivating K+ current but did not block the rapidly activating, rapidly inactivating A-type current. A combination of fluoxetine and 1 mM 4-aminopyridine blocked all the outward current in a PZ calyx terminal. Despite the strong blocking effect on calyces, Fluoxetine (100 μM) had no effect on K+ currents in type I hair cells (n =3).
Conclusions: Our data demonstrate that Fluoxetine eliminates Na+ currents and partially blocks outward K+ currents in vestibular calyx terminals. Fluoxetine blocks a non-inactivating K+ current and reveals fluoxetine-resistant A-type current, which is abolished by 4-aminopyridine. Our data therefore show that Fluoxetine has a direct effect on the electrophysiological properties of neurons in the peripheral vestibular system. Furthermore, at micromolar concentrations fluoxetine would likely inhibit action potential firing in vestibular afferents and reduce sensory input to the central nervous system.
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T208. Characterizing Oculomotor, Vestibular, and Reaction Time Testing (OVRT) in Acute Pediatric mTBI

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Category: Vestibular: Basic Research and Clinical
Background: The diagnosis of pediatric mTBI is challenging due to the limited availability of objective assessments. Accurate and reliable diagnostic tools are essential for providing sensible management plans, including evidence-based guidelines for return to play or school. As in the adult population, the current standard of care relies on self-reported symptoms, subjective clinical exams, and comparison of pre/post-injury neurocognitive assessments, which are rarely available outside of athletics. Previously, our group has characterized normative values for oculomotor, vestibular, and reaction time testing (OVRT) in adult mTBI. Thus, the goal of this study was to examine if a pediatric OVRT testing battery (for which normative values were previously collected) can consistently detect acute mTBI in a pediatric population.
Methods: Pediatric participants (M/F [6/7]) between 7-16 years of age (n=13; currently enrolling up to n=50) were recruited from the concussion clinic at the University of Miami Health System within 10 days of their injury. Following written informed consent/assent, all participants completed OVRT testing with a portable, compact, 3D head-mounted videooculography system (Neurolign USA, LLC). For our analysis, the complete test battery (a total of 18 tests) was stratified into 8 distinct testing domains: 1) Nystagmus, 2) Optokinetics, 3) Smooth Pursuit, 4) Saccades, 5) Cognitive, 6) 3D Vergence, 7) Reaction Time and 8) Vestibular. Each domain is comprised of 1-4 sub-tests (e.g., the Cognitive domain includes self-paced saccades, anti-saccades, and predictive saccades). Percent abnormal was assessed according to existing normative values previously described by our group.
Results: Overall, participant race and ethnicity were nearly evenly distributed with half identifying as Hispanic and half identifying as White. The mean age among all participants was 13.8 years (2.4). Across the eight testing domains, we observed abnormalities in 84.6% of individuals during smooth pursuit testing, 53.8% in optokinetic nystagmus, 46.2% in both vergence testing and horizontal/vertical saccades, 38.5% in

vestibular (subjective visual vertical/horizontal), and 30.8% during spontaneous nystagmus testing. All seven testing domains examined were found to be highly indicative of pediatric TBI. In particular, we found that abnormalities were most commonly observed among the following assessments: smooth pursuit vertical at 0.1 Hz (61.5%), saccade and reaction time testing (46.2%), vergence steps (46.2%), horizontal saccades (46.2%) and optokinetic nystagmus at 20d/s (46.2%).
Conclusions: These preliminary findings highlight the diagnostic value of the OVRT testing battery for acute pediatric mTBI. All participants exhibited abnormal values among at least one of the 18 tests across all 7 domains.

T209. Postural Sway in Autism (ASD) CNTNAP2 Mouse Model

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Category: Vestibular: Basic Research and Clinical
Background: There is a need for improved translational research performed in humans and in model organisms to study behaviors known to be conserved and germane to autism spectrum disorder (ASD). One such directly translatable behavior is postural sway, which involves the vestibular, proprioceptive, and visual sensory systems. Previous studies have consistently shown that autistic individuals exhibit greater postural sway than non-autistic individuals (for review, Lim et al., 2017). In fact, postural asymmetry during 2-legged standing was predictive of the presence and severity of repetitive behaviors (and anxiety) in ASD (Rodgers et al., 2012). In addition, others have shown that both the frequency and intensity of repetitive behaviors in ASD is correlated with postural sway area (Radonovich et al., 2013). In summary, a core symptom of ASD, the presence of repetitive behaviors, is evident in postural dynamics. Moreover, impact of motor difficulties in childhood can be severe, as they may contribute to reduced participation during play and sports, and consequently may hamper social interaction and development.
Methods: Postural sway can be quantified by Center-of-Pressure (CoP) area, using an AMTI force platform to record ground reaction forces with customized software to compute an ellipse encompassing 95% of sway area and from that ellipse CoP area, and anterior-posterior (AP) and medial-lateral (ML) sway can be computed.
Results: In these studies we assessed postural sway in 14F/14M CNTNAP2 (-/-) and 13F/12 M unaffected control (CNTNAP(+/+) mice. Both lines are congenic for the C57BL/6 strain. We found that both male and female CNTNAP2 (-/-) mice exhibit greater Center-of-Pressure (CoP) sway areas than unaffected control mice.
Conclusions: These findings suggest that postural sway can be a translatable behavior, and may serve as a biomarker or potential outcome measure in future ASD research and interventions.

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T210. Postural Sway in a Mouse Model of Alzheimer's Disease (AD)

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Category: Vestibular: Basic Research and Clinical
Background: Currently, 5.5 million Americans (~10%) over age 65 have Alzheimer’s disease (AD), and this number will more than double by 2050. AD is definitively diagnosed from post-mortem analyses of neurodegeneration with the presence of beta-amyloid (Ab) containing plaques and tau-containing neurofibrillary tangles. As Ab accumulation in the brain can precede cognitive impairment by decades, treatments aimed at facilitating clearance of Abare most effective at early stages, when the disease is difficult to diagnose. The inability to detect AD early enough to apply meaningful interventions is a major barrier to AD’s successful management. AD patients have a higher prevalence of vestibular impairment relative to healthy age-matched controls, with postural sway serving as an excellent predictor of the overall cognitive

assessment score in AD. Gait metrics discriminated better between patients with mild cognitive impairments and healthy controls, more so than verbal-fluency tests. Additionally, patients with AD have difficulty suppressing incongruent brain signals when maintaining balance compared to age-matched controls. Preclinical models have been extremely useful to test hypotheses about AD pathophysiology and to assess putative interventions. However, it is unknown if current AD mouse models exhibit alterations in postural sway and if there are increased Aβ deposition occurs in the vestibular subcortical areas of AD mouse models, similar to post-mortem histological evidence of early Ab deposition in brainstems of patients with little evidence of cognitive impairment.

Methods: We measured postural sway in the 5X FAD mouse model crossed to CBA mouse line without any age-related hearing loss. We assessed postural sway in both male and female 5XFAD*CBA (+/-) mice and non-affected 5XFAD*CBA littermate controls. Postural sway can be quantified by center-of-pressure (CoP) area using an AMTI force platform, which can record ground reaction forces, and CoP elliptical area can be generated from many CoP measurements generated by a standing mouse.
Results: At three months of age we found that the female but not male mice exhibited greater sway in the 5xFAD*CBA line (+/-) than unaffected littermates. However, by six and nine months of age there was no difference in sway area between the groups, suggesting a tight window of early detection in the 5X FAD*CBA line.
Conclusions: We are currently assessing other AD mouse lines for postural sway such as mice carrying the APOE4 gene. We will also determine if there is plaque deposition, demyelination, or inflammation in subcortical vestibular CNS areas of these AD mouse lines. We hope that these sway findings may pave the way to develop and test future therapeutics for pre-dementia AD.

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T211. Open Board

T212. Transmembrane Channel-Like (Tmc) Subunits Contribute to Frequency Sensitivity in the Zebrafish Utricle

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Category: Vestibular: Basic Research and Clinical
Background: Information about dynamic head movements is transmitted by sensory receptors, known as hair cells, in the labyrinth of the inner ear. The sensitivity of hair cells to fast or slow movements of the head differs according to cell type. Whether the mechanotransduction complex that converts mechanical stimuli into electrical signals in hair cells participates in conveying frequency information is not clear. Here we find that the transmembrane channel-like (tmc) 1/2 genes, which encode a central component of the complex, are differentially expressed in the utricle and contribute to frequency sensitivity in zebrafish.
Methods: Tmc1/2a/2b are differentially expressed in the extrastriolar and striolar region of the zebrafish utricle. As the striolar region is predicted to be more sensitive to high frequency stimuli, we developed a simple, noninvasive method to assess eye movements in zebrafish larvae in response to high frequencies. We based our assay on previous reports demonstrating that bone conducted vibration or air-conducted sounds at 500 Hz selectively activate central zone afferents. Our customized device was constructed to measure the torsional eye movements of zebrafish larvae in a dorsal up position induced by a controlled vertical vibration. We tracked rotation of the eye by measuring the movement of centroids marking individual pigment cells within the outer layer of the retina using a custom script and plotted responses to various frequencies and intensities.
Results: To determine the contribution of the Tmc1/2 subunits to high frequency sensitivity, we assessed vibration induced eye movements (VibEMs) in response to vibrational stimuli of 300 - 1800 Hz at various intensities in the tmc mutants. We observed no differences in homozygous mutants tmc1, and tmc2b with WT siblings at any of the frequencies or intensities tested. whereas, tmc2a mutants showed decreased responses at the higher frequencies of 1500 and 1800 Hz. With the exception of a subset of tmc2a-positive hair cells within

the striolar region, individual hair cells of the utricle show co-expression or expression of all three tmc genes. We therefore tested VibEMs in tmc double mutants. In tmc1/2a double homozygous mutants, we observed that responses were decreased at 1500 and 1800 Hz, similar to a tmc2a single homozygous mutant. In tmc1/2b mutants, we did not observe differences with WT sibling responses. In contrast to the other two double mutants, mutants that are homozygous for both tmc2a and tmc2b have pronounced deficits at all frequencies. **Conclusions:** Our study suggests that there is a division of labor among the three tmc genes in the zebrafish utricle, which is reflected in their differential expression pattern and functional differences among the tmc mutants. Our findings also support the notion that mechanotransduction in hair cells requires specialization at the molecular level for optimal detection of stimuli.

T213. Interaction Between Noise Exposure and Aging on Rat Vestibular Sensory Epithelia

Marie Anderson[1], Ariane Kanicki[1], Richard Altschuler[2], W. Michael King[1], Courtney Stewart*[3]

[1]*University of Michigan, Kresge Hearing Research Institute,* [2]*University of Michigan, Kresge Hearing Research Institute and VA Ann Arbor Healthcare System,* [3]*VA Ann Arbor Healthcare System*

Category: Vestibular: Basic Research and Clinical

Background: Previous work has highlighted the short-term consequences of noise exposure on calyx only afferent terminals on vestibular sensory epithelia. Although some changes in the vestibular periphery have been documented, changes in the number of calyx-only afferent terminals at key timepoints in the rat's lifespan have not been shown, and the effect that noise exposure may have on the aging vestibular periphery is unclear. We hypothesize that a decline in calretinin immunolabeling of calyx-only afferent terminals will occur with age, and that this effect will be greater in rats that have been exposed to 120dB SPL 1.5 kHz 3-octave band noise (3OBN).

Methods: Rats participating in longitudinal studies of vestibular-dependent behaviors and vestibular evoked potentials were exposed to 120dB SPL 1.5kHz 3-octave band noise continuously for up to 6-hours. Upon completion of experiments ears were either immediately collected or rats were maintained in the vivarium until a target age was reached. At this time, rats' ears were collected and immunostained with antibodies against combinations of antibodies including calretinin, myosin7a, beta-3 tubulin, and neurofilament. Whole vestibular sensory epithelia were then mounted on slides and imaged using a confocal microscope. The focus of these analyses was on a previously characterized 100x200 micron region of interest at the bend of the striola in the saccule and the center of the striolar zone in the utricle. Calyceal terminals, including calyx-only afferents were quantified for all rats and categorized by age and noise exposure history. Counts across age groups for noise-exposed and control rats were compared.

Results: Overall, noise exposure was related to lower calyx-only afferent terminal counts regardless of age. There was also a decrease in mean calyx-only afferent terminals due to age in control rats in the saccule and utricle, but a greater difference was observed between older rats and younger rats with the same noise exposure history. The combined effect of noise and aging was greater in the saccule than the utricle.

Conclusions: These data suggest that while there is a small decrease in calyx-only afferent terminals related to natural aging, noise exposure decreases the number of calyx-only afferent terminals in the saccule and utricle to a greater extent. Although the dose of noise used to demonstrate this effect is significant, it demonstrates the potential impact of extreme noise exposure on a population of afferents with particular importance for rapid responses that may stabilize the body during an abrupt perturbation.

DISCLAIMER: The views expressed do not necessarily reflect the official policies of the Department of Health and Human Services, nor does mention of trade names, commercial practices, or organizations imply endorsement by the U.S. Government. This work was supported by R01 DC018003-01 (King), 1IK2RX003271-01A1 (Stewart), I01RX003250-02 (Altschuler), R01 AG073157 (Stewart).

T214. Role of Vestibular Insults on Peripheral and Central Vestibular Function

Syed Naqvi[1], Sukesh Gupta*[1], Rod Braun[1], Alejandro Ponce-Sepulveda[1], Avril Genene Holt[1]

[1]*Wayne State University School of Medicine*

Category: Vestibular: Basic Research and Clinical

Background: The vestibular system is crucial for posture, gait, and the perception of head and body position in space. Impairments to this system can manifest as dizziness, imbalance, and poor postural control leading to the inability to respond appropriately in challenging terrain, increased risk of falls, and premature death. Recent studies suggest that the vestibular system can be damaged by noise overstimulation. However, over a lifetime these vestibular end organs may be subjected to multiple and varied insults, the impact of which is unknown, our recent studies suggest that mechanical overstimulation can result in the dysfunction of vestibular irregular afferents centrally. The combined effect of noise and mechanical overstimulation is explored. Vestibular short-latency evoked potentials (VsEPs) can be generated in response to linear acceleration and used to assess vestibular pathway function. Manganese-enhanced magnetic resonance imaging (MEMRI) is a method that uses manganese (Mn2+) as a paramagnetic calcium surrogate that accumulates in active neurons and can be visualized using MRI. Therefore, we combined VsEP and MEMRI to evaluate vestibular function and neuronal activity in central neurons following overstimulation (repetitive stimulation and noise).
Methods: Male Sprague Dawley rats were exposed to a 1/3 octave band noise centered at 1.5 kHz at 120 dB SPL for 6 hours. Twenty-four hours later, MnCl2 was administered just prior to a jerk stimulation paradigm consisting of 6,000 jerks divided equally across three blocks. Each block had five trials (200 up and 200 down jerks) in the naso-occipital plane (640 g/s or 3,300 g/s,). Recorded VsEPs were analyzed using custom MATLAB scripts with ANOVAs and T-tests used as appropriate. To assess Mn2+ uptake in vestibular nuclei T1 maps were collected using MRI.
Results: Noise-exposed rats stimulated at 3,300 g/s had significant VsEP waveform alterations (p less than 0.05) with increased latencies at 3,300 g/s. The least Mn2+ uptake was observed after 640 g/s, but also, while the greatest was at 3,300 g/s (p less than 0.05). Interestingly, the 640 g/s stimulated group, had more Mn2+ uptake than the 3,300 g/s group after noise exposure. Following noise exposure, P1 amplitude significantly increased and P2 amplitude significantly decreased from baseline after 4000 jerks at the intensity of 640g/s. After 4,000 jerks in the 3,300 g/s group, VsEP P2 amplitude was significantly decreased (p less than 0.05).
Conclusions: Irregular afferent fiber activity peripherally (P1) appeared to remain constant in response to repetitive linear acceleration but increased after a mild jerk intensity following noise exposure. Diminished synaptic activity and delayed responses following intense stimulation or noise exposure were preferentially observed centrally (P2). Our results demonstrate that different forms of overstimulation produce damage in vestibular pathways that is detectible with VsEP and MEMRI.

T215. Frequency Tuning Characteristics of Bone-Conduction Ocular Vestibular-Evoked Myogenic Potentials in Healthy Individuals

Chandan Suresh*[1], Isabel Valdovinos[1], Arthur Ni[1], Alaina Bassett[1]

[1]*California State University, Los Angeles*

Category: Vestibular: Basic Research and Clinical
Background: Ocular vestibular evoked myogenic potentials (oVEMPs) assess the function of the utricle and its associated afferent pathways of the superior vestibular nerve. These are contralateral responses indicated by a negative excitatory peak (N1) at approximately 10ms and a positive peak (P1) at approximately 15ms. The clinical utility of oVEMPs includes assessing the utricle and superior portion of the vestibular nerve in addition to testing for third window conditions (TWCs). Rosengren et al., (2019) reported that bone conduction (BC) oVEMPs might be a better screener for detecting TWC with high sensitivity and specificity than air conduction (AC) oVEMPs. Fröhlich et al., (2021) indicated that BC elicits higher amplitudes and lower thresholds of oVEMP than AC stimulation. BC is also the preferred mode of stimulation for patients with conductive pathologies. The newer B-81 bone oscillator creates less harmonic distortion and higher dynamic range at lower frequencies over the B-71 (Jansson et al., 2015). Frequency tuning is essential for differentiating normal and pathological conditions. This study aims to characterize the frequency tuning of BC oVEMP responses in healthy participants using a B-81 bone oscillator.
Methods: The study recruited participants with no history of neurologic disease, chronic noise exposure, vestibular disorders, and conductive hearing loss. For oVEMP recording, the positive electrode was placed on the right inner canalith, the negative electrode on the contralaterally inferior oblique, and the ground electrode on the sternum. oVEMP recording was performed while participants fixated on a point in the wall, approximately 60 cm from the participant at a 30-degree angle from an optimal gazing position. Tone burst stimuli were randomly presented at 500 Hz, 750 Hz, 1,000 Hz, 2,000 Hz, and 4,000 Hz. The initial intensity

presentation was 75 dBnHL for 500 Hz, 750 Hz, 1,000 Hz, and 2,000 Hz, and 70 dBnHL for 4,000 Hz. The modified Hughson-Westlake method was utilized at each frequency to measure oVEMP thresholds. The oVEMP response metrics, such as N1, P1 latency, and N1-P1 peak-to-peak amplitude, are considered for analysis.

Results: The preliminary findings from 40 ears indicated that oVEMPs were present in all participants between 500-1000 Hz. No participants had present oVEMPs between 2,000-4,000Hz. The responses had maximum amplitude, longer N1, and P1 latencies, and lowest thresholds at 500 Hz than other frequencies evaluated. The average threshold at 500 Hz, 750 Hz, 1,000 Hz were 65 dBnHL, 71 dBnHL, and 73 dBnHL respectively. The average amplitude at the high-intensity presentation is approximately two times bigger at 500Hz compared to 1000Hz.

Conclusions: Similar to previous studies using AC stimulation and BC stimulation with B-71, oVEMPs are 500 Hz tuned responses with no incidence of responses 2,000-4000Hz. The study's findings can be considered when diagnosing pathological conditions such as TWC.

Symposium 10 - Activity-Driven Mechanisms of Auditory Development, Regeneration, and Repair

1:45 p.m. - 3:45 p.m.

Grand Ballroom Salon E

Activity-Driven Mechanisms of Auditory Development, Regeneration, and Repair

Chair: Thomas Coate*, Georgetown University*

Co-Chair: Zoe Mann*, King's College London*

Co-Chair: Travis Babola*, JHMI*

Session Description: In the nervous system, action potentials and metabolic pathways drive synaptic development, and can promote regeneration and repair of damaged systems. In this symposium we will explore recent experimental findings related to these concepts in the auditory system. This symposium will be kicked off by a 30-minute presentation by Dr. Dwight Bergles, one of the co-discoverers of spontaneous activity in the auditory system. His talk will cover new findings on molecular mechanisms that generate spontaneous activity in mammals, how activity sets the gain and frequency selectivity of auditory circuits, and how activity promotes the refinement of circuits in the brain. Following this, Dr. Andrea McQuate will focus our attention on the hair cell/afferent synapse, and how activity regulates mitochondrial dynamics in both compartments. Her talk will showcase spectacular ultrastructure data from the zebrafish lateral line. Dr. Satish Ghimire will next discuss how Semaphorin-5A, which is most well known as an axon guidance regulator, regulates patterns of spontaneous activity generated in spiral ganglion neurons. To cap off the auditory periphery, Dr. Zoe Mann will describe recent findings on how metabolic pathways shape positional identity of hair cells in the chick basilar papilla. We will next venture into the auditory CNS where Dr. Travis Babola will describe new findings from innovative imaging and physiology studies showing how activity drives tonotopic specificity in the cortex. Next, Dr. Saman Hussain will take us back to the zebrafish lateral line where she will demonstrate, using advanced imaging techniques, how activity drives the formation and maintenance of ribbon synapses – structures that are degraded following acoustic insult. Finally, the session will conclude with Dr. Jonathan Gale, one of the other co-discoverers of spontaneous activity in the auditory system. In his talk, he will focus on how activity may drive different aspects of epithelial repair following damage.

Diverse Roles of Developmental Spontaneous Activity in Maturation of the Auditory System

Dwight Bergles

Johns Hopkins University

Individual Abstract: Spiral ganglion neurons in the developing cochlea fire bursts of action potentials in a highly stereotyped pattern prior to the onset of hearing. These bursts of activity pass through developing auditory centers in the brain, providing the means to induce maturation of sound processing neurons and refinement of auditory circuits. Burst events are initiated within the cochlea when inner supporting cells release ATP, which initiates a cascade of events culminating in the release of potassium, causing depolarization and calcium action potential generation by nearby inner hair cells. To define the roles of this intrinsically generated activity in auditory system development, we selectively disrupted the ability of supporting cells to induce hair cell depolarization by genetically inactivating the calcium-activated chloride channel TMEM16A, which is required to trigger potassium efflux from these cells. Loss of this channel abolished spontaneous inward currents in both supporting cells and inner hair cells prior to hearing onset. In vivo imaging of neural activity from these mice revealed that spontaneous activity in the inferior colliculus was also greatly suppressed. Nevertheless, auditory brainstem responses to pure tones and clicks recorded just after hearing onset had similar thresholds, indicating that mechanotransduction and acoustic transformation were preserved in the absence of developmental spontaneous activity. However, several abnormalities were present in these spontaneous activity deficient mice. The amplitude of neural responses to a given stimulus were larger, neurons responded to a broader range of frequencies, and sound responsive areas in the inferior colliculus and the auditory cortex were reduced in size. These results indicate that supporting cell induced spontaneous activity in the developing cochlea is responsible for setting the appropriate gain of auditory circuits, refining the frequency sensitivity of auditory neurons, and establishing sound processing domains in the brain during a crucial developmental period before ear canal opening.

SEMA5A Regulates Spontaneous Neural Firing in the Prehearing Cochlea
Satish Ghimire

Georgetown University

Individual Abstract: Semaphorins (SEMAs) are a large family of secreted or membrane-bound ligands that signal through Neuropilin and/or Plexin receptors in diverse contexts like axon guidance, synaptic pruning and plasticity. SEMA5A is a transmembrane Semaphorin shown to regulate circuit assembly in the developing retina. Our data suggest that SEMA5A acts as an inhibitor of spontaneous activity in the developing cochlea. In the pre-hearing cochlea, the ATP released from the cochlear epithelium and subsequent excitation of hair cells followed by glutamate release results in spontaneous firing of spiral ganglion neurons (SGNs). SGN spontaneous firing patterns during embryonic stages appear infrequent and uncoordinated but become more frequent and coordinated by early postnatal stages. In-situ hybridization and scRNAseq studies show that Sema5a is expressed by SGNs and otic mesenchyme in the developing cochlea. In Ca2+ imaging studies using Snap25-GCaMP6s and cochlear explants, SEMA5A-Fc had dramatic and immediate effects on SGN spontaneous activity: the area of activity, frequency, and coordinated events were all significantly reduced compared to baseline events, whereas application of control IgG had no effect. Analyses of cochlear explants from Sema5a-/-;Snap25-GCaMP6s+ mice showed increased fluorescence levels of Ca2+ compared to littermate controls. We also observed increased phosphorylated CREB in SGNs and cochlear epithelial cells from Sema5a-/- samples as an indicator of elevated activity. Supporting these findings, we also observed reduced terminal branches of type I SGNs in Sema5a-/- cochleae suggesting a homeostatic mechanism. A compelling possibility is that SEMA5A interacts with one or more channels, which directly regulate SGN excitability. Interestingly, SEMAs were shown to have a short amino acid sequence conserved with hanatoxin, a naturally occurring inhibitor of voltage-gated K+ and Ca2+ channels. We have found that the hanatoxin analog HpTx2 inhibits SGN Ca2+ transients, similar to the effects of SEMA5A-Fc. We are currently examining the effects of hanatoxin domain (HD) mutants of SEMA5A in the context of SGN spontaneous activity and protein pull-down experiments designed to discover SEMA5A binding partners.

Metabolic Tuning of Positional Identity in the Developing Chick Cochlea: A Sweet Story
Zoe Mann

King's College London

Individual Abstract: In vertebrates with elongated auditory organs, mechanosensory hair cells (HCs) are organised such that complex sounds are broken down into their component frequencies along a basal-to-apical long (tonotopic) axis. This process relies on unique morphological and physiological properties of HCs along to the axis, which are specified during development. Acquisition of tonotopic morphology requires that HCs interpret their positional identity within their local niche at different positions along the cochlea. The complex signalling within the auditory organ between a developing HC and its local niche along the cochlea is poorly understood. Using NAD(P)H fluorescence lifetime imaging (FLIM) to image metabolism in living cells, we identify tonotopic differences in the fate of cytosolic glucose along different branches within the glycolytic network. We further show that re-shaping these glycolytic gradients, by modulating glucose catabolism, disrupts Bmp7 and Chordin like-1 signalling and abolishes the normal gradient in HC morphologies along the basal-to-apical axis. We propose a necessary and causal link between graded morphogen signalling and cytosolic glucose flux in specifying positional identity in developing auditory HCs.

Illuminating the Depths of Auditory Cortex: The Role of Subplate Neurons in Developing Circuits

Travis Babola

JHMI

Individual Abstract: To form circuits that process information, make decisions, and execute complex behaviors, billions of brain cells must establish and refine trillions of appropriate connections during development. A major step in this process involves pioneering axons from the thalamus traversing to layer 4 (L4), cementing the ability of the periphery to communicate with the cortex. Surprisingly, thalamic axons do not proceed directly to L4, but rather synapse first onto subplate neurons (SPNs) located beneath L6. In the visual system, early ablation of SPNs disrupts ocular dominance column formation, suggesting that SPNs exert a powerful influence over early large-scale network activity and connectivity, however, the role of SPNs in shaping auditory cortical circuits has remained underexplored, prompting questions about how or if these neurons are involved in shaping emergent tonotopic organization. To explore the role of the SPNs in cortical development, we generated a new mouse model using CRISPR/Cas9-mediated insertion of CreER into the endogenous Cplx3 locus, allowing for unprecedented access to SPNs. In order to understand what types of activity these cells display before hearing onset, we crossed this line to a Cre-dependent GCaMP6s line and used two-photon imaging in awake (non-anesthetized) mouse pups. We observed highly coordinated spontaneous activity among groups of SPNs that we hypothesized could reflect activity generated within the cochlea, as previous experiments demonstrated that early sensory-independent activity propagates form the cochlea to the cortex at this age (PND 7). To address this, we simultaneously imaged auditory SPNs and midbrain neurons using widefield imaging and observed a high degree of correlation between the two areas, suggesting that the periphery drives SPN activity at this time. Further, robust responses to pure tone stimuli could be seen in SPNs using widefield and two-photon imaging in mice after hearing onset, providing additional evidence that peripheral activity can engage SPNs. Ongoing experiments seek to alter SPNs during this critical developmental period by either genetic ablation (Cre-dependent DTA) or silencing (Cre-dependent tetanus toxin) strategies to understand how these neurons contribute to cortical development. We hope that the proposed studies lead to fundamental knowledge of how these early circuits coalesce into their mature state so that we can better understand early developmental auditory processing disorders (APDs) in children, whose etiology and mechanisms remain unknown.

Activity Regulates Mitochondria Morphology in Hair Cells and Afferent Neurons of the Zebrafish Lateral Line

Andrea McQuate

University of New Mexico

Individual Abstract: The hair cell (HC) to afferent neuron (AN) synapse, the first synapse of the auditory system, is highly vulnerable to insults, including noise overexposure and excitotoxicity. Loss of these synaptic connections can lead to a “hidden” hearing loss, where complex auditory information is lost without change to auditory threshold. Therefore, it is of great interest to understand molecular mediators that can preserve synaptic health. Mitochondria play crucial roles in all synapses by buffering calcium and providing ATP. A

growing body of literature suggests that mitochondria adopt specific morphologies that directly impact their function. We predict that activity at the HC to AN synapse over development drives differentiation of unique mitochondrial morphologies in both HCs and ANs, which subsequently impacts synaptic transmission. Using serial block-face scanning electron microscopy (SBFSEM) and HCs of the zebrafish lateral line, we recently demonstrated that presynaptic HCs develop a specific mitochondrial architecture in an activity-dependent manner. This HC mitochondrial architecture includes two mitochondrial populations: small mitochondria apically, and large, reticular mitochondrial networks at the basolateral end of the HC. Preliminary data now suggest that AN postsynaptic terminals also contain specialized mitochondria, including both networked mitochondria, and elongated mitochondria that bridge multiple synaptic terminals. These AN mitochondrial morphologies also appear activity-dependent. This mitochondrial crosstalk between different synapses is a novel concept and has larger implications for the regulation of synaptic transmission between these cells. Overall, we show a role for activity in driving the development of specialized mitochondrial morphologies at the HC to AN synapse.

Visualizing Ribbon Synapse Maturation In Vivo Using Zebrafish

Saman Hussain

NIH/NIDCD

Individual Abstract: The presynaptic ribbon is a hallmark of synapses in sensory hair cells. In hair cells, the ribbon synapse enables fast and precise neurotransmission that is crucial for proper hearing and balance. In previous work we investigated how spontaneous activity generated intrinsically in hair cells impacts ribbon synapse formation. Here, we found that spontaneous influx of calcium enters hair cells via presynaptic Cav1.3 channels and is then loaded into mitochondria. Block of this calcium exchange results in larger ribbons and fewer complete synapses. To study synapse assembly, this study and others have relied on immunohistochemistry or electron microscopy methods in fixed preparations. Thus, how activity shapes the ongoing dynamics underlying ribbon synapse formation is unclear. In this work, we study the dynamics of ribbon synapse formation using transgenic zebrafish expressing live markers that label the presynaptic ribbon (Ribeye-mCherry) and the post-synaptic density (FingR-PSD95-GFP). To image ribbon synapse formation in these lines, we are using both light-sheet and high-resolution Airyscan confocal microscopy, which enables us to track synapse development at different spatiotemporal scales. Using these imaging approaches, we find that early in development ribbon precursors are transported to and localize at the cell base in a microtubule-dependent manner. During transport and at the active zone, ribbon precursors can fuse to form larger densities. Soon after ribbons are present at the cell base, hair cells extend out processes containing developing ribbons; postsynapses within the innervating afferent migrate unidirectionally towards developing ribbons. After pre- and post-synapses meet, hair cell processes retract and newly formed synapses can fuse to form larger synapses. Currently we are exploring how activity (Cav1.3-mitochondrial calcium) impacts these dynamic processes. Overall, this work will provide fundamental knowledge of ribbon synapse maturation. This knowledge is important to reform synapses when they are lost in age-related or noise-induced hearing loss.

Supporting Cell Activity and the Regulation of Inner Ear Sensory Epithelial Repair

Jonathan Gale

UCL Ear Institute

Individual Abstract: When hair cells are damaged repairing the epithelium is a critical process that is required for the maintenance of any future sensory capability. The mechanisms of cellular and organ repair are therefore fundamental to our understanding of how to maintain healthy hearing and balance. Inner ear sensory epithelia are constantly subjected to cellular stress, be it mechanical, metabolic or excitotoxic. Hair cells are essential for the sensory capacity of the organ but it is the supporting cells, the cochlea's glial-equivalent cells, that minimise and prevent damage to the sensory epithelium. In response to damage, supporting cells maintain the barrier function of the epithelium, clear damaged cells by phagocytosis and minimise excitotoxicity by removing excess glutamate. The regulation of supporting cell activity and the cellular and molecular mechanisms underlying their critical functions during epithelial repair will be described and discussed.

Podium Session 19 - A Bundle of Mechanisms Contributing to Stereocilia Development and Function

1:45 p.m. - 3:45 p.m.

Platinum Salon 6

ER Stress-Induced Outer Hair Cell Stereocilia Fusion – Molecular Changes Involved

Ulla Pirvola*[1], Saija Leinonen[1], Tuuli Lankinen[1], Kuu Ikäheimo[1]

[1]*University of Helsinki*

Category: Hair Cells: Anatomy and Physiology

Background: Loss of the structural integrity of the stereocilia bundle of hair cells is a major cause of sensorineural hearing loss. We have shown that endoplasmic reticulum (ER) stress, caused by Cadherin 23 missense mutation together with depletion of a component of the ER proteostasis machinery, triggers progressive degeneration of the hair bundle of high-frequency outer hair cells (OHCs) and leads to a strong stereocilia fusion phenotype (Ikäheimo et al, 2021, Life Sci Alliance). We define stereocilia fusion as lifting of the apical cell surface membrane to cover multiple stereocilia cores. Here, we studied the molecular changes in the fused OHC stereocilia in a mouse model of ER stress. In addition, we studied if stereocilia fusion and the molecular changes therein are associated with aging OHCs and with the OHC response to noise overexposure.

Methods: Mouse models used: Manf fl/fl;Pax2-Cre, C57BL6 (B6) background, age 8 weeks; wild type B6 mice, age 6 months as a model of age-related hearing loss; wild type B6 mice exposed to chronic, moderate-level noise. We applied immunofluorescence on cochlear wholemounts and Apotome optics for light microscopy detection. For super-resolution imaging, we used expansion microscopy.

Results: We used the FM1-43FX dye to probe the functionality of the mechanotransduction channels in fused stereocilia. Using BAPTA-treated specimens as control for background fluorescence, quantification showed a significant decrease in FM1-43 fluorescence in OHCs of the high-frequency region of cKO cochleas compared to wild type cochleas. Immunofluorescence for the Ca2+-efflux-channel PMCA2 was strongly reduced in fused stereocilia, accompanied with similar changes in its obligate partner, neuroplastin. The upper-tip-link-density-protein Myosin 7 and the F-actin-membrane linker BAIAP2L2 were detected in fused stereocilia, yet with abnormal localization and abnormally low levels. Quantification revealed a significant increase in the levels of the Ca2+-buffering-protein oncomodulin in OHCs with fused stereocilia. These molecular changes and stereocilia fusion morphology were not evident in the aging wild type mice. Interestingly, mice exposed to chronic, low-level noise showed restricted stereocilia fusion accompanied with changes in oncomodulin expression.

Conclusions: Our findings identify ER stress as a mechanism causing a unique and severe OHC hair bundle dysmorphology, stereocilia fusion, that is coupled with unique molecular changes. The results show that a common gene mutation causing progressive hearing loss becomes devastating together with a concomitant failure in the maintenance of the ER proteostasis machinery. Our results point to problems in calcium homeostasis in the fused OHC stereocilia, and chronic noise exposure appears to trigger partly similar OHC hair bundle alterations, yet in a more subtle fashion compared to the mutant mouse model of ER stress used.

Enzymatic Characterization of MYO7A Isoforms Localized to the Stereocilia Upper Tip-Link Density

Jinho Park*[1], Sihan Li[2], Jung-Bum Shin[2], Jonathan Bird[1]

[1]*University of Florida,* [2]*University of Virginia*

Category: Hair Cells: Anatomy and Physiology

Background: The molecular motor myosin 7a (MYO7A) is expressed in hair cells and photoreceptors, and mutations in MYO7A cause Usher Syndrome type 1 (USH1B) and autosomal recessive hearing loss, DFNB2.

MYO7A has multiple functions in cochlear hair cells, being essential for normal development of hair bundle architecture, as well as concentrating at the upper tip link density (UTLD) where it helps tension the mechanoelectrical transduction (MET) complex. Hair cells produce multiple isoforms of MYO7A that contribute to these diverse and essential functions. At the UTLD, a canonical isoform (MYO7A-C) is detected in addition to an isoform (MYO7A-N) with an alternative N-terminal domain. In this study, we explore the hypothesis that altered ATPase activities of MYO7A-C and MYO7A-N contribute towards tuning mechanical activity at the UTLD.
Methods: Utilizing a multiple promoter baculovirus system (biGBac) system, we engineered baculovirus that contains the ATPase domain of either MYO7A-C or MYO7A-N with five IQ motifs or truncated two IQ motifs, in addition to co-expressing a myosin specific chaperone (UNC45A), and candidate light chains: CALM1, CALML4, MYL6 and MYL12B. MYO7A-C and MYO7A-N baculovirus were transfected to produce recombinant protein in Sf9 cells. MYO7A-C and MYO7A-N proteins were captured from whole cell lysates using FLAG-affinity chromatography and further purified with sequential anion exchange and size exclusion chromatography. Purified MYO7A-C and MYO7A-N were further evaluated by a steady-state NADH ATPase assay and single-molecule mass photometry.
Results: SDS-PAGE results show that recombinant MYO7A-C and MYO7A-N is greater than 99% pure and forms a pentamer, stably binding to all four light chains (CALM, CALML4, MYL6, MYL12B). Purified MYO7A-C and MYO7A-N were monomeric as measured by mass photometry with masses of 183±18 kDa (94%) and 183±27 kDa (92%), respectively. MYO7A-N had a reduced maximum ATPase rate (kcat = 0.10±0.01 s-1) and requires higher actin concentration for the half maximal ATPase activity (KATPase = 10.2±2.9 μM) compared with MYO7A-C (kcat = 0.38±0.02 s-1 , KATPase = 7.0±1.0 μM), indicating the N-terminal domain reduces the motor activity of MYO7A. Work is ongoing to generate truncated IQ domains to map light chain binding to MYO7A-C and MYO7A-N to understand how they contribute the ATPase cycle.
Conclusions: n conclusion, our results show that the N-terminal domain can regulate the ATPase activity of MYO7A. The reduced kcat of MYO7A-N suggests that it can generate tension on actin filaments for an extended period compared to MYO7A-C. The altered ratio of MYO7A isoforms may therefore tune mechanical activity at the UTLD, thus influencing tension on the tip-link.

Myosin 15 Isoform 3 Traffics the Elongation Complex to the Tips of Row 1 Stereocilia and is Required for Their Maintenance in the Adult Hair Cells

Ghazaleh Behnammanesh*[1], Shadan Hadi[2], Abigail Dragich[2], Xiayi Liao[3], Mi-Jung Kim[1], Lijin Dong[4], Benjamin Perrin[3], Shinichi Someya[1], Gregory I Frolenkov[2], Jonathan Bird[1]

[1]*University of Florida,* [2]*University of Kentucky,* [3]*Indiana University - Purdue University Indianapolis,* [4]*National Eye Institute, NIH*

Category: Hair Cells: Anatomy and Physiology
Background: Actin-based stereocilia assemble into the hair bundle with rows of precisely graded heights, and maintenance of this “staircase” architecture is essential for normal mechanotransduction. Mutations in MYO15A, encoding the molecular motor myosin 15, disrupt the hair bundle and cause hereditary hearing loss, DFNB3. MYO15A influences hair bundle architecture via multiple protein isoforms. MYO15A-1 maintains shorter row stereocilia that harbor functional MET channels. MYO15A-2 controls stereocilia elongation during development by trafficking the elongation complex (EC), WHRN, EPS8, GNAI3, and GPSM2, and potentially by directly stimulating actin polymerization. Here, we study a newly reported isoform, MYO15A-3, and explore its function in the cochlea.
Methods: Using CRISPR-Cas9 genomic editing, we generated a new mouse model lacking only isoform 3 of Myo15a (Myo15a-Δ3). To assess hearing, we measured auditory brainstem response (ABR) thresholds at 8, 16, and 32 kHz and distortion product otoacoustic emission (DPOAE) amplitudes at 8, 16, and 32 kHz in Myo15a-Δ3 mutant mice and littermates. Myo15a-3 expression was quantified using qPCR and BaseScope in situ hybridization. To determine MYO15A-3 protein targeting, P4 cochlear explants were injectoporated to express MYO15A-3-EGFP. Hair cells were labeled using antibodies recognizing MYO15A and EC proteins and imaged with spinning-disk confocal microscopy. Hair bundle ultrastructure was examined using scanning electron microscopy (SEM).

Results: Myo15a(Δ3/Δ3) mice had normal ABR thresholds at P17. However, by P30, Myo15a(Δ3/Δ3) mice had ABR thresholds greater than 90 dB SPL, indicating a rapidly progressing and profound hearing loss. In wild-type cochleae at P1, Myo15a-3 expression was not present, but increased postnatally from P7 onwards and was specifically detected in IHCs and OHCs. Exogenous EGFP-tagged MYO15A-3 localized strongly to the tips of stereocilia, consistent with being identical to MYO15A-2 except for a small 6 kDa N-terminal domain. We hypothesized that MYO15A-3 could traffic the EC in postnatal hair cells, similar to the activity of MYO15A-2 in neonatal hair cells. To test this, we examined EPS8 and WHRN immunofluorescence in Myo15a(Δ3/Δ3) hair cells and littermate controls. At P4, when MYO15A-2 is present in stereocilia, the distribution of EPS8 and WHRN were indistinguishable between Myo15a(Δ3/Δ3) hair cells and controls. By P21, when MYO15A-3 is dominant, there was a significant and robust decrease in WHRN and EPS8 labeling at the tips of Myo15a(Δ3/Δ3) stereocilia. SEM analysis of Myo15a(Δ3/Δ3) cochleae at P21 revealed distinctive thinning and changes in the shape of tips of row 1 stereocilia in IHCs, consistent with abnormalities of underlying structures.
Conclusions: Our data show that the EC complex is initially trafficked to the tips of stereocilia by MYO15A-2, before switching to MYO15A-3 postnatally. Whilst the reason for this handover is unclear, our results argue that the combined presence of MYO15A-3 and EC proteins are critical for maintaining the essential cytoskeletal elements at the stereocilia tips.

Proteins Required for Stereocilia Elongation During Hair Cell Development Ensure Precise and Steady Heights in Adult Life

Elli Hartig*[1], Matthew Day[1], Amandine Jarysta[1], Basile Tarchini[1]

[1]*The Jackson Laboratory*

Category: Hair Cells: Anatomy and Physiology
Background: The hair cell sensory organelle is comprised of stereocilia, actin-based membrane projections organized into rows of graded heights. For proper function, developing stereocilia must grow to precise dimensions and establish differential row identity. In contrast, mature stereocilia are highly stable barring a ~0.5 µm region at the distal tip defined by continuous actin turnover. Our current knowledge of stereocilia is biased toward development, and less is known about their life-long preservation in mammalian hair cells. We hypothesized that proteins required for stereocilia elongation during development remain active in maturity to ensure stable height. To test this idea, we focused on the GPSM2-GNAI protein module, which enriches the MYO15A-driven elongation complex in developing stereocilia fated to become the tallest row (row-1).
Methods: To allow normal hair cell development, we inactivated mouse Gpsm2 or Gnai3 at 6 weeks using conditional alleles and Cre recombinase delivered either surgically (AAV-Cre) or using a tamoxifen-inducible Gfi1-CreERT strain. We used immunolabeling and Airyscan confocal microscopy to assess protein localization and levels, and to measure stereocilia height in inner hair cells. To estimate GPSM2 longevity at tips, we measured decay of immunolabeled protein in conditional mutants, and generated a HaloTag-Gpsm2 strain for pulse-chase approaches in vivo using HaloTag ligands. To assess how adult Gpsm2 inactivation impacts hearing, we recorded auditory brainstem responses (ABRs) and Distortion Production Otoacoustic Emissions (DPOAEs).
Results: We first confirmed that GPSM2-GNAI and other elongation complex proteins remain enriched at the tip of row-1 stereocilia in mature cochlear hair cells. Following Gpsm2 inactivation, row-1 stereocilia showed a progressive height collapse that totaled ~600 nm after 12 weeks, regardless of cochlear position (12-17% of height). Inactivation of Gnai3 showed a more modest ~130 nm height reduction over the same period, likely due to functional redundancy among GNAI proteins as observed during development. We found that GPSM2 protein is long-lived at stereocilia tips, with both immunolabeled protein decay and Gpsm2HaloTag pulse-chase curves estimating a half-life of 9-10 days. Adult Gpsm2 inactivation had a modest impact on ABR thresholds and DPOAEs, consistent with the absence of hair cell death or stereocilia loss. Interestingly, ABR deficits were biased towards lower frequencies, with significantly reduced wave 1 amplitudes at 4-12 KHz, but not 16 KHz. Finally, we showed that GPSM2 is required for normal enrichment of elongation complex proteins MYO15A, WHRN, EPS8 and GNAI at stereocilia tips, mirroring their co-dependence during development.

Conclusions: The elongation complex remains essential after development to maintain precise and stable stereocilia height in adult hair cells, and to ensure detection of a full range of sound frequencies. How adult stereocilia maintain their integrity remains under-studied; new insight will define the extent of normal housekeeping and damage repair, informing therapeutic approaches toward hearing preservation.

Staircase Formation During Embryonic Development of Mouse Inner Hair Cells

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Category: Hair Cells: Anatomy and Physiology

Background: Hair-bundle assembly depends on differential growth of stereocilia to produce the staircase architecture necessary for mechanotransduction. During postnatal development, stereocilia in each row differentially widened and lengthened under the control of transduction and the accumulation of a MYO15A-dependent complex at the tips of row 1 stereocilia. To examine factors controlling initial formation of the hair bundle staircase, we characterized embryonic stereocilia growth of inner hair cells in wild-type mice and in mutant mice lacking MYO15A.

Methods: Using lattice structured illumination microscopy (SIM) and scanning electron microscopy (SEM), we quantified stereocilia growth in C57BL/6 mouse inner hair cells (IHCs) between the ages of embryonic day 15.5 (E15.5) and postnatal day 7.5 (P7.5). To examine the role of MYO15A in embryonic stereocilia growth, we performed the same set of measurements in Myo15a^sh2/sh2 mutant mice. Measurements of stereocilia or fluorescent beads oriented in xy or z planes were used to quantify measurement errors.

Results: Stereocilia in all rows started to lengthen at approximately the same time during embryonic development, with apical IHCs initiating elongation later than mid and basal cells. Stereocilia in all rows lengthened linearly, maintaining an even staircase with roughly equivalent step spacing. At the end of this period (E18.5 in mid and basal IHCs), stereocilia rows 2-4 started to shorten. Row 1 stereocilia of basal IHCs shortened, whereas row 1 lengths in apical and mid IHCs remained stable.

In Myo15a^sh2/sh2 mutant mice, stereocilia lengths were nearly equivalent between heterozygotes and knockouts at early stages of growth and only started to diverge after E18.5 (apical or mid IHCs) or after E17.5 (base). In heterozygotes, as in C57BL/6 mice, apical IHC stereocilia continued to lengthen until P0.5, after which stereocilia rows 2-4 shortened or disappeared, with row 1 length stabilizing until P7.5. In knockouts, however, row 1 stereocilia shortened significantly between P0.5 and P7.5, along with rows 2-4, producing a bundle with 4-5 rows of stereocilia with nearly equivalent lengths.

Conclusions: Despite morphological differences between mature IHCs along the tonotopic axis, all IHCs undergo the same extent of early stereocilia lengthening, diverging only in when lengthening starts and the timing and extent of stereocilia shortening. Stereocilia form a shallow staircase during the initial lengthening phase (stage IIa); the staircase is then refined through differential shortening of the shorter rows rather than differential lengthening of row 1 (stages IIb and III). MYO15A does not contribute to lengthening in stage IIa but it does during stage IIb lengthening. The short stereocilia of Myo15a^sh2/sh2 mutant mice derive not from a failure of initial staircase formation, but rather from retraction of all stereocilia rows. These results suggest that MYO15A and its row 1 tip complex partners may protect row 1 from shortening during early hair-bundle development.

The Endolymphatic Potential Can Control the Gating Force of the Hair Cell's Transduction Channels.

Achille Joliot[1], Pascal Martin*[2]

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Category: Hair Cells: Anatomy and Physiology

Background: Mechanosensitivity of the hair cell results from direct mechanical activation of ion channels by the tip links of the hair bundle. Deflection of the hair bundle modulates tip-link tension, resulting in channel gating and a transduction current. By virtue of mechanical reciprocity, channel gating feeds back on tip-link

tension, producing an internal force called the “gating force”. The gating force is a fundamental determinant of hair-cell mechanosensitivity. Its magnitude indeed sets the maximal slope of the relationship between the transduction current and the hair-bundle displacement. Moreover, the gating force can be large enough to reduce the hair bundle’s stiffness and even produce negative stiffness, fostering spontaneous hair-bundle oscillations and amplification of sinusoidal stimuli. Here, we report that varying the electric potential in endolymph bathing the hair bundles can serve as a control parameter of the gating force.
Methods: We used an excised preparation of the frog saccule with two ionic compartments that mimicked ex vivo the endolymphatic and perilymphatic compartments found in vivo. Under such conditions, the hair bundles displayed spontaneous oscillations. By applying steps or slow ramps of a transepithelial current within a range ±10 μA, the potential into the artificial endolymph that bathed the hair bundles was varied within a range of ±100 mV with respect to the potential in the perilymphatic compartment. We estimated the transduction channels’ gating force from gating compliance in the force-displacement relation of individual hair bundles and studied how it varied upon application of a transepithelial current.
Results: We found that the magnitude of the gating force displayed a strong negative correlation with the transepithelial current, I. The gating force could be reduced to hardly detectable levels upon application of a positive current. Conversely, it could nearly double upon application of a negative current of growing magnitude until, beyond a threshold value (I≤-5 μA), the gating force abruptly dropped to a low level, resulting in linear force-displacement relations and, correspondingly, in the disappearance of spontaneous hair-bundle oscillations. In addition, when descending and ascending ramps of transepithelial currents were applied in succession, the transition between strong and weak gating forces displayed hysteresis. Finally, the hair-bundle stiffness at large displacements, that is outside the region of gating compliance in the force-displacement relation, was only weakly affected by transepithelial currents. All these effects were fully reversible. All these effects were fully reversible.
Conclusions: We conclude that the endolymphatic potential may serve as a control parameter of the transduction channel’s gating force and in turn of hair-cell mechanosensitivity. Our work indicates that the molecular movement associated with channel gating—the gating swing—can be of variable magnitude in the same hair cell depending on electrical conditions.

Single-Molecule Microscopy in Live Hair Cells Reveals a Regulatory Mechanism of MYO7A-Driven Cargo Transport in a Stereocilium

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Category: Hair Cells: Anatomy and Physiology
Background: Unconventional myosins are essential for developing and maintaining functional stereocilia. Among the myosins expressed in hair cells, MYO7A is crucial for transporting and anchoring components of the tip-link complex and also for its association with retinal dysfunction manifested as Usher syndrome type 1. While a number of proteins have been identified as cargo of MYO7A, little is known about how its trafficking is regulated in a stereocilium. Proteins in the MYO7A interactome can provide three types of machinery to allow myosins to move in a stereocilium: dimerization, anchoring to the plasma membrane and binding to F-actin. Here, we introduce our novel workflow for single-molecule microscopy in live hair cells and demonstrate our application to MYO7A trafficking in a stereocilium.
Methods: We expressed HaloTag-fused proteins in vestibular hair cells using a Helios® gene gun and fluorescently labeled them using a low concentration of JFX554-conjugated ligands. Transfected hair cells were imaged using the light-sheet illumination of a dual-inverted selective plane illumination microscope (diSPIM). For dimerization, HaloTag-fused MYO7A head (motor + neck region; HaloTag-MYO7A-HMM) was fused with the p.F36V mutant of FK506-binding protein 12 (FKBP) to the C-terminus and crosslinked by an FK506 derivative, AP20187. Anchoring to the plasma membrane or to the F-actin core was achieved by using a heterodimerization technique using FKBP, the FKBP-rapamycin binding domain (FRB) and a crosslinking Rapalog, AP21987. We fused either FKBP or FRB to the C-terminal of HaloTag-MYO7A-HMM

and the unused one to the anchoring proteins, such as the Tac membrane antigen and the THDII F-actin binding domain of MYO3A, depending on the structure of anchoring proteins.
Results: Under the single-molecule microscopy, HaloTag-MYO7A-HMM-FKBP showed processive movements toward stereocilia tips only when dimerized by AP21087. Anchoring to the plasma membrane (via Tac antigen) or to the F-actin (via THDII of MYO3A) did not cause movements of HaloTag-MYO7A-HMM (with FRB or FKBP for anchoring; see Methods) in a stereocilium. However, the positive controls, MYO10 and MYO3A heads, moved in stereocilia using these two mechanisms. To test if MYO7A can dimerize in a physiological condition, we imaged HaloTag-fused MYO7A with two mutations in the second MyTH4-FERM domain (MYO7A-M/F2*), by which the tail-mediated motor autoinhibition is removed. Interestingly, a small number of HaloTag-MYO7A-M/F2* molecules showed processive movements on the F-actin core without any treatments. We also found that MYO7A accumulates at stereocilia tips when co-expressed with USH1G indicating that USH1G may be a key molecule to activate MYO7A trafficking in a stereocilium.
Conclusions: Using single-molecule microscopy, we enabled real-time observation of myosin trafficking in a stereocilium. Our data suggest that the MYO7A-HMM has kinetic properties tuned to function via dimerization in a stereocilium and that MYO7A can dimerize on the F-actin core in a physiological condition when its motor domain is exposed.

The Role of MYO7A Isoforms in Tuning Hair Cell Function

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Category: Hair Cells: Anatomy and 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 the molecular motor that tensions the 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: 5' RACE and RT-PCR was performed to identify MYO7A isoforms expressed in hair cells. Isoform specific MYO7A deletion or affinity tagged mouse lines were generated by using CRISPR/Cas9. 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.
Results: By conducting 5'RACE on cochlea cDNA, we identified two major isoforms of Myo7a in the auditory system: a widely studied canonical isoform (MYO7A-C) and an unreported novel isoform (MYO7A-N). The expression patterns of these two MYO7A isoforms were tested on isoform specific tagged mice, which demonstrated that IHCs predominantly express MYO7A-C, and with a significantly lower level of MYO7A-N. In OHCs, MYO7A-C and MYO7A-N showed an opposing gradient along the tonotopic axis. Moreover, the simultaneous deletion of both MYO7A-C and MYO7A-N nearly abolishes MYO7A immunoreactivity in all hair cells, accompanied by disorganized hair bundle and profound hearing loss. Therefore, we conclude that MYO7A-C and MYO7A-N are the major isoforms in hair cells. Our electrophysiology experiments on Myo7a-ΔC and hair cells showed a significant reduction of resting open probability in IHCs, consistent with the proposed role of MYO7A in generating tip-link tension. We also observed hearing loss of Myo7a-ΔN mice. Lastly, we tested motor activities of MYO7A-C and MYO7A-N isoforms. We have preliminary evidence 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. For future experiments, cryo-EM will be conducted to resolve the structure of MYO7A isoforms. We also created and

will analyze mouse lines in which the MYO7A isoforms are genetically switched without affecting overall expression level.
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 motor activity differences 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.

Podium Session 20 - Inner Ear: Drug Delivery

1:45 p.m. - 3:45 p.m.

Platinum Salon 5

Rational Design of Magnetic Nanoparticles as Cochlear Drug Delivery Vehicles

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Category: Inner Ear: Drug Delivery
Background: To support emerging biologic therapeutics (e.g. proteins and nucleic acids) for the treatment of sensorineural hearing loss, there is a critical need to develop a robust, minimally-invasive, re-doseable delivery vehicle that can be applied to large clinical populations. Magnetic nanoparticles (MNPs) are able to penetrate the round window membrane (RWM) after intratympanic injection and magnetically-assisted transport, but their translational development as cochlear delivery vehicles require highly interdisciplinary investigation. We present a framework for the rational design of MNPs by using an ex vivo guinea pig RWM model and specifically study the relationship between particle size and magnetically-assisted transport across the RWM.
Methods: Custom synthesis of core-shell MNPs and their physical-chemical characterization were performed with precisely controlled particle sizes. Size-dependent transport across ex vivo guinea pig RWM was characterized in a modified Ussing chamber with and without magnetic field gradient. A continuous flow model was constructed to study MNP diffusivity within RWM as a function of MNP hydrodynamic size and compared across various cellular and acellular tissue types. The histologic basis of MNP transport was studied using confocal and transmission electron microscopy and the epithelial transport in RWM was probed using various endocytotic inhibitors and enhancers.
Results: Monodispersed, PEG-coated superparamagnetic magnetic nanoparticles of 7nm core size and 44, 66, and 89 nm hydrodynamic sizes were synthesized and characterized. Relative to acellular semiporous porcine intestinal submucosal (SIS) membrane, ex vivo guinea pig RWM demonstrated increased transport of MNPs with and without magnetic field gradient. Transport was maximized for hydrodynamic diameter of 66nm. To control for differential membrane thickness, continuous flow model demonstrated MNP effective diffusivities of 28.2 ± 0.8 x10-11, 7.0 ± 0.1 x10-11, and 12.9 ± 0.6 x10-11 m2/s across SIS, guinea pig RW, and guinea pig endothelial (vein) membranes under a magnetic field gradient. Inhibition of clathrin-mediated endocytosis via chlorpromazine demonstrated the largest effect on epithelial transport in RWMs compared to other endocytotic pathways, while application of sodium caprate, a junctional modulator, was found to enhance transport by 61% under magnetic field gradient. Electron microscopy demonstrated MNP distribution across all cellular layers and confocal microscopy demonstrated lysosomal-dependent transport of MNPs within the epithelial layer with the outer and inner layers serving as primary transport energy barriers.
Conclusions: We present a framework for the systematic investigation of MNP transport across guinea pig RWM to allow rational development as cochlear drug delivery vehicles. In contrast to non-magnetic nanoparticles and molecules, we find that the ratio of MNP hydrodynamic to core size is more important than overall size alone in affecting transport across RWM and that the transcellular transport across the outer epithelial cell layer represents the rate-limiting step of RWM transport, which can be augmented via a magnetic field gradient.

Magnetic Nanoparticles for Cochlear Drug Delivery: Mechanisms and Modulation of Epithelial Transport in the Round Window Membrane

Sarek Shen*[1], Mukund Madhav Goyal[2], Zimin He[2], Mohamed Lehar[1], Chao Wang[2], Daniel Sun[1]

[1]*Johns Hopkins School of Medicine,* [2]*Johns Hopkins University*

Category: Inner Ear: Drug Delivery

Background: The round window membrane (RWM) serves as an important anatomical barrier to local application of therapeutics to the inner ear. Superparamagnetic iron oxide nanoparticles (SPIONs) have emerged as a potential vehicle to improve transmembrane drug delivery. These particles are comprised of an iron oxide core and can be coated with a biocompatible polymer layer, allowing for both magnetically assisted transport as well as controlled drug elution. Evidence suggests that the outer epithelial cell layer containing tight junctions is the rate-limiting step. However, little is known about the cellular transport mechanisms underlying SPION transport across the RWM. Understanding of SPION uptake pathways in the RWM will aid in the rational design of these magnetically assisted drug delivery vehicles.

Methods: Monodispersed SPIONs with core size of approximately 7nm were synthesized in organic solution and coated with poly(ethylene glycol) MW 3000, to make core-shell nanoparticles (NP-PEG3000). Transport of these particles across freshly explanted guinea pig RWM was investigated both with and without the presence of a magnetic field gradient using a modified Ussing chamber. The mechanism of PEG-SPION transcellular transport was further studied by inhibition of specific endocytosis pathways. To augment the permeability of the RWM, a variety of junctional modulators were applied. Confocal laser scanning microscopy and transmission electron microscopy were utilized for histological analysis of the RWM.

Results: 20.1±1.9% of NP-PEG3000 were transported across the native guinea pig RWM over a 4-hour period. Lysosomal-dependent transport was verified via colocalization on confocal microscopy. Inhibition of clathrin-mediated endocytosis reduced transport by 64.1% (7.4±0.3% delivered, 95%CI: [8.9–16.2]). No significant change to delivery was found with caveolin-mediated endocytosis inhibition (21.1±1.1% delivered, 95%CI: [-2.4–3.1]) or micropinocytosis inhibition (16.0±2.2% delivered, 95%CI: [-4.2 – 5.8]). Placement of the RWM within a 0.7T magnetic field gradient increased SPION delivery by an average of 42.9% (Range: 27.8% - 53.3%). Application of the tight junction modulators sodium caprate and collagenase within a magnetic field gradient further augmented delivery to 61.5% (32.3±1.8% delivered, 95%CI:[8.5-14.2]) and 67.1% (33.6±4.5% delivered, 95%CI [7.8-17.2]) respectively. On imaging analysis, these modulators induced transient gaps in intercellular spaces within the outer epithelial layer of the RWM. However, SPION transport remained largely transcellular in a lysosomal-dependent manner.

Conclusions: Clathrin-mediated endocytosis serves as the primary internalization mechanism for SPIONs at the outer epithelial layer of the RWM. The addition of a magnetic field significantly increases particle translocation, while junctional modulators have a smaller effect on transmembrane delivery. Physiochemical modification of SPIONs may further augment transcellular uptake and improve overall delivery.

Surgical Procedure of Transtratympanic Injection and Inner Ear Drug Kinetic Simulation in Domestic Pigs

Adele Moatti*[1], Shannon Conard[1], Novietta De Beritto[1], William Dunn[2], Ken Hutson[2], Douglas Fitzpatrick[2], Alec Salt[3], Carlton Zdanski[2], Alon Greenbaum[1]

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Category: Inner Ear: Drug Delivery

Background: The study addresses the challenge of delivering drugs to the inner ear, a complex task that requires a reliable model for optimization. Systemic delivery is hindered by blood labyrinth barriers. Local techniques like cochleostomy and semicircular canal injection, though viable, carry risks and yield variable outcomes. The transtympanic injection is safer but less efficient as the drug must traverse the round window membrane and diffuse to the cochlear apex. Understanding the cochlea's anatomical size is crucial for preclinical drug kinetics. Some studies have aimed to enhance transatympanic delivery in small animal models, yet direct translation to human clinical trials is hindered by anatomical differences. This necessitates validation in a large animal model.

Methods: The study focuses on delivering drugs to the middle ear via intra/transtympanic injection in domestic pigs, a large animal model. A detailed transcanal surgical protocol is described, addressing the

challenges posed by the narrow and tilted external canal. Evaluation metrics include drug concentration in perilymph via mass spectrometry and auditory brainstem responses (ABR) before and after injection. Volumetric analysis of porcine inner ear data and drug kinetics simulations in comparison to human inner ear using Fluidsim software are provided.
Results: The method successfully delivered dexamethasone to the inner ear. ABR tests pre- and post-injection revealed no adverse effects on hearing threshold due to surgery. The piglets were monitored for two weeks post-surgery, displaying no behavioral changes. The simulation demonstrated similar drug diffusion time from the base to the apex of the cochlea in humans.
Conclusions: This predictable drug delivery model in large animals like pigs holds immense promise for translational research in otology. The selection of an appropriate large animal model is pivotal in drug dosage and timing optimization due to inner ear characteristics. Pigs, with similar cochlear attributes to humans, such as length, hair cell count, and round window membrane thickness, prove to be an ideal model for inner ear drug delivery studies. The established transtympanic delivery method not only enhances drug delivery efficiency but also enables the testing of a wide array of potential therapeutics in a predictable preclinical setting. Additionally, the technique's confirmation of survival adaptability in domestic pigs allows for safety and immune response testing.

Novel Microneedle Device for Transcanal Round Win-dow Membrane Access via Endoscope and Flexible Actuation Mechanism

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[1]*Haystack Medical,* [2]*Columbia University*

Category: Inner Ear: Drug Delivery
Background: Safe and reliable access to the cochlea is a significant barrier in the field of otology. Our laboratory has developed microneedles that safely perforate the round window membrane (RWM) for diagnostics and delivery in the guinea pig model. In this study, we introduce a novel microneedle-endoscope design, which facilitates the deployment of microneedles to the RWM via a transcanal approach.
Methods: 100-µm diameter hollow microneedles were fabricated using two-photon polymerization lithography and mounted on a custom-designed mechanism consisting of stainless-steel and polyimide tubing. Microneedle-tubing assembly was housed within a modified curved suction tube of a Colibri micro-endoscope that allowed visualization of the microneedle tip (3NT Medical, NJ). A 3D-printed spring-loaded actuation mechanism was attached to the endoscope at its proximal end. Fixed human cadaveric temporal bones were used to visualize and advance the microneedle towards the RWM as a proof of principle in situ.
Results: The endoscope-microneedle was introduced into the middle ear of a human cadaveric temporal bone sample through a posterior tympanomeatal flap. The device was placed near the round window niche and the microneedle was oriented and actuated towards the RWM. The microneedle was retracted into the curved tube and the endoscope was removed from the middle ear safely.
Conclusions: Our results demonstrate the use of a novel microneedle actuation system which works in conjunction with ENT micro-endoscopes. The provided access to the RWM under full visual guidance and the ability to advance and retract microneedles in a controlled manner bolster the potential of microneedles for minimally invasive inner ear diagnosis and treatment procedures.

Development of Laser-Activated Nanoparticles for Controlled Release of Neural Differentiation Factors in Inner Ear Regenerative Therapy

Celine Abueva[1], Sung Ryeong Yoon*[1], Nathaniel Carpena[1], So-Young Chang[2], Jin-Chul Ahn[1], Ji-Eun Choi[3], Jae Yun Jung[3], Min Young Lee[3]

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Category: Inner Ear: Drug Delivery
Background: Nanoparticles present promising research in inner ear dysfunction treatment and possess unique properties that enable targeted delivery of therapeutic or neuroprotective agents directly to affected inner ear structures to promote repair and regeneration. Neurotrophic factor cargo can be released through light

irradiation. Light is a particularly appealing tool for on-demand release due to its noninvasive nature, ease of application, breadth and depth strategies, and spatio-temporal control. The aim was developing laser-activated nanoparticles for targeted delivery and controlling release of differentiation factors to damaged neurons in the inner ear. Neural progenitor cells (NPCs) and Scarpa's ganglion neuronal cell (SGNC) spheroids were utilized for nanoparticle evaluation in vitro. Ouabain, a known Na/K-ATPase inhibitor, was used on SGNC spheroids as in vitro damage model. The Mongolian gerbil with similar ear anatomy to humans was used as an animal model in vivo.

Methods: Light-responsive polymer comprised of a hydrophobic dye molecule sensitive to near-infrared light and a polyethylene glycol polymer backbone. Nanoparticle was fabricated using the light-responsive polymer via solvent-assisted self-assembly, encapsulating brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) neurotrophic factors. BDNF/NT-3 amount released upon laser irradiation at 808 nm wavelength, 200 mW/cm2 power, and different energy densities (30, 60, and 90J/cm2) were monitored. For inner ear dysfunction model, SGNC spheroids were treated with ouabain (100uM). Nanoparticles were delivered, laser treated and cell viability was confirmed after 24h. Additionally, nanoparticles were injected into the inner ear of Mongolian gerbils through the round window membrane for monitoring and implantation assessment.

Results: Fabricated nanoparticles respond to 633-808 nm laser irradiation to release encapsulated BDNF and NT-3. Light-controlled BDNF and NT-3 release upon laser irradiation was observed from 30 to 90J/cm2 using an 808 nm laser with optimum energy density at 60J/cm2. The nanoparticles were found to be noncytotoxic to NPCs and SGNC spheroids in culture. To exclude laser effects, cell viability assay was performed without nanoparticles in ouabain treated spheroids. No significant change in viability was observed with 30 and 60 J/cm2, but significantly increased at 90 J/cm2 laser irradiation compared to control. Cell viability in non-irradiated nanoparticles was not different compared to control, but cell viability in nanoparticles with laser significantly increased, showing potential regeneration after damage with released BDNF/NT-3. The nanoparticles fluoresce upon excitation, allowing for monitoring of particle location and confirming the release of load. Stained sectioned tissues from the implanted cochlea confirmed the successful migration and localization of the nanoparticles within the inner ear.

Conclusions: The development of light-sensitive nanoparticles that can self-assemble for growth factor encapsulation and controlled release has been achieved. These nanoparticles demonstrate non-cytotoxicity and have potential applications for delivering growth factors within the inner ear, serving as a regenerative therapy for inner ear disorders.

Magnetic Field-Enhanced Delivery of Mesenchymal Stem Cells in a Mouse Model of Ototoxic Hearing Loss

Yeji Ahn*[1], Seonmin Choi[2], Jaehong Key[2], Young Joon Seo[1]

[1]*Yonsei University Wonju College of Medicine,* [2]*Yonsei University Mirae*

Category: Inner Ear: Drug Delivery

Background: Stem cells are unique in their ability to self-renew and differentiate into many specialized cell types. These cellular entities can maintain their stemness or differentiate into more specialized cell lineages. Researchers are interested in mesenchymal stem cell (MSC) cell therapy due to its ethical feasibility, ease of isolation, and abundance. In vitro and in vivo, MSCs have anti-inflammatory and immunomodulatory properties, they inhibit T-cell proliferation and function. These cells can also migrate to the injury site and activate native cells for tissue regeneration and reconstruction. Although stem cells can migrate, targeting complex anatomical regions such the inner ear regions remains challenging.

Methods: A magnetic field and a novel magnetic nanoparticle improve stem cell transport to the cochlea. The nanoparticle has a cluster core of iron oxide nanoparticles surrounded by Poly(D, L-lactide-co-glycolide). Laser microscopy visualization is enhanced by conjugating the nanoparticle with cyanine 5.5. Creating specialized static magnets generated the magnetic field. Two magnets in opposing orientations in a metallic cylinder generate the desired magnetic force. To increase magnetic flux propagation over a wide range, the cylinder has a conical end. The cylindrical magnet produced has a magnetic flux density of 535.2 mT and measures 1 cm by 7 mm. A magnetic flux density with a repulsive force was created by aligning two magnets. The configuration nullified magnetic fields at a specific location, creating a repulsive magnetic field. MSCs

were labeled with nanoparticles and exposed to a magnetic field in vitro and in vivo settings to determine migratory capacity and therapeutic efficacy.
Results: Transwell migration assay was utilized to evaluate the magnetic attraction and repulsion. The nanoparticle-labeled MSCs exhibited comparable responses to magnetic fields, indicating their susceptibility to magnetic forces. However, the introduction of stromal cell-derived factor-1 resulted in a twofold increase in cell migration within the repulsive group. Indicating that the magnetic repulsive force exhibits greater strength than the attractive force. The hearing threshold was assessed after the implantation of mesenchymal stem cells using in vivo outcome auditory brainstem response (ABR) click stimuli. The magnetic repulsive force exhibited a 40% efficacy than magnetic attraction force, in terms of restoring hearing within a span of 7 days subsequent to the injection of stem cells.
Conclusions: In this study we have developed nanoparticles and magnetic devices to deliver stem cells to the inner ear. We evaluated the therapeutic effect of nanoparticle labeled MSCs delivered to the inner ear and observe changes in inflammatory factors. Restoration of hearing as a consequence of administration of nanoparticles labeled MSCs is a significant finding of this study. Visualization of delivered MSCs and quantitative and qualitative investigation of associated mechanisms will help establish an evaluation framework for an efficient inner ear delivery method and will further aid hearing loss treatment.

Intracochlear Distribution and Accumulation of Locally Applied Dexamethasone Formulations in a Large Animal Model

Matthias Gerlitz*[1], Anselm Joseph Gadenstaetter[1], Lukas Landegger[1], Clemens Honeder[1], Eric Lehner[2], Arne Liebau[2], Stefan Plontke[2], Erdem Yildiz[1], Christoph Arnoldner[1]

[1]*Medical University of Vienna,* [2]*Martin Luther University of Halle-Wittenberg*

Category: Inner Ear: Drug Delivery
Background: At present, glucocorticoids stand as the benchmark for addressing a range of inner ear disorders. However, their respective distribution and accumulation inside the inner ear after local delivery is still inadequately understood. Large animal models possessing inner ear dimensions resembling those of humans can therefore help to shed light on intracochlear pharmacokinetics and enhance our understanding of how drugs spread within the cochlea. In a translational research approach, we topically applied dexamethasone (DEX) or dexamethasone phosphate (DEX-P) in four different formulations to the middle or inner ears of piglets and analyzed its distribution in the porcine cochlea.
Methods: Up to 250µl of DEX-P in its liquid form [4mg/ml] or bound to a thermoreversible hydrogel [6% DEX] was injected to piglets intratympanically. Moreover, a novel inner ear catheter [40µl DEX-P] and a custom-made DEX-loaded, biodegradable poly(lactic-co-glycolic acid) (PLGA) implant [10% DEX] was used for direct intracochlear drug delivery via the round window. DEX distribution in the porcine inner ear was subsequently determined via apical scala tympani perilymph (PL) sampling at different time points after application using high-performance liquid chromatography.
Results: Utilizing an endaural approach, successful application of fluid and hydrogel-bound DEX-P and DEX, along with their intracochlear application through the round window using the inner ear catheter or PLGA implant, was feasible. After 2 hours, overall PL concentrations of DEX were significantly increased in the inner ear catheter group compared to the hydrogel-IT ($p \leq 0.0001$) and the fluid-IT groups ($p \leq 0.000.1$). After 24 hours, the hydrogel-IT group displayed reduced DEX concentrations in comparison to the sampling conducted after 2 hours ($p \leq 0.0001$). Notably, DEX could not be detected in the apical regions of any of the animals after administering the DEX-loaded PLGA implant. However, the highest DEX concentrations in the cochlear basal regions after PLGA implantation were observed after 7 days, which was significantly different from the levels detected at 2 hours ($p=0.0013$) and 3 days ($p=0.0119$).
Conclusions: Our work presents the first data on glucocorticoid distribution in the inner ear in a large animal model. The similarity of the porcine cochlea to the human inner ear with respect to spatial dimension supports the clinical translation of our findings and can lay the foundation on how the treatment of inner ear disorders can be optimized in the future.

Manipulation of Notch Signaling to Regenerate Type I and Type II Hair Cells With Restoration of Vestibular Function

Hanae Lahlou*[1], Hong Zhu[2], Wu Zhou[2], Albert S. B. Edge[1]

[1]*Massachusetts Eye and Ear Infirmary - Harvard Medical School,* [2]*University of Mississippi Medical Center*

Category: Inner Ear: Drug Delivery

Background: Notch signaling plays multiple roles in otic specification of the developing inner ear 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 pharmacological and genetic approaches to modulate Notch signaling in a murine model of damaged utricle. We targeted hair cells for ablation using a mouse line (Pou4f3-DTR) that expresses the diphtheria toxin receptor in hair cells. A gamma-secretase inhibitor was administrated locally via the round window and the extent of spontaneous vs drug-induced regeneration was compared between the treated and untreated (contralateral) ear. We also deleted Notch in Sox2-expressing cells to explore the effect of Notch conditional knockout on hair cell regeneration. We employed lineage tracing and quantitative analysis to assess changes in cell fate in response to altered Notch signaling.

Results: Our findings reveal that targeted manipulation of Notch leads to a significant increase in cells expressing hair cell marker, Myo7A. Drug treatment resulted in regeneration of 48% of the normal number of hair cells after 1 month as compared to 25% restoration of hair cells in the damaged ear by spontaneous regeneration without drug treatment. Regenerated hair cells with drug treatment displayed both type I and type II phenotypes, while spontaneously regenerated hair cells were exclusively type II. Similar results were observed in Notch conditional knockout. The changes in hair cell number were associated with a significant functional improvement as assessed by the vestibuloocular reflex and single fiber recordings from vestibular afferent neurons.

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.

Honorary Symposium - Mechanisms of Neural Encoding in the Inferior Colliculus and Beyond, A Symposium in Honor of George D. Pollak

4:00 p.m. - 6:00 p.m.

Platinum Salon 6

Mechanisms of Neural Encoding in the Inferior Colliculus and Beyond: A Symposium in Honor of George D. Pollak

Chair: R. Michael Burger*, Lehigh University*

Co-Chair: Achim Klug*, University of Colorado School of Medicine*

Co-Chair: Laura Hurley*, Indiana University*

Cholinergic Input Modulates Sound Encoding in the Superior Olive

R. Michael Burger

Lehigh University

Individual Abstract: Acetylcholine (ACh) is a prevalent neurotransmitter throughout the nervous system and is widely regarded as a potent neuromodulator. ACh signals are conferred through a variety of receptors that influence a broad range of physiological phenomena such as neurotransmitter release or neural excitability. In the auditory pathway, ACh modifies neurons' response to stimuli across multiple levels of processing. These factors may enable individual neurons to rapidly adapt to the dynamics of complex sensory stimuli. In the

auditory system, anatomical and physiological evidence shows that acetylcholine receptors (AChRs) are expressed at virtually every level of the ascending auditory pathway. In this presentation, I highlight our recent studies revealing the mechanisms, anatomy, and functional impact of cholinergic modulation in the superior olive.

Beyond Just Localization: How Fast Glycinergic Inhibition Helps Us Survive a Cocktail Party

Achim Klug

University of Colorado School of Medicine

Individual Abstract: Sound localization is one of the two principal tasks that our auditory system performs and is initially accomplished by a circuit which compares interaural time and intensity differences that a given sound creates between the two ears. This circuit resides in the brain stem and consists of two principal localization nuclei which are the lateral superior olive (LSO) and the medial superior olive (MSO) plus several accessory nuclei. One of these is the medial nucleus of the trapezoid body (MNTB) which provides fast and well-timed neural inhibition to the localization process. While the role of this circuit in sound localization is well established, it is less well appreciated that the same circuit also likely plays a role in situations where multiple sound sources are active at the same time, such as crowded restaurants, cocktail parties, classrooms or busy public areas. In such situations, these brainstem circuits perform the initial separation of these simultaneous sounds based on their location, thereby being the lowest brain area in the ascending auditory system that plays a role in cocktail party performance. There are several common conditions where alterations in the brainstem could likely lead to decreased abilities to listen in such acoustically busy environments, with aging being the most common condition. Our most recent work suggests that several age-related changes in the sound localization pathway can lead to a decreased ability to both localize sound and detect acoustic targets in background noise. One of these alterations is a change in myelination along the afferent pathway to MNTB. This heavily myelinated pathway which ends at the calyx of Held, a type of giant synapse, is known for temporally precise and fast action potential propagation. During the aging process, the myelination pattern along these fibers changes, leading to slower and less temporally precise neural activity. This in turn leads to less temporally precise firing among MNTB neurons and thus less precise outgoing neural inhibition from MNTB, which then compromises the precision by which nuclei such as the LSO and MSO extract information about spatial location, a likely cause of decreased ability to listen in acoustically crowded environments. Our data suggest that these alterations may occur in both human subjects and animal models.

What is Serotonin Doing in the Auditory System? The Social Feedback Model of Auditory Neuromodulation

Laura Hurley

Indiana University

Individual Abstract: Auditory processing is influenced by a wide range of non-auditory inputs, including projections from centralized neuromodulatory systems. One hypothesis about the function of these systems is that they integrate information on behavioral context, and modulate how sensory neurons respond to social cues, ultimately contributing to contextually appropriate behaviors. Serotonergic projections to the inferior colliculus (IC) conform to multiple predictions of this hypothesis. The activity of IC-projecting serotonergic neurons and serotonin release within the IC correspond to social behaviors. Serotonin also alters the responses of IC neurons to sound, including social vocalizations. Here, we test the prediction that serotonergic modulation in the IC can affect behavioral responses to vocal signals, using the recently described split cage assay, which measures male responses to the playback of human-audible female vocal signals (squeaks). Serotonin levels were pharmacologically manipulated through both the systemic injection of the serotonin precursor 5-hydroxytryptophan (5-HTP) and the local injection into the IC of the serotonin releaser fenfluramine. Both manipulations increased the amount of USV suppression during the playback of squeaks. 5-HTP injection also strongly affected male investigation of a small window in the barrier separating males from their female social partners: males injected with 5-HTP decreased investigation during and after playback, but males injected with saline did not. Neither 5-HTP nor fenfluramine had pronounced effects on

the non-social behaviors of digging and grooming. These findings suggest that serotonergic effects within the IC cause males to become more responsive to female vocal signals, consistent with the social feedback model.

Cholinergic Circuitry and the Inferior Colliculus

Brett Schofield

Northeast Ohio Medical University

Individual Abstract: George Pollak conducted many studies directed at understanding how individual neurons integrate information from different sources to analyze sound. His interests included the role of neuromodulators and their effects on neural processing in the inferior colliculus (IC). Acetylcholine, a neuromodulator that affects a majority of IC neurons, is believed to play a role in a wide variety of auditory functions, but little is known about the underlying circuitry. In this talk, I will summarize some of our work on cholinergic circuits that innervate the IC. First, we found that the IC receives input from cholinergic neurons in multiple regions: the pedunculopontine tegmental nucleus (PPT), the laterodorsal tegmental nucleus (LDT) and the lateral paragigantocellular nucleus (LPGi). Projections from the PPT and LDT terminate throughout the IC whereas projections from the LPGi terminate primarily in the extra-lemniscal IC (i.e., dorsal cortex and lateral cortex). The cholinergic inputs appear to contact both glutamatergic and GABAergic IC neurons, suggesting modulation of excitatory and inhibitory IC circuits. Finally, cholinergic neurons that innervate the IC appear to receive input from multiple auditory areas, including descending projections from the auditory cortex. We conclude that a multitude of sources for cholinergic innervation of the IC is consistent with multiple cholinergic functions, including modulation associated with arousal, sleep-wake cycle, sensory gating and reward. The targeting of both glutamatergic and GABAergic neurons suggests that cholinergic inputs modulate both excitatory and inhibitory IC circuits. Finally, direct projections from auditory cortex to cholinergic cells that innervate the IC provide a substrate for top-down cholinergic modulation of acoustic processing in the midbrain.

Regulation of Recurrent Excitation in the Auditory Midbrain

Michael Roberts

University of Michigan

Individual Abstract: One of the major themes of George Pollak's work was that inhibition critically shapes auditory computations in the inferior colliculus (IC). Synaptic inhibition in the IC comes from both ascending inputs and local inhibitory circuits, but the neurons and synaptic mechanisms underlying local inhibition in the IC have been difficult to address experimentally. Using a multifaceted approach, we identified NPY neurons as the first molecularly identifiable class of inhibitory neurons in the IC. NPY neurons are GABAergic, express the signaling peptide neuropeptide Y, and project extensively within the IC and to long-range targets including the auditory thalamus and nucleus of the brachium of the IC. In this talk, we will discuss how NPY neurons are poised to regulate network excitability in the IC through a combination of GABAergic and NPYergic signaling. Our data show that most glutamatergic IC neurons express NPY Y1 receptors (Y1R), a class of Gi/Go-coupled receptors, and that application of a Y1R agonist induces hyperpolarization in Y1R+ neurons. Using targeted optogenetics, we found that Y1R-expressing neurons form highly interconnected circuits in the IC that are prone to driving recurrent excitation. This recurrent excitation is strongly inhibited by activation of Y1Rs, suggesting that NPY neurons regulate local circuit activity in the IC through a combination of GABAergic and NPYergic signaling. Thus, our results provide novel cellular and synaptic mechanisms that expand the repertoire of ways that inhibitory circuits in the IC shape auditory processing.

Brain Mechanisms of Acoustic Communication and Emotion

Jeffrey Wenstrup

Northeast Ohio Medical University

Individual Abstract: Analysis of the emotional content in social vocalizations depends on brain networks within and beyond the auditory system. Using mice and bats as models, our work describes integrative roles of the basolateral amygdala (BLA). Studies of vocal responses in the auditory midbrain and higher centers establish the raw material for processing in the amygdala. Within BLA, many neurons respond to social vocalizations in a highly selective manner, based on extracellular recordings. These neurons integrate a broader set of auditory inputs, as shown by post-synaptic potentials in intracellular recordings, to form the selective outputs. Responses to vocalizations but not to other acoustic stimuli depend on non-auditory sensory inputs that provide meaning to the vocal signals (e.g., olfactory cues). Additional contextual information, related to internal state, arises from neuromodulator inputs to BLA that are activated differentially by emotional vocalizations associated with different behaviors. Overall, this work carries on a core focus evident in the work of George Pollak and his colleagues, seeking neuronal and circuit-level explanations for highly developed acoustic behaviors.

Identification of Functional Circuits in the Inferior Colliculus Using Intersectional AAV Viruses
Nace Golding

University of Texas at Austin

Individual Abstract: One of the major contributions of George Pollak to auditory neuroscience was to show that the central nucleus of the inferior colliculus (ICC) not only inherits information processed at earlier stages in the auditory pathway but computes information de novo as well. Our understanding of such computations within the ICC has been hampered by a difficulty in defining clear cell types as well identifying cells' local and long-distance connections. To understand ICC computations at the level of cells and circuits we have developed novel intersectional recombinant AAV viruses (rAAVs) that provide access to two distinct, major ICC cell types in Mongolian gerbils, a rodent model with low frequency hearing similar to humans. Our experiments combining anatomical, optical and electrophysiological approaches have identified a population of excitatory, cholecystokinin-expressing neurons (CCK neurons) with dendrites oriented along isofrequency laminae. These neurons have both excitatory and inhibitory counterparts and exclusively target the ventral division of the medial geniculate body (vMGB). The excitatory cells comprise ~60% of the excitatory ascending lemniscal pathway to the MGB, and in vitro and in vivo recordings reveal that these excitatory neurons provide powerful, driving excitation to vMGB neurons. Finally, we will discuss a second population of excitatory ICC neurons that exhibit stellate morphology, span isofrequency laminae and display electrophysiological firing features distinct from CCK neurons. Both CCK and stellate neuron populations exhibited diverse tuning and temporal responses in vivo, suggesting that both populations of cells process diverse patterns of synaptic inputs.

Wednesday, February 7, 2024

Symposium 11 - Circuits and Function of Auditory Midbrain and Auditory Brainstem

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

Grand Ballroom Salon E

Circuits and Function of Auditory Midbrain and Auditory Brainstem

Chair: Marina Silveira*, University of Texas at San Antonio*
Co-Chair: Alexandria Lesicko*, University of Pennsylvania*

Neuropeptide Y Signaling Regulates Recurrent Excitation in the Auditory Midbrain
Marina Silveira

University of Texas at San Antonio

Individual Abstract: Neuropeptides play key roles in shaping the organization, function, and computations of neuronal circuits. We recently found that Neuropeptide Y (NPY), which is a powerful neuromodulator across many brain regions, is expressed in the inferior colliculus (IC) by a distinct class of GABAergic neurons. The IC is localized in a central position the auditory midbrain, integrating information from numerous auditory nuclei making the IC an important hub for sound processing. In the IC, NPY neurons project locally and send long range inhibitory projections outside the IC. Previous studies showed that most neurons in the IC have local axon collaterals, suggesting that the IC is rich in local circuits. However, the organization and function of local circuits in the IC remains largely unknown. We previously found that neurons in the IC can express the NPY Y1 receptor (Y1R+) and application of the Y1R agonist, [Leu31, Pro34]-NPY (LP-NPY), decreases the excitability of Y1R+ neurons. However, how Y1R+ neurons and NPY signaling shape local IC circuits is unknown. Here we found that Y1R+ neurons represent nearly 80% of glutamatergic neurons in the IC, providing extensive opportunities for NPY signaling to regulate excitation in local IC circuits. Next, to investigate how Y1R+ neurons and NPY signaling contribute to local IC circuits, we used optogenetics to activate Y1R+ neurons while recording from other Y1R+ neurons as well as neurons that do not express the NPY Y1 receptor (Y1R- neurons) in the in the transfected side of the IC. We found that Y1R+ neurons provide excitatory input to most other Y1R+ and Y1R- neurons in the IC and therefore form highly interconnected networks within local IC circuits. Additionally, Y1R+ neuron synapses exhibit moderate short-term synaptic plasticity, suggesting that local excitatory circuits maintain their influence over computations during sustained stimuli. We further found that application of LP-NPY decreases recurrent excitation in the IC, suggesting that NPY signaling strongly regulates local circuit function in the IC. Together, our data show that Y1R+neurons are highly interconnected in the local IC and their influence over local circuits is tightly regulated by NPY signaling.

Past, Present, and Future? What the Barn Owl's Inferior Colliculus Teaches us About Experience-Dependent Plasticity

Roland Ferger

Albert Einstein College of Medicine

Individual Abstract: The Barn owl has been a model in sound localization for decades. Despite difference to humans and other mammals, especially in the detection of interaural time differences (ITD), major computational principles are the same across the animal kingdom and circuits in the inferior colliculus (IC) serve comparable purposes. We found owl's IC has shown plasticity across different time spans, from rapid spike frequency adaptation within tenths of milliseconds after stimulus onset, over response adaptation influenced by the most recent stimulus history and habituation to repeated stimuli – specific to some stimulus cues but not others – all the way to experience-dependent changes of frequency tuning across the auditory map in the owl's IC during an individual's development. Many if not all of these forms of plasticity appear to serve one purpose: Optimized reliability and precision for sound localization, improving the efficiency of a sensory system vital to this species' fitness. The external nucleus of the owl's IC (ICX) shows a topographically organized map of auditory space, where neurons respond maximally to combinations of ITD and ILD (interaural level difference), which – thanks to asymmetric outer ears – roughly correlate with azimuth and elevation, respectively. This organization can also be found in the optic tectum, the avian homologue of the superior colliculus and major projection target of the ICX, from where projections to the forebrain likely go along with a change in coding scheme from a place code to a rate code. In both regions, the topography enables probing of plastic changes to the responses of individual neurons as well effects on the population-wide response patterns in the context of stimulus history. Open questions remain on the influences of factors such as concurrent sounds and attention.

Functional Implications of a Patch/Matrix-Like Compartmental Organization in the Mouse Inferior Colliculus

Alexandria Lesicko

University of Pennsylvania

Individual Abstract: The inferior colliculus (IC) is an obligatory relay station and massive convergence center for auditory information. In addition to its role in sound processing, the IC receives inputs from diverse multisensory and neuromodulatory structures and is implicated in acoustico-motor behavior. The lateral cortex of the IC, a multisensory region, contains a network of neurochemical modules that subsect this structure into discrete processing regions. Somatosensory inputs to the IC target these modules, which stain heavily for markers of inhibition, plasticity, and metabolic processing, while auditory inputs target complementary extramodular zones. While these auditory inputs have been shown to mediate diverse functions, including predictive processing and flight behavior, the role of somatosensory inputs to the IC is unknown. Previous studies have shown that inputs from the somatosensory cortex target inhibitory colliculo-thalamic projection neurons in the neurochemical modules, leading to suppression of auditory responses in the auditory thalamus. These results suggest that modular regions of the IC may serve as somatosensory-driven gating regions for auditory information. To test this hypothesis, we trained mice to perform a go/no-go task in which they lick for a water reward after presentation of a noise target, with the goal of then selectively activating somatosensory-inputs to the IC on a subset of behavioral trials to determine how this affects target detection. We also performed anterograde trans-synaptic labeling of somatosensory-recipient neurons in the IC. In addition to assessing the functional role of somatosensory inputs to the IC, we used two-photon imaging to determine the sound response properties of neurons in modular and extramodular regions of the IC. Movement behavior during imaging was recorded and analyzed using FaceMap and DeepLabCut. Preliminary data suggest that mice can learn the go/no-go task paradigm with high accuracy following ~3 weeks of training. Axon fibers from trans-synaptically labeled somatosensory-recipient IC neurons were found in known targets of the lateral cortex, including the medial geniculate body, laterodorsal tegmental nucleus, contralateral lateral cortex, and the superior colliculus. Two-photon imaging of IC responses to pure tones, FM sweeps, noise, and vocalizations was successfully performed. Sound, motion, and sound/motion responsive units were parsed using a generalized linear model. The results of the experiments will determine what effect somatosensory input to the IC has on sound processing and target detection and will show whether modular and extramodular regions of the IC have distinct sound and movement processing features.

Central Feedback From Lateral Olivocochlear Neurons Protects the Cochlea From Acoustic Trauma

Gabriel Romero

Harvard Medical School

Individual Abstract: In response to stressful stimuli, we adapt to avoid harm by modulating the protective capacities and performance of key systems. For instance, the auditory system must rapidly act to preserve its function in the presence of traumatically loud sound. Excessively intense sound damages the cochlea, leading to sensorineural hearing loss (SNHL). The descending auditory system offers protection from SNHL through multiple efferent systems that directly project to the organ of Corti from the brainstem. One of these systems, comprised of lateral olivocochlear neurons (LOCs), is implicated in multiple roles related to audition—including protection from SNHL. To directly test this, we specifically ablated intrinsic LOCs bilaterally in mice using a chemogenetic approach and then assayed their cochlear function after traumatic noise exposure. We found that LOCs protect the peripheral auditory system from noise-induced damage, even within 24 hours of exposure. Even though the mechanism underlying this protective effect is unclear, LOCs dynamically alter their transmitter expression in a context dependent manner, which gives us insight into their function. LOCs, which are primarily cholinergic, express tyrosine hydroxylase (TH), a precursor for dopamine, in response to intense sounds, likely facilitating protection from subsequent acoustic trauma by reducing auditory nerve activity. We recently revealed that acoustic trauma also induces an upregulation in expression of signaling molecules implicated in mammalian stress responses and neuroprotection: urocortin (Ucn), calcitonin gene related peptide (CGRP) and neuropeptide Y (NPY). This suggests that the central nervous system may use LOCs to produce a variety of effects in the cochlea; however, to determine their action we must first answer a set of more basic questions: What stimuli activate LOCs and initiate peptide expression, and what is the time course of transmitter upregulation after acoustic trauma? To tackle these questions, we took a multifaceted approach using single-nucleus RNA sequencing, transgenic tools, and classic immunohistochemistry. We found that LOCs are activated by both continuous tones and broadband noise, and that transcripts and protein for NPY, CGRP, Ucn, and TH are rapidly upregulated within 2 to 24 hours in response to traumatic sound

exposure. After one week, transmitter protein levels remained upregulated, but few transcriptional differences persisted. Overall, our data suggest that LOCs are primed to detect when sound reaches traumatic levels of intensity, and in response alters their output in the cochlea to grapple with ongoing and impending sound stressors.

Shell Inferior Colliculus Population Activity Encodes Behavioral Choice During an Operant Task
Gunnar Quass

University of Michigan

Individual Abstract: Active listening requires not only correctly identifying primary sound features, but also learning their associated behavioral relevance. Behaviorally relevant representations are abundant in auditory cortex and thalamus, but whether similar activity is present earlier in the auditory pathway is unclear. The non-lemniscal nuclei of the inferior colliculus (shell IC) receive a variety of acoustic, multi-sensory and neuromodulatory signals, suggesting in integratory role in perceptual learning. Indeed, many of the non-lemniscal IC's targets - higher order regions of the medial geniculate - famously code for both sounds and behavioral outcomes and constitute major components of goal-oriented behavior. However, whether this joint coding of acoustic and behavioral information arises locally, or is already present in upstream IC neurons, remains unknown. We used multiphoton Ca2+-imaging, machine learning, and a reward-based discrimination task in mice to test if behaviorally relevant signals are present in shell IC neurons. We trained 11 CBA/C57 Bl-6J mice to detect the presence of amplitude modulation in a bandpass noise stimulus using a GO/NOGO paradigm. Using 2-photon microscopy, we tracked GCaMP fluorescence signals in the same dorsal shell IC neurons over several days, while modulation depth was varied to obtain different behavioral outcomes to identical sounds. We analyzed the population- and individual neuron activity during psychometric trials and used PCA-based neural trajectory analysis and a support vector machine (SVM) classifier to predict task-related variables and behavioral outcomes from neural population activity. In line with previous results, we found a strong task-modulation of sound responses in a significant number of neurons. We further observed stark trial-outcome-dependent differences in population processing during sound presentation of equal sounds. This activity consists of separate sound- and movement-related parts that a SVM classifier can use to decode and even predict an animal's behavior already before a task-relevant action is taken. Remarkably, the processing of non-auditory, task-related variables continues after the sound and even after the reward period, suggesting that the shell IC integrates learned associations with sound stimuli on a scale of seconds. Thus, shell IC activity contains all the building blocks necessary for sound-behavior association – sound encoding, movement encoding, and retrospective processing, thus implying a role for the shell IC in processing stimulus-reward associations and task-driven behavior outside of cortico-thalamic networks.

Spatially Clustered Neurons Encode Vocalization Categories in the Bat Midbrain
Jennifer Lawlor

Johns Hopkins University

Individual Abstract: Categorical perception of sensory inputs, including human speech, enables adaptive behavior and is thought to emerge in the sensory cortex. There would be significant computational advantages, however, to functional specialization before cortical processing. To what extent do categorical representations emerge earlier in the auditory hierarchy? To address this, we reasoned that the ideal species would exhibit expert auditory sensing and a rich repertoire of vocalizations, akin to human listening and speech. The big brown bat, Eptesicus fuscus, stands out in the animal kingdom for its acoustic communication for self-navigation (through echolocation) and for social interactions (with conspecifics). Eptesicus fuscus move in three dimensions through their environment and must rapidly distinguish between vocalizations intended for navigation and those intended for social interaction. Here, we used two-photon calcium imaging in the awake big brown bat, to enable large-scale (7,868 neurons in three bats), spatially resolved recordings in the inferior colliculus (IC) during auditory playback. We discovered a novel, superficial tonotopy in the IC that was orthogonal to spatially clustered representations of social and navigation vocalizations. Population decoding revealed sharp boundaries across, but not within, these categories. Auditory models for perceptual categorization rely on the idea that the periphery and midbrain possess mostly a feedforward and filter-bank

role. Our data support a revised view of categorical perception in which ethologically relevant sensory streams are spatially segregated early in the auditory hierarchy and provide parallel channels of organized information to downstream regions.

Top-Down Modulation of the Dorsal Cochlear Nucleus by Descending Projections From Inferior Colliculus Neurons

Tim Balmer

Arizona State University, School of Life Sciences

Individual Abstract: An animal's survival depends on their accurate perception of biologically relevant sounds in a complex hearing environment that is awash with background noise. Top-down contextual information modulates the processing of bottom-up sensory signals to improve hearing accuracy, but the neural mechanisms underlying this essential function remain unclear. One potential source of higher-level contextual cues is the massive top-down projection from the inferior colliculus (IC) to the dorsal cochlear nucleus (DCN). The IC cell types that send their axons to DCN remain unknown. This presentation will introduce concepts of top-down modulation of sensory signals, report new results regarding the IC cell types that convey descending signals to DCN, and show preliminary data on the role of these cells in shaping auditory responses in DCN in vivo.

Podium Session 21 - Transduction and Channels and More, Oh My!

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

Platinum Salon 5

Novel Heterozygous USH1C Mutation Impacts Hair Cell Mechanotransduction and Causes Progressive Hearing Loss

Yanyan Jia[1], Wenyan Li*[1]

[1]*The EENT Hospital of Fudan University*

Category: Hair Cells: Anatomy and 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.

Evaluation of Lhfpl5b Loss-Of-Function on Lateral Line Mediated Behavior, Hair Cell Synapse Morphology, Mitochondrial Homeostasis, and Vulnerability to "Noise" Damage

Keziah-Khue Nguyen*[1], Melanie Holmgren[1], Lavinia Sheets[1]

[1]*Washington University School of Medicine*

Category: Hair Cells: Anatomy and Physiology

Background: Mechanoelectrical transduction (MET) channels contain accessory protein LHFPL Tetraspan Subfamily Member 5 (Lhfpl5) which couples the tip-link protein Protocadherin 15 (PCDH15) to the pore-forming subunit Transmembrane channel-like protein 1/2 (TMC1/2). While the roles of Lhfpl5 on MET channel assembly and function have been evaluated, the impact of Lhfpl5 loss-of-function on hair synapse morphology and vulnerability to damage has not been described. Here we assess lateral line function and hair cell synaptic morphology in zebrafish lhfpl5b mutants, which have normal, functional inner ear hair cells but dysfunctional lateral line hair cells.

Methods: All experiments were performed on genotyped lhfpl5b-/- mutants and wildtype siblings. Rheotaxis behavior experiments were conducted at 6-7 days-post-fertilization (dpf) using a flow stimulus regime (10s pre-stim, 20s stim, 10s post-stim, v = 3.75-7.5mm/s), and resulting swim behaviors were recorded and analyzed. To assess hair cell synaptic vesicle populations, fixed fish were immunolabeled with antibodies to Vglut3. To evaluate hair cell mitochondrial function, live fish were treated with Mitotracker CMXROS. To induce lateral line "noise" damage, 7 dpf fish were exposed to intense water wave stimulus (60 Hz, 40.3 +/- 0.5 m/s2, 2.3 hours total) to produce damage to lateral line organs comparable to damage in noise-exposed ears. Subsequently, immunohistochemical processing was used to label hair cells and their synaptic structures (presynaptic ribbons and postsynaptic densities (PSD)) in exposed fish and unexposed controls.

Results: Analysis of lhfpl5b-/- mutant rheotaxis behavior show an inability to station hold, shorter mean and total duration of rheotaxis events, and a reduced proportion of mutants performing rheotaxis over the duration of stimulus. The number of hair cells per neuromast was significantly fewer in mutants, but the number of synapses per hair cell were comparable to wildtype. The morphology of synaptic structures also differed, as mutant fish had significantly smaller synaptic ribbon volume and significantly larger PSD volume. Additionally, lhfpl5b-/- mutants had significantly reduced Vglut3 average immunolabel intensity per neuromast. Regarding mitochondrial membrane potential, mutants demonstrated depolarized hair cell mitochondria. This observation was not due to a lack of MET, as BAPTA treated fish, i.e., fish with broken tip links, displayed similar patterns of Mitotracker CMXROS intensity. Unexpectedly, when exposed to mechanical overstimulation mimicking noise damage, lhfpl5b-/- mutants showed significantly greater hair cell synapse loss relative to wildtype siblings.

Conclusions: Lhfpl5b loss-of-function mutant fish displayed deficits in rheotaxis behavior, differences in hair cell synaptic morphology, reduced levels of Vglut3, and depolarized hair cell mitochondria. The exacerbated hair cell synapse loss observed in lhfpl5b-/- mutants after exposure to intense water wave stimulus was surprising, as mutant lateral-line hair cells would not be expected to transduce signal. Further studies aim to determine how "noise" exposed lhfpl5b-/- mutants lose hair cell synapses.

The Speed of Activation of Mechanotransducer Channels in Mammalian Auditory Hair Cells Depends on Intracellular Potential

Isabel Aristizabal*[1], Ana I. Lopez-Porras[1], A. Catalina Velez-Ortega[1], Gregory I. Frolenkov[1]

[1]*University of Kentucky*

Category: Hair Cells: Anatomy and Physiology
Background: Mammalian auditory hair cells can detect sounds up to ~100 kHz, implicating that the mechano-electrical transduction (MET) channels are gated in less than 10 µs. Typically, MET currents are evoked by hair bundle deflections with stiff probes driven by piezoelectric actuators. The command signals driving these actuators are filtered at relatively low frequencies (~10-15 kHz) to avoid mechanical resonances. Therefore, with only one notable exception of a very experimental probe (Doll et al., 2012), conventional probes can resolve MET channel activation only in non-mammalian auditory hair cells (Ricci et al., 2005; Corey and Hudspeth.,1983). These non-mammalian studies suggested that the time constant of MET channel activation depends on the amplitude of bundle deflection, extracellular and intracellular Ca2+, and temperature but does not depend on membrane potential. Whether this is true for mammalian auditory hair cells is unknown.
Methods: We built a stiff probe using a smaller piezoelectric chip with a higher unloaded resonant frequency compared to conventional piezo stacks (~600 kHz vs ~140 kHz, respectively). The movement of the stiff probes was monitored by high-speed (90,000 fps) video recordings, while MET currents were recorded with conventional whole-cell patch clamp technique using pipettes with as small access resistance as possible. Both the voltage command to the piezo and the MET currents were low-pass filtered at 30 kHz. Recordings were obtained from outer hair cells of young postnatal mice (P4-P8) in the middle of the apical cochlear turn. Voltage effects on MET channel activation were determined by holding the same cell at -90 and +90 mV. Since activation kinetics of many mechanosensory channels depend on lipid environment, we also measured the time constant of MET channel activation after pharmacological blockage of MET channels, known to alter membrane-bound proteins at the tips of stereocilia.
Results: Video analysis revealed that the resonant frequency of the assembled probe in physiological solution is above 30 kHz and, therefore, about three times faster movements can be achieved (~4.4 µs for ~1 µm step deflection) compared to the conventional stack actuators. The time constants of these probe movements were significantly faster than the ones of MET current activation. Consistent with initial models of MET channel gating, the MET current onset was best described with a double-Boltzmann fit, unlike the single-Boltzmann fit for the probe's movement. Surprisingly, unlike published data in non-mammalian cells, the time constant of MET channel activation was almost independent of the amplitude of the bundle deflection but did change at positive holding potentials and after 24h of MET channel blockage.
Conclusions: We concluded that the activation kinetics of the MET channels in mammalian outer hair cells may differ from that in non-mammalian hair cells.

Phospholipid PIP2 is the Key Mediator of Mammalian Hair Cell Slow Adaptation

Giusy Caprara[1], Ye-Ri Kim[1], Sujin Jun[1], Anthony Peng*[1]

[1]*University of Colorado Anschutz Medical Campus*

Category: Hair Cells: Anatomy and Physiology
Background: The mechano-electrical transduction (MET) process allows the transduction of mechanical information from sound and head movements into electrical signals. MET occurs at the hair bundle level and is triggered by stereocilia deflection. During a sustained displacement, the receptor current peaks and then decays, indicating a gradual decrease in MET channel open probability, a process that is called "adaptation". Adaptation shifts the operating range of the MET process and might be necessary for preserving the system's sensitivity and filtering (Crawford et al., 1989; Eatock et al., 1987, Ricci et al., 2005). The slow adaptation process operates with a time constant on the order of 10-100 ms and requires Ca2+ entry through the MET channels and the activity of myosin motors, in particular Myosin1c (Myo1c) in the vestibular system (Holt et al., 2002; Yamoah and Gillespie, 1996; Caprara et al., 2020). Recently, we demonstrated that the mechanism of slow adaptation does not involve the upper tip-link insertion movement as hypothesized by the motor model (Caprara et al., 2020), questioning its molecular mechanism.
Methods: Using electrophysiological recording in mouse vestibular and cochlear hair cells, we tested an alternative hypothesis involving the activity of myosin, PIP2, and TMIE to regulate slow adaptation. PIP2 inhibition affects slow adaptation in bullfrog saccular hair cells (Hirono et al., 2004), and TMIE is an essential subunit of the MET channel and contains charged amino acids that mediate binding to phospholipids, including PIP2 (Cunningham et al., 2020).

Results: Our data support a model where PIP2 is the key mediator of the slow adaptation process, and it's binding to the MET complex through TMIE is required to mediate slow adaptation. The data also supports that myosin motors are not important for direct participation in slow adaptation, however, myosin motors are required to setup the environment necessary for PIP2 to mediate slow adaptation.
Conclusions: Our results provide the first data describing a new model os slow adaptation.

In Silico Insights into TMC Function in Sensory Perception

Wei-Hsiang Weng[1], Harsha Mandayam Bharathi[1], Shounak Mukherjee[1], Travis Harrison-Rawn[1], Marcos Sotomayor*[1]

[1]*The Ohio State University*

Category: Hair Cells: Anatomy and Physiology
Background: The poorly characterized family of transmembrane channel-like (TMC) proteins, consisting of eight members in mammals, is loosely related to the TMEM16 family of membrane proteins that can function as lipid scramblases as well as cation or anion channels. Two vertebrate TMC proteins, TMC1 and TMC2, are expected to be cation channels mediating inner ear mechanotransduction, whereas mouse TMC4 has been recently proposed to function as an anion channel involved in taste perception. In addition, mouse TMC1 might mediate lipid scrambling. Although several experimental studies have been carried out to back these claims, a molecular picture of conduction mechanisms and possible scramblase activity is still missing.
Methods: Here we use experimentally supported AlphaFold2 models of TMC proteins along with equilibrium and non-equilibrium all-atom molecular dynamics simulations to predict conduction properties and function of various vertebrate TMC family members.
Results: Simulations show robust cation conduction for TMC1 and TMC2, whereas other members are predicted to have low, non-selective or anion-selective ion conductance. In addition, simulations show lipid scrambling and aminoglycoside uptake by TMC1.
Conclusions: Molecular dynamics simulations of TMC proteins are shedding light on ion and aminoglycoside conduction pathways as well as on important residues for activation and ion selectivity across family members.

Voltage-Dependent Movements of Mammalian Inner Hair Cell Bundles

Jamis McGrath*[1], Anthony Ricci[1]

[1]*Stanford University*

Category: Hair Cells: Anatomy and Physiology
Background: Sensory hair cells have organelles, called "hair bundles", comprised of a staircase like array of microvilli-like protrusions, stereocilia, interlinked by extracellular connectors. Deflections (by sound induced vibrations) modulate mechanically sensitive ion channels transforming the motion into an electrical signal, a process termed mechano-electrical transduction (MET). The classical hair bundles of turtle and frog move as a unit whereas mammalian cochlea hair bundles do not. Classical hair bundles also show three movements in response to depolarization; a fast step toward the shorter stereocilia, followed by a slower motion toward the taller stereocilia rows and finally the slower motion can reverse when the hair bundles are biased toward the taller stereocilia. The slower motions require MET current while the faster motions require intact tip links but not current. Our goal was to determine whether inner hair cell hair bundles (IHCHBs) had these similar mechanical responses to voltage.
Methods: We used whole cell patch clamp to voltage clamp rat IHCs at postnatal ages 7-9. We used high-speed imaging to measure IHCHB motion in response to voltage steps. We analyzed individual stereocilia and whole hair bundle motion using custom developed software in MATLAB with components from Image J. Mechanical stimulation of the hair bundle was done using the narrow probe technique that leaves the majority of stereocilia stimulated through their connections with neighboring stereocilia
Results: Freestanding IHC bundles moved similarly to turtle or frog bundles. First, stereocilia rapidly (less than 300 μs) moved toward the shorter rows. The motion was fast but slower than the voltage clamp speed. Second, stereocilia moved slower (~5 ms) back toward their resting position. The slower motion was smaller than that observed in turtle, rarely crossing the baseline, whereas the faster motion was comparable to those

reported in turtle. The slower motion, like in turtles, was sensitive to open channel block, in this case tubocurarine, suggesting ion flux, was important for generating the movement. Using the narrow probe to offset a portion of the IHC hair bundle produced the reversal response similar to that observed in turtle, albeit smaller in magnitude. Previous work in IHC bundles revealed a stereocilia motion in response to the narrow probe stimulus with a rapid peak and then decline to steady-state, reminiscent of the notch seen in frog hair bundles, we show that this response was independent of voltage and insensitive to channel block.
Conclusions: Data reveal three motions in IHC hair bundles like those found in turtle auditory cells. The major difference was that the slower motion, dependent on ion flux was proportionately much smaller, consistent with the idea of reduced calcium-dependent adaptation. Reversal motion in response to the narrow probe stimulus is insensitive to voltage, supports a non-calcium driven response.

LOXHD1 Interacts With Subunits of the Mechanotransduction Complex

Katharine Miller*[1], Pei Wang[1], Nicolas Grillet[1]

[1]*Stanford Medicine*

Category: Hair Cells: Anatomy and Physiology
Background: Mutations in LOXHD1 cause hearing loss in humans, mice, and dogs, but the exact function of LOXHD1 has not previously been elucidated. We recently revealed that LOXHD1 is located at the tips of transducing stereocilia and is required for mechanotransduction (Wang et al., under review). Therefore, we wanted to investigate how the loss of LOXHD1 might affect the proteins involved in mechanotransduction. After creating a complete knockout of LOXHD1 in mice (Wang et al., under review), we utilized our novel SUB-immunogold scanning electron microscopy (SEM) method (Miller et al., in preparation) to nanometrically localize the mechanotransduction complex proteins. We found that when LOXHD1 is absent, TMC1 is mislocalized from its normal position at the tips of the transducing stereocilia. This suggests that LOXHD1 may interact with protein subunits of the mechanotransduction complex.
Methods: We performed co-immunoprecipitation experiments on HEK293T cells co-transfected with LOXHD1 and individual subunits of the mechanotransduction complex. We resolved protein interactions using SDS-PAGE and western blot.
Results: LOXHD1 interacts with subunits of the mechanotransduction complex.
Conclusions: LOXHD1 is found at the tips of transducing stereocilia and is required for proper maintenance of TMC1's localization. Biochemistry experiments reveal that LOXHD1 interacts with subunits of the mechanotransduction complex, emphasizing the importance of LOXHD1 for hearing function.

Piezo 1 and 2 May Constitute Part of the Mechano-Sensitive Channel Complex in Hair Cells

Ebenezer Yamoah*[1], Jeong Han Lee[1], Maria C. Perez-Flores[1], Seojin Park[1], Hyo Jeong Kim[1], Yingying Chen[1], Mincheol Kang[1], Jennifer Kersigo[1], Jinsil Choi[1], Phung N. Thai[1], Ryan Woltz[1], Dolores Columba Perez-Flores[1], Guy Perkins[1], Choong-Ryoul Sihn[1], Pauline Trinh[1], Xiao-Dong Zhang[1], Padmini Sirish[1], Yao Dong[1], Wayne Wei Feng[1], Isaac N. Pessah[1], Rose E. Dixon[1], Bernd Sokolowski[1], Bernd Fritzsch[1], Nipavan Chiamvimonvat[1]

[1]*University of Nevada School of Medicine*

Category: Hair Cells: Anatomy and Physiology
Background: The sound and balance gateways to the brain are through MET-channel activation in HC stereocilia. However, the components forming the HC MET channel have been an enigma, the identities of which have been slowly and painstakingly forthcoming.
Methods: The prevailing data suggest TMC may be the pore-forming protein, but it only exists in a liposomal membrane as a reconstituted pore-forming protein. Moreover, whereas mutations of TMC1 in HCs may block conventional MET current, the anomalous current is spared. Experiments implicating Tmc1 as a pore-forming protein in HCs did not rule out; none did contemplate an allosteric role of Tmc on another channel. Previous

evidence shows us that altering protein mutations or varying their concentrations may only provide evidence for an accessory protein rather than the sought-after pore-forming protein.
Results: Here, we report that the mouse utilizes Piezo1 (Pz1) and Piezo2 (Pz2) isoforms as components of the MET complex. The Pz channel subunits are expressed in HC stereocilia, are co-localized and co-assembled, and are essential components of the MET complex in vitro and in situ, including integration with the transmembrane channel (Tmc1/2) protein. Mice expressing non-functional Pz1 and Pz2, but not wildtype Pz1 at the ROSA26 locus under the control of HC promoters, have impaired auditory and vestibular traits that suggest the Pz channel is integral to the MET complex.
Conclusions: We propose that Pz protein subunits constitute part of the MET complex and that interactions with other MET components yield a functional hair-cell MET current.

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Podium Session 22 - Attention, Cognition, and Learning in Speech Perception

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

Platinum Salon 6

Attentional Modulation of the Cortical Contribution to the Frequency-Following Response Evoked by Continuous Speech

Alina Schüller*[1], Achim Schilling[2], Patrick Krauss[3], Stefan Rampp[4], Tobias Reichenbach[1]

[1]*Friedrich-Alexander-University,* [2]*Neuroscience Lab, University Hospital Erlangen,* [3]*Neuroscience Lab, University Hospital Erlangen; Pattern Recognition Lab, Friedrich-Alexander-University,* [4]*University Hospital Erlangen; University Hospital Halle (Saale); University Hospital Erlangen*

Category: Speech Perception
Background: Selective attention allows us to concentrate on relevant information while filtering out distracting signals. In addition, selective attention enables us, for instance, to understand one speaker's voice even in the presence of multiple distracting speakers. When investigating the neural mechanisms of selective attention to speech, research has primarily focused on low-frequency responses to speech rhythms such as set by the rate of phonemes, syllables and words in the auditory cortex. However, EEG studies also revealed the attentional modulation of subcortical neural responses [1]. These subcortical activities emerge in response to the fundamental frequency of speech and its higher harmonics (speech-FFR). While studies using MEG have recently identified cortical contributions to the speech-FFR [2, 3, 4], it remains unclear if these cortical contributions are also modulated by selective attention.
Methods: In the present study, we employed Magnetoencephalography (MEG) followed by source reconstruction to investigate how selective attention influences the cortical contributions to the speech-FFR. We recorded MEG data from 22 healthy, normal-hearing subjects listening to continuous speech stimuli of two male speakers of a duration of 40 minutes, with attention shifting regularly between the two speakers [5]. We then analyzed the speech-FFR through computing the neural responses at the fundamental frequency using neural source estimation followed by the computation of source-level TRFs. We computed the speech-FFR to each of the two voices, both when they were attended and when they were ignored.
Results: We found a strong impact of selective attention on the cortical contribution to the speech-FFR. When participants focused on a speaker, the neural response was notably higher compared to when that speaker was ignored. This attentional modulation emerged both on the population level and on the level of individual subjects. Additionally, we observed that, irrespective of attention, the speaker with a lower fundamental frequency elicited a larger cortical contribution to the speech-FFR than the speaker with the higher fundamental frequency.
Conclusions: Our findings demonstrate that attentional modulation affects not only the subcortical contribution to the speech-FFR, but also the cortical portion.

[1] Antonio Elia Forte et al. The human auditory brainstem response to running speech reveals a subcortical mechanism for selective attention. eLife, 6:e27203, 2017.

[2] Emily BJ Coffey et al. Neural correlates of early sound encoding and their relationship to speech-in-noise perception. Frontiers in Neuroscience, 11:479, 2017a.

[3] Joshua P Kulasingham et al. High gamma cortical processing of continuous speech in younger and older listeners. NeuroImage, 222:117291, 2020.

[4] Alina Schüller et al. Early subcortical response at the fundamental frequency of continuous

speech measured with MEG. bioRxiv, doi: 10.1101/2023.06.23.546296, 2023.

[5] Alina Schüller et al. Attentional modulation of the cortical contribution to the frequency-following response evoked by continuous speech. Journal of Neuroscience, in print, bioRxiv, doi: 10.1101/2023.07.03.547608, 2023.

Short- And Long-Term Experience-Dependent Neuroplasticity Interact During the Perceptual Learning of Concurrent Speech

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

Jessica MacLean*[1], Jack Stirn[1], Alexandria Sisson[1], Gavin Bidelman[1]

[1]*Indiana University*

Category: Speech Perception

Background: Plasticity from auditory experiences shapes brain encoding and perception of sound. However, whether such long-term plasticity alters the trajectory of short-term plasticity during speech processing has yet to be investigated. Additionally, it is unclear how and where in the auditory system (cortical vs. subcortical) learning excerpts neuroplastic effects. Here, we explored the neural 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 minute training sessions recorded simultaneously with high-density EEG. We analyzed frequency-following responses (FFRs) and event-related potentials (ERPs) to investigate neural correlates of learning at subcortical and cortical levels, respectively. FFRs were analyzed in the time and frequency domains. ERPs were analyzed in both latency and amplitude at the electrode and source level to identify when and where changes in brain activity map to successful learning outcomes.

Results: While both groups showed rapid perceptual learning in accuracy and reaction time with training, musicians showed faster behavioral decisions than nonmusicians overall. Learning-related changes were not apparent in brainstem FFRs. However, plasticity was highly evident in cortex, where ERPs revealed unique hemispheric asymmetries between groups suggestive of different neural strategies (musicians: right hemisphere bias; nonmusicians: left hemisphere). Source reconstruction and the early (150-200 ms) time course of these effects localized learning-induced cortical plasticity to auditory-sensory brain areas.

Conclusions: Our study replicates earlier work by demonstrating rapid improvements in perceptual learning arise surprisingly early in auditory-sensory brain areas. However, we extend prior studies by showing behavioral gains in speech processing and its neural underpinnings vary according to a listeners' prior auditory experience, with stronger learning-related changes in musicians. Our findings confirm domain-general benefits for musicianship but reveal successful speech sound learning is driven by a critical interplay between long- and short-term mechanisms of auditory plasticity that first emerge at a cortical level.

Examining the Benefits of Categorical vs. Continuous Listening Strategies on Speech in Noise Perception

Rose Rizzi*[1], Gavin Bidelman[1]

[1]*Indiana University*

Category: Speech Perception

Background: Acoustic information in speech signals changes continuously, yet listeners form discrete perceptual categories to ease the demands of perception. Being a more discrete/categorical as opposed to continuous/gradient listener may be further advantageous for parsing speech in noise assuming phonetic categories (a higher-level code) are more robust to noise than physical surface features of the speech signal. The degree to which a listener's responses to a continuum of speech sounds are categorical versus continuous can be quantified using visual analog scaling (VAS) during speech labeling tasks.

Methods: Here, we recorded event-related brain potentials (ERPs) to vowels along an acoustic-phonetic continuum (/u/ to /a/) while listeners made categorical judgments in both clean and noise (-2.5 dB SNR) conditions. Behavior was assessed under standard two alternative forced choice (2AFC) and VAS paradigms to evaluate categorization under task structures that promote discrete (2AFC) vs. continuous (VAS) hearing, respectively.

Results: Behaviorally, identification curves were steeper under 2AFC vs. VAS categorization but were relatively immune to noise, suggesting robust access to abstract, phonetic categories even under signal degradation. We used cluster-based analyses applied to the topographic scalp data to identify neural differences between tasks across space and time. We found left frontal scalp regions differentiated task-related changes in the speech-ERPs within ~250 ms after vowel onset for both clean and noise-degraded speech. ERPs were also generally larger under VAS vs. 2AFC categorization, suggesting additional neural resources are deployed when categorizing under more continuous listening scenarios. Interestingly, listeners' strength of categorical hearing (identification slopes) was correlated with ERP responses.

Conclusions: Our results demonstrate that listening strategy (i.e., being a discrete vs. continuous listener) modulates the categorical organization of speech and behavioral success in speech in noise perception.

Influence of Social and Semantic Context in Processing Speech in Noise

Etienne Abassi*[1], Robert Zatorre[1]

[1]*McGill University, Montreal Neurological Institute*

Category: Speech Perception

Background: Social interactions occupy a substantial part of our life. Not only interacting with others, but also listening to others' interactions serves critical functions in understanding our social world. Yet, current audiologic tests only use isolated words or sentences to evaluate hearing loss, lacking abilities to assess communication and understanding in real-world social situations. Although the role of semantics in speech comprehension has already been studied, the role of social context, and its interactions with semantics, is unknown. We conducted a series of online perceptual experiments to better understand processing of multiple-speaker conversations from third-person viewpoint by manipulating social and semantic context of a conversation.

Methods: We created stimulus sets consisting of two-speaker dialogues or one-speaker monologues (factor: social context) arranged in intact or sentence-scrambled order (factor: semantic context). The content of dialogues and monologues was counterbalanced across conditions. Each dialogue or monologue comprised five sentences, with the fifth sentence embedded in multi-talker babble noise. This fifth sentence was subsequently repeated without noise, with a single word altered or unchanged. We presented the stimuli over headphones to healthy young adult listeners, who were asked whether the repeated sentence was same as or different from the previous sentence-in-noise.

Results: In experiment 1 we manipulated signal-to-noise ratio (SNR) of sentence five to define optimal SNR for our task. One group of participants (n=38) was exposed to dialogues exclusively, while another group (n=34) encountered only monologues. Group 1 exhibited higher accuracy for intact versus scrambled dialogues at an SNR of -4 dB, while group 2 displayed a trend toward lower RTs for intact versus scrambled dialogues at the same SNR. Consequently, we selected an SNR of -4 dB for subsequent experiments. In Experiment 2 (n=65), we explored the impact of semantic context by comparing performance between intact and scrambled presentations within separate blocks of dialogues and monologues. RTs showed significant effect of semantic context, while accuracy exhibited similar trend. Experiment 3 (n=62) focused on the influence of social context by directly comparing performance between dialogues and monologues, randomly

presented within same blocks. RTs revealed effects related to both social and semantic contexts, although no significant interaction was observed. Interestingly, we also observed lower RTs for intact dialogues compared to all other conditions.
Conclusions: Taken together, our findings highlight that both semantic and social aspects of a conversation can modulate the processing of this conversation. Furthermore, our results suggest the existence of facilitation mechanisms favoring the processing of prototypical conversations, such as intact dialogues, over other similar auditory stimuli. These results underscore the importance of considering both social and semantic components of speech when assessing hearing impairments. They furthermore raise new questions regarding the predictive or other mechanisms that may be present when perceiving speech in social contexts.

Investigating the Hierarchical Neural Mechanisms Underlying Auditory Attention Switching

Sara Carta*[1], Emina Alickovic[2], Johannes Zaar[2], Alejandro López Valdes[1], Giovanni Di Liberto[1]

[1]*Trinity College Dublin,* [2]*Eriksholm Research Centre*

Category: Speech Perception
Background: Speech perception in the real-world often involves noisy scenarios with simultaneous speech streams, in which listeners selectively attend to the speaker of interest and ignore competing speakers. Previous research has shown that neural signals encode attended and ignored speech streams in a different manner, enabling the robust identification of the attended speaker from non-invasive electroencephalography (EEG) and magnetoencephalography (MEG) signals.

In real-world multi-talker scenarios, listeners can sustain their attention to a particular speaker, but they can also rapidly re-orient their focus of attention at will. Previous studies have examined speech attentional switching across spatially separated speakers. Research on attentional switching was also conducted in the context of real-time attention decoding. However, there remains considerable uncertainty on the neural underpinnings of attentional switching.

Here, we will present recent EEG results investigating how the attention switching phenomenon unfolds across different stages of the speech processing hierarchy. Our study probes both acoustic and linguistic features of speech (acoustic envelope and semantic surprisal), investigating two central questions: 1) how rapidly can the attention switching be detected from EEG signals? And 2) does the switching mechanism require longer time for more abstract linguistic features?

Methods: EEG signals were recorded from twenty-four native English speakers as they performed a listening task in an immersive multi-talker scenario. Participants were asked to switch their attention between two spatially separated speech streams when receiving a visual cue. Using the multivariate Temporal Response Function (TRF) and Canonical Component Analysis (CCA), we examined how the cortical tracking of acoustic and semantic information disengages from one speech stream and engages to the other.
Results: Using a sliding-window with a duration of 7-seconds and models relying on the sound envelope, we could decode attention with an average accuracy of ~61% with TRF and ~65% with CCA. The sliding window was then used to assess the temporal dynamics of attention switching, showing a robust tracking of the switching of attention. Next, the neural encoding of lexical level information was assessed to identify what exact linguistic context is used to build lexical predictions immediately after switching attention. Preliminary results indicate that, when switching from an auditory stream A to a second stream B, attention switching interrupts the accumulation of context from A, re-activating previous context from B.
Conclusions: In conclusion, this study demonstrated how the neural encoding of acoustic and linguistic properties of speech is affected by attention switching, shedding light on the temporal dynamics of the switch of attention as well as on how linguistic context is reset and restored in dynamic listening scenarios.

Effect of Crossmodal Divided Attention on Continuous Speech Processing in Noise

Grace Frerking[1], Anya Chatani[1], Zilong Xie*[1]

[1]*Florida State University*

Category: Speech Perception

Background: Speech-in-noise perception is an essential skill for our everyday life, which can be compromised by aging and hearing loss. Speech-in-noise perception frequently occurs in multisensory contexts, where listeners often perform other nonauditory tasks concurrently (i.e., multitasking), e.g., reading the food menu while conversing at a restaurant. Listeners must allocate attention across modalities (i.e., crossmodal divided attention) for successful multitasking. Many studies have examined speech-in-noise perception with natural continuous speech and revealed how auditory selective attention disproportionately affects acoustic to linguistic processing. However, few have investigated the effect of crossmodal divided attention on continuous speech processing in noise. The current study aims to determine the extent to which crossmodal divided attention impairs acoustic to linguistic processing of continuous speech in noise.

Methods: Young adults with normal hearing performed an audiovisual dual task consisting of a primary visual n-back task on a sequence of blue squares and a secondary auditory task involving listening to audiobook stories in cafeteria noise and answering comprehension questions. The visual n-back task was to judge whether each square in the sequence appeared at the exact location of a target square. The target square was either fixed (always the first square in the sequence; 0-back) or variable (the square 3-positions back relative to the current square; 3-back), corresponding to low and high cognitive load conditions.

Results: Preliminary results showed that behaviorally, increased cognitive load (3- vs. 0-back) was associated with lower accuracy and slower response time on the visual task and lower accuracy on the auditory comprehension task. Data collection will continue to simultaneously record behavioral and electroencephalographic (EEG) data during the task. Multivariate temporal response function encoding models will be used to assess the neural processing of acoustic to linguistic representations of continuous speech from EEG data and determine their changes with the dual-task cognitive load.

Conclusions: Our findings have implications for elucidating mechanisms of crossmodal attention on natural speech processing and understanding how older listeners and listeners with hearing loss manage speech-in-noise perception in multisensory scenarios.

Effects of Cognitive Ability and Semantic Context on Word Report in Noise

Joseph Rovetti*[1], Stephen Van Hedger[2], Ingrid Johnsrude[1]

[1]*Western University,* [2]*Huron University*

Category: Speech Perception

Background: Measuring listeners' cognitive ability can account for additional variability in speech-in-noise (SIN) understanding, beyond that explained by audiometric measures. Cognitive models of SIN understanding usually focus on working memory (WM) as the primary supporting function. However, work in our lab and elsewhere has demonstrated that performance on tests of non-verbal problem solving (e.g., Raven's Advanced Progressive Matrices [RAPM]) also predicts degraded speech understanding, perhaps even more than WM does. Cognitive ability might benefit SIN understanding by supporting the use of contextual information. If so, then individual differences in cognitive ability should predict the difference in SIN understanding between semantically coherent sentences rich in context (e.g., "Her new skirt was made of denim") and semantically anomalous sentences low in context (e.g., "Her good slope was done in carrot").

Methods: One hundred nineteen participants, consisting of younger and older adults with normal hearing, listened to sentences (coherent or anomalous) presented in babble at different signal-to-noise ratios (SNRs; clear, +3 dB, or -1 dB). They then completed a short version of RAPM (Arthur and Day, 1994, Educ. Psychol. Meas.) as a measure of fluid intelligence, as well as the Reading Span (RSPAN; Daneman and Carpenter, 1980, J. Verbal Learn. Verbal Behav.) as a measure of WM capacity.

Results: Word-report accuracy exceeded 95% for the clear sentences, and it declined as the SNR decreased. This decline with SNR was shallower for coherent sentences than anomalous sentences. Although higher RSPAN scores predicted higher word-report accuracy, this was a small effect and it did not appear to differ by context level or SNR. In contrast, lower RAPM scores predicted steeper slopes as a function of SNR, seemingly irrespective of context level. It is unclear whether the effects of RAPM score and context on word report as a function of SNR are additive or interactive.

Conclusions: As the SNR became less favourable, greater sentence context allowed listeners to maintain understanding, presumably enabling the prediction of degraded utterances. Higher scores on the RAPM, but not on the RSPAN, also had a marked protective effect on SIN understanding at less favourable SNRs. These results suggest that the RAPM, unlike the RSPAN, may be tapping into some cognitive ability that allows for the signal to be recovered in noise. More research will be needed to evaluate whether the contribution of RAPM score depends on the level of sentence context.

The Effect of Voice Identity Training and Talker Identity Information on Speech in Noise Perception

Aditi Nayak*[1], Joseph Rovetti[1], Ingrid Johnsrude[1]

[1]*Western University*

Category: Speech Perception

Background: Noisy environments make it difficult to understand speech, but familiar voices such as those of a spouse or friend are more intelligible than novel voices in noise. However, we still do not have a complete understanding of how a voice becomes familiar and more intelligible. One possible factor that may influence voice learning is the degree to which a voice is associated with an individual identity. Perhaps the more a talker's voice is associated with personhood, the more effectively the listener will learn the voice and the more intelligible it will be.

Methods: In an online experiment with recruitment via Prolific, we recruited 64 younger adults with normal hearing. Participants, in three different groups, were first trained on two talkers' voices by listening to 276 sentences from each voice. Group 1 listeners received no talker identity information and instead indicated whether each sentence contained a reference to a person. Group 2 listeners were trained to associate a name with each talker and indicated which of the two talkers had spoken each sentence. Group 3 listeners performed the same talker discrimination task as the second group and were also given a short biography and a picture of each talker before the training began. After training, all three groups listened to the same closed-set matrix sentences (Kidd et al., 2008) spoken by trained and novel voices masked with speech in a novel voice at two different SNRs(+3 dB/-6dB). The intelligibility of target speech was measured with a word-report task.

Results: Despite the small sample size, our preliminary data indicate that speech was more intelligible at the more favourable SNR compared to the less favourable SNR, and trained voices were more intelligible than novel voices. The difference in intelligibility between trained and novel voices was greater at an SNR of +3 dB compared to -6 dB. Furthermore, there appears to be an emerging intelligibility benefit where the group given only the name of the talker found voices to be more intelligible than the group given no talker identity information. We did not see this intelligibility benefit for the group additionally given the biography and picture of each talker. Recruitment is ongoing.

Conclusions: Trained voices are more intelligible than novel voices, and listeners specifically trained on talker identity appear to benefit more than those trained with no identity; although results are preliminary, and no effects were seen for the richest identity condition. Identity information may play a role in facilitating the learning of familiar voices.

Podium Session 23 - Inner Ear: Noise Induced Hearing Loss, Models and Therapies

10:15 a.m. - 12:15 p.m.

Platinum Salon 5

Evaluation of Metformin as a Noise-Induced Hearing Loss Therapeutic

Benjamin Shuster*[1], Catherine L. Kennedy[2], Beatrice Milon[1], Reza Amanipour[1], Priya Patel[3], Ran Elkon[4], Ronna Hertzano[1]

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Category: Inner Ear: Damage and Protection
Background: A previous study from our laboratory and collaborators profiled transcriptomic changes that occur in the cochlea following exposure to a permanent threshold shift (PTS)-inducing noise. While the molecular response to noise was largely cell type-specific, an upregulation of immune-related gene was identified across all cell types. Of note, an immune response in the cochlea is characteristic following PTS-inducing noise exposures but not following less severe noise exposures that produce only temporary elevations in auditory thresholds. By cross-referencing the dysregulated genes with the DrugCentral database, we identified FDA-approved therapeutics predicted to counter the molecular response to PTS-inducing noise. Here we demonstrate that metformin, the top-ranking candidate, protects against noise-induced hearing loss (NIHL) in male but not female mice. Using bulk RNA-sequencing, we then show that metformin treatment in male mice counteracts the upregulation of immune-related genes following exposure to a PTS-inducing noise.
Methods: To evaluate metformin as a candidate otoprotective agent, male and female B6CBAF1/J mice were obtained at 7-8 weeks of age. A subset of female mice underwent bilateral ovariectomy (OVX) at 8-weeks of age to mimic menopause and eliminate the effect of protective ovarian estrogens. At 9-weeks of age, baseline auditory brainstem response (ABR) thresholds were established. Mice were then administered metformin (200 mg/kg) or a saline control in their drinking water for the remainder of the study. At 10-weeks of age, mice were exposed to a PTS-inducing noise (102.5-105 dB SPL, 8-16 kHz, 2h). ABR threshold shifts were quantified 1-day and 1-week post-exposure. Following the 1-week ABR, the mice were euthanized to collect cochlear tissue for histological analysis.

To understand the effect of metformin treatment on gene expression in the cochlea following exposure to a PTS-inducing noise, male B6CBAF1/J mice were obtained at 8-weeks of age and administered metformin or a saline control as described above. The mice were noise-exposed at 10 weeks of age. Twenty-four hours following the exposure, cochlear tissue was collected and processed for bulk RNA-sequencing.

Results: Our data demonstrate that metformin treatment reduced hearing loss and high-frequency outer hair cell (OHC) loss in male mice following the noise exposure. Metformin treatment did not ameliorate hearing loss or OHC loss in the gonadally intact or ovariectomized female mice. In the noise-exposed male mice treated with metformin, we observed a blunting of the cochlear canonical upregulation of immune-related genes and pathways following PTS inducing noise.
Conclusions: Metformin treated ameliorates hearing loss and OHC death in male but not female mice following exposure to PTS-inducing noise. Systemic metformin administration represses immune-related gene expression in the cochlea following PTS-inducing noise.

A Cell-Penetrating Peptide Containing a Neurotrophin Receptor TrkB-FL Sequence as a Novel Therapeutic Approach for Sensorineural Hearing Loss

Elena Torres Campos*[1], Margarita Díaz-Guerra González[1], Isabel Varela-Nieto[1]

[1]*IIBSM, CSIC-UAM*

Category: Inner Ear: Damage and Protection
Background: Sensorineural hearing loss (SNHL) is a heterogeneous condition due, among others, to genetic causes, ototoxic drugs, noise exposure, or aging. Normal hearing requires exquisite communication between the different types of cells that constitute the organ of Corti. Hair cells (HCs) are responsible for mechanoelectrical transduction by releasing neurotransmitters (glutamate) at synapses formed with spiral ganglion neurons (SGNs). Efficient communication between inner HCs (IHCs) and SGNs also depends on trophic factors, such as brain-derived neurotrophic factor (BDNF) or neurotrophin-3 (NT-3). They activate tyrosine kinase receptors on SGNs membranes, respectively TrkB and TrkC, promoting neuronal survival and synapse formation. Synaptic connections between HCs and SGNs degenerate early after auditory damage, a central mechanism being exacerbated glutamate release from damaged IHCs which overactivates SGN glutamate receptors and induces excitotoxicity. HCs and SGNs are postmitotic cells that cannot be replaced after their loss. However, cochlear synaptopathy reparation is feasible if HCs, SGNs and their central axons still survive. Thus, neurotrophin-mediated otoprotection is a promising strategy, although it might be compromised by calpain-degradation of active receptor TrkB-FL induced by excitotoxic damage as previously shown in cortical neurons and stroke models.

Cell-penetrating peptides (CPPs) are highly innovative and promising therapeutic tools that overcome the intrinsic difficulty of introducing drugs into the nervous system. They use the transduction properties of natural proteins, such as the HIV-1 Tat protein, to facilitate passage across the blood-brain and blood-labyrinthine barriers of different cargoes. The CPP developed by our group, MTFL457, contains a short TrkB-FL sequence and is able to interfere with TrkB-FL degradation and recover BDNF/TrkB signalling. The result is a decrease in neuronal death in models of excitotoxicity in vitro and stroke.

Methods: We have used cochlear explant cultures from P1-2 mice pups, consisting of a portion of the organ of Corti with its corresponding spiral ganglion, to investigate MTFL457 therapeutic potential.
Results: Our results show that this peptide is capable of penetrating into the explants, accumulating in both HCs and SGNs. Excitotoxic treatments (NMDA-glycine, kainate and NMDA-glycine-kainate) induce calpain activation, reduced TrkB-FL levels, neurodegeneration and synaptopathy. Pre-treatment with MTFL457 prevents TrkB-FL downregulation, preserving the neurotrophin-mediated signaling and reducing the evidences of neurodegeneration and Golgi fragmentation (a hallmark of neurodegeneration) induced by excitotoxicity. Additionally, cochlear synapses are notably preserved due to the stabilization of the levels of the postsynaptic scaffolding protein, PSD-95. Our peptide, MTFL457, also interferes with the synaptopathy induced by excitotoxicity when used after damage initiation.
Conclusions: The neuroprotective effects of MTFL457 in an ex-vivo model of excitotoxicity and synaptopathy suggest a great potential of this CPP in SNHL treatment.

Combinatorial Protection of Cochlear Hair Cells: Not too Little, but not too Much

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[1]*University of California San Diego*

Category: Inner Ear: Damage and Protection
Background: A number of strategies have been proposed to prevent the loss of hair cells, based on cellular processes that can mediate or reduce cellular damage. This includes treatment with survival-promoting growth factors, antioxidants, and inhibitors of apoptosis, autophagy, death pathways, or proteinases. Individual treatments targeting these pathways have been demonstrated to reduce hair cell damage in animals and/or in vitro models. However, the translation to humans has often been disappointing. One reason for this may be the complexity of intracellular damage processes, which includes many parallel and interacting pathways. We hypothesized that manipulating only one aspect of cell damage would insufficient to produce maximal hair cell protection, but that combined treatments would be more effective.
Methods: Using data from several screens of compounds targeting different aspects of hair cell damage, we identified inhibitors targeting five different cell damage pathways, and one survival-promoting growth factor. Each was effective in protecting against hair cell loss in an in vitro mouse model of gentamicin toxicity. We then tested all 64 possible combinations of these factors to identify an optimal formulation, using dosages below those required for hair cell protection.
Results: Increasing protection was observed for combinations of compounds that consisted of two to four factors, all though not all combinations were equally protective. The optimal combination of four compounds consisted of an antioxidant, an apoptosis inhibitor, an autophagy inhibitor and a growth factor. Increasing the number of factors to five or six resulted in decreasing levels of protection.
Conclusions: The results support the hypothesis that targeting multiple cellular damage or survival pathways provides more effective hair cell protection. However, they also indicate that blocking too many processes impairs functions that are critical to hair cell survival, resulting in decreased protection and even toxicity.

ERK1/2 Inhibitor, Tizaterkib, Protects Mice From Noise-Induced Hearing Loss While Modulating the Innate Immune Response

Richard Lutze*[1], Matthew Ingersoll[1], Alena Thotam[1], Anjali Joseph[1], Joshua Fernandes[1], Kristina Ly[1], Tal Teitz[1]

[1]*Creighton University*

Category: Inner Ear: Damage and Protection
Background: Damaging noise exposure is one of the most common causes of hearing loss, yet there are no Food and Drug Administration (FDA)-approved drugs to shield from this common disability. We and others have shown that activation of the mitogen activated protein kinase (MAPK) pathway occurs after noise exposure, and inhibition of this cellular pathway protects from hearing loss. Tizaterkib, formerly AZD-0364, is a novel, highly selective, orally bioavailable ERK1/2 inhibitor that is currently in Phase-1 clinical trials. Infiltration of immune cells following noise exposure could be a possible mechanism that is leading to hearing loss, and some studies suggest that activation of the MAPK pathway could be regulating this immune response. In this study, we show that low doses of the drug that are equivalent to the doses tested now for cancer treatment, protect mice from NIHL and explore the mechanism of protection that the drug is working through.
Methods: Different treatment schedules and dosages of tizaterkib were tested to determine the drug's optimal regimen, and minimum effective dose to protect from NIHL. The auditory brainstem response (ABR) was utilized to measure overall hearing function in mice, and distortion product otoacoustic emission (DPOAE) was used to measure outer hair cell function after noise and tizaterkib treatment. Whole mount cochlear sections were stained with anti-myosin VI and anti-Ctbp2 antibodies to measure noise-induced synaptopathy. Cochlear cryosections were stained with anti-CD45 antibody and DAPI, and cochlear western blots were probed with anti-CD45 and CD68 antibodies to determine the effect that tizaterkib has on immune cell infiltration following noise exposure.
Results: Tizaterkib significantly protects 2 different strains of mice from permanent NIHL with a dose of 0.5 mg/kg administered twice a day for 3 days, starting 24 hours after noise exposure of 100-dB SPL or 106-dB SPL for 2 hours. Tizaterkib-treated mice have significantly lower ABR (average 20-25 dB in three frequencies) and DPOAE threshold shifts compared to noise alone mice. Additionally, mice treated with tizaterkib have more ctbp2 puncta per IHC and larger ABR wave 1 amplitudes compared to noise alone mice. Furthermore, tizaterkib treatment significantly lowers the number of CD45 and CD68 positive cells in the cochlea on days 4 and 6 following noise exposure. Tizaterkib treatment was also confirmed to protect mice from NIHL through inhibition of the MAPK pathway by utilizing the KSR1 KO genetic mouse model.
Conclusions: 0.5 mg/kg given twice a day is the mouse equivalent to the doses that humans are currently receiving in Phase-1 clinical trials. Tizaterkib has a therapeutic window greater than 50 in mice to protect from NIHL. Tizaterkib offers significant protection from NIHL while lowering the number of immune cell infiltrates into the cochlea which could be a possible protective mechanism of MAPK inhibition.

Sleep Fragmentation Increases Noise-Induced Hearing Damage via Reductions in Cochlear Efferent Signaling to Outer Hair Cells

Lingchao Ji[1], Beatriz Borges[1], Marina Silveira[1], Alejandra Mondino[2], Giancarlo Vanini[2], Ana Belen Elgoyhen[3], Michael Roberts[1], Gabriel Corfas*[1]

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Category: Inner Ear: Damage and Protection
Background: Noise-induced hearing loss affects about 320 million people worldwide, with 10% of the world's population exposed to potentially damaging sounds daily. The risk for hearing loss varies among individuals, but the mechanisms mediating these differences remain poorly understood. While genetic factors probably contribute to this, it is likely that behaviors and other health conditions are also involved. Obstructive sleep apnea (OSA), a sleep disorder involving frequent episodes of breathing cessation during sleep, is a prime example of a condition that increases the probability of hearing loss. The mechanisms mediating this comorbidity remain unknown, with some speculating it is due to inner hair cell damage caused by intermittent hypoxemia, while others suggest that high-volume snoring (80 dB SPL) frequent in OSA patients may play a role. Here we tested if chronic sleep fragmentation (SF), a cardinal clinical feature of OSA, alters cochlear function and influences susceptibility to noise using a well-established mouse model of chronic SF.
Methods: SF was induced by housing mice 24/7 in cylindrical cages with a horizontal bar sweeping just above the cage floor powered by a near-silent electrical motor system with the speed set at 2-min intervals, resulting in a sleep disruption rate (30/h) like that seen in humans with moderate-to-severe sleep apnea. This chronic

SF paradigm increases sleep debt without altering circadian rhythms. Control mice were housed in cages without the bar but with an active motor.
Results: Two or four weeks of chronic SF do not alter ABR and DPOAE thresholds, or ABR peak 1 amplitudes, but induce a significant increase in the amplitude of the DPOAE and a large decrease in contralateral DPOAE suppression. SF also reduced the volume and intensity of vesicular acetylcholine transporter (VAChT) immunostaining in outer hair cell efferent synaptic terminals, suggestive of a reduction in the levels of cholinergic transmission in MOC terminals. Importantly, the VAChT staining in MOC terminals and DPOAE function recover if mice are allowed to sleep normally for 2 weeks after a period of SF, showing that the changes in OHC efferent innervation are due to the sleep disturbance and are reversible. Furthermore, mice carrying a mutation that enhances the function of the OHC's α9 nicotinic acetylcholine receptors also exhibit reduction in OHC efferent innervation but do not present increased susceptibility to noise-induced hearing loss.
Conclusions: Together, these results suggest that SF contributes to hearing loss by increasing the sensitivity of the inner ear to noise-induced damage due to decreased efferent function and that treatments that improve sleep patterns in OSA patients or increase OHC cholinergic function could help reduce the incidence of hearing loss. These findings also provide a new model to study the mechanisms by which SF alters neural circuits and synapses.

Exploring Synaptopathy in Swine Models of Noise and Blast-Induced Hearing Loss

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[1]*Mass Eye and Ear, Harvard Medical School,* [2]*Blast-Induced Neurotrauma Branch, Center for Military Psychiatry and Neuroscience, WRAIR*

Category: Inner Ear: Damage and Protection
Background: Noise-induced hearing loss (NIHL) is a global health concern. The enduring consequences of NIHL, and its more severe counterpart, blast-induced hearing loss (BIHL), encompass a spectrum from mild hearing loss to total deafness. These impairments impact communication and the perception of crucial sounds and manifest persistent psychological and cognitive challenges. While small animal models have significantly contributed to NIHL research, there has been a scarcity of studies involving large animals, particularly swine. Swine, with hearing ranges and susceptibility to NIHL similar to humans, offers a compelling model due to the striking anatomical resemblance of their inner ears to human counterparts, providing a unique platform for developing inner ear delivery procedures applicable to humans.
Methods: Micro-Yucatan pigs aged 7-10 weeks were exposed to 120 dB white noise for 90 minutes (N1) or two 90-minute noise episodes 24 hours apart (N2), with three pigs in each group. Auditory brainstem responses (ABR) were monitored before noise exposure (NE), and at 7, 14, 21, and 28 days post-NE. Sound frequencies at 4, 8, and 16 kHz were systematically measured. Blast exposure (BE) under double 22.8 psi pressure was administered to three pigs, and test ABR at the same time points. We conducted the pathological evaluation immunohistochemical staining using antibodies against MYO7A, CtBP2, and NF200. H and E staining was utilized to analyze the morphology. The comparison of Micro-CT between wild-type pig and human was analyzed.
Results: Following NE1, ABR thresholds exhibited a shift ranging from 4 to 32 kHz at 7, 14 days post-exposure. NE2 displayed ABR thresholds significantly increasing across all tested frequencies (4 kHz to 32 kHz), persisting for 28 days. The Micro-CT readout of comparison between swine and humans showed remarkable similarity. Histological analysis of the swine cochlea in the N1 group revealed an increased presence of MYO7A-negative IHCs distributed throughout the cochlea. Inner hair cell (IHC) nuclear shrinkage and synapse loss were also observed post-NE, while outer hair cells (OHC) displayed no significant impact following NE1 and NE2.In contrast, the BE group exhibited dramatic reductions in IHCs in the basal turn, mild reductions in the middle turn, and no changes in the apex turn compared to the wild type. Importantly, OHCs and Ctbp2/per IHC were markedly decreased in the basal turn but remained unaffected in the apex and middle turns. Additionally, H and E staining results consistent with the immunostaining results.
Conclusions: Our study underscores the relevance of establishing noise exposure parameters in swine models for temporary, permanent NIHL, and BIHL. This animal model sheds light on how NE and BE affect hair

cells and synapses and provides a powerful tool for translating drug-mediated protective interventions against NIHL from animals to humans.

ECochG Measurements in the Course of Cochlear Implantation to Evaluate the Insertion Trauma in a Large Animal Model With an Extended Follow-Up

Erdem Yildiz*[1], Matthias Gerlitz[1], Anselm Joseph Gadenstaetter[1], Rudolf Glueckert[2], Hinrich Staecker[3], Lukas D. Landegger[1], Clemens Honeder[1], Christoph Arnoldner[1]

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Category: Inner Ear: Damage and Protection

Background: With its human-like inner ear dimensions, the minipig represents a highly relevant animal model in translational hearing research. Compared to rodents, in most cases it can be assumed that the knowledge gained in this large animal model can be better transferred to humans. Thus, we used cochlear implants that are typically utilized in a clinical setting for human patients in said porcine inner ears in vivo. To observe and characterize long-term sequelae after electrode insertion, we performed electrocochleography (ECochG) measurements during and after cochlear implantation surgery.

Methods: The inner ear dimensions of three different pig breeds were measured with segmentations of high-resolution micro-CT scans. Subsequently, cochlear implants (Flex20 and Flex24, MED-EL, Austria) were surgically inserted into minipigs under sterile conditions. ECochG, precisely cochlear microphonic (CM), auditory neurophonic (AN), and acoustic compound action potential (CAP) measurements, were recorded before, during, and after electrode array insertion to accurately define the elicited hair cell trauma within the inner ear. Prior to implantation, baseline ECochG recordings via a gold-wire electrode that was placed on the round window membrane, were performed. Intracochlear telemetric ECochG recordings via the inserted electrode array were performed after partial and full insertion (n=6). In addition, acoustic auditory brainstem response (ABR), electrically evoked CAP (eCAP), electrically evoked ABR (eABR), and impedance measurements were performed. Threshold and amplitude changes were monitored during the extended follow-up of six months after implantation.

Results: ECochG measurements provided timely feedback on the functional status of the inner ear during electrode array insertion. Long-term follow-up measurements with ECochG presented residual hearing four months after cochlear implantation with the decrease of CM thresholds emphasized between two weeks and 1.5 months after implantation. 1kHz CM and AN amplitudes increased in the majority of subjects over time in comparison to baseline and intraoperative recordings. Acoustic ABR threshold shifts remained increased with thresholds ranging from 103 ± 19 dB SPL and were increased in comparison to CM and AN recordings (57 ± 25 dB SPL). Recorded eABR thresholds were not significantly increased over time after implantation. Consecutive increases in impedances and eCAP thresholds were observed two weeks, 1.5, 3 and 4 months after implantation.

Conclusions: Cochlear implantation using clinically certified biomedical devices is feasible in minipigs. Our explorational study presents a large animal model with an electrode-insertion trauma mimicking the human cochlea, and gives insights to the integrity of cochlear health over a period of six months. Our longitudinally collected data provide novel insights aimed at enhancing implantation outcomes, particularly with respect to the preservation of residual auditory function in affected patients. Conducting precise, functional ECochG evaluations enables the exploration of prospective therapeutic optimizations, including local drug delivery of potentially otoprotective agents to improve functional hearing outcome.

Podium Session 24 - Clinical Otology: Pediatric Diseases and Animal Models

10:15 a.m. - 12:15 p.m.

Platinum Salon 6

Unraveling the Phenotypic Spectrum of ACTG1-Related Disorders: Insights From Genotype Associations

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Category: Genetics A: Genomics and Gene Regulation

Background: Variants in ACTG1 are associated with nonsyndromic hearing loss at the DFNA20/26 locus and Baraitser-Winter syndrome, type 2 (BWS2), a craniofacial syndrome with variably expressed phenotypes including facial dysmorphism, intellectual disability, and hearing loss. A smaller number of patients have been reported to exhibit seemingly nonsyndromic hearing loss with mild features consistent with BWS2. To date, no consistent genotype-phenotype correlation has been described.

Methods: Comprehensive genetic testing of all exons and flanking intronic sequences of known hearing loss-associated genes was completed in patients referred for clinical diagnostic testing using the OtoSCOPE targeted genomic enrichment and massively parallel sequencing panel. Genetic variants reported in PubMed, DECIPHER, ClinVar, and the Deafness Variation Database were reviewed and categorized as DFNA20/26 (non-syndromic), mild syndromic (or variably reported phenotypes), or BWS2. Audiometric data were reviewed to determine age of onset and pattern of hearing loss progression.

Results: Variants in ACTG1 were causative of hearing loss in 29 of 2616 (1.1%) of patients referred for testing. 2559 records reviewed from PubMed, ClinVar, DECIPHER, and the DVD identified 84 likely causative genetic variants in ACTG1 in these databases and the OtoSCOPE cohort. The proportion of variants causing DFNA20/26 varied by ACTG1 exon ($p = 0.002$) and was greatest in exon 5. The pattern of hearing loss progression varied by subdomain ($p = 0.0007$).

Conclusions: ACTG1-related diseases include a highly variable spectrum of craniofacial and neurologic manifestations in addition to progressive sensorineural hearing loss. Detection of an ACTG1 missense variant in a proband with hearing loss should prompt close evaluation for syndromic features.

Patients With Pathogenic MYO15A Variants Presenting Phenotypes Mimicking Auditory Neuropathy Spectrum Disorders

Pei-Hsuan Lin*[1], Pey-Yu Chen[2], Cheng-Yu Tsai[3], Yu-Ting Chiang[4], Tien-Chen Liu[1], Chuan-Jen Hsu[5], Pei-Lung Chen[6], Chen-Chi Wu[7]

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Category: Clinical Otolaryngology and Pathology

Background: Auditory neuropathy spectrum disorder (ANSD) primarily affects the auditory pathway from the inner hair cell synapse to the brainstem nuclei. Traditionally, ANSD has not been associated with pure hair cell pathologies causing cochlear hearing loss. However, our recent identification of patients with pathogenic variants in the MYO15A gene presenting with ANSD-like features challenges this assumption. These patients exhibited cochlear microphonics and abnormal auditory brainstem response (ABR) waveforms, despite the fact that MYO15A variants have historically been associated with stereocilia-related pathologies. To comprehensively investigate these auditory features, we performed a thorough analysis of audiological data in patients carrying bi-allelic pathogenic MYO15A variants.

Methods: We enrolled 26 unrelated patients with bi-allelic pathogenic MYO15A variants and collected their basic characteristics and genotypic information. Serial audiological evaluations were performed, including behavioral audiometry, ABR, auditory steady-state response (ASSR), distortion product otoacoustic emissions, and cortical auditory evoked potential (CAEP).

Results: Our study cohort consisted of 10 boys and 16 girls with a mean age of 4.5±4.6 years and a mean hearing threshold of 87.3±29.1 dB. The most common MYO15A variant was c.3524dupA. Sixteen patients (61.5%) with a mean age of 1.7±1.3 years showed cochlear microphonics at their first ABR examination,

while the remaining ten patients (38.5%) with a mean age of 8.6±4.7 years did not. ABR and ASSR thresholds were consistent with behavioral thresholds in all patients. ABR waveforms were abnormal in 17 patients (65.4%) with bilateral profound hearing loss, of whom 11 (64.7%) had cochlear microphonics. The remaining nine patients (34.6%) with bilateral moderate-to-severe hearing loss had normal ABR waveforms, including five (55.5%) with cochlear microphonics. The P1 latencies on the CAEP test fell within age-adjusted normal ranges for all subjects. Notably, young age was associated with the presence of cochlear microphonics ($p = 0.002$), whereas genotypes, normal ABR waveforms, and P1 latency were not. One patient had cochlear microphonics at six months but not at nine years of age, and he also had normal otoacoustic emissions from six months to 1.5 years but not at two years of age or older.

Conclusions: Approximately 60% of patients in our cohort had cochlear microphonics, and younger age correlated with their presence. Our findings demonstrate that the audiological phenotype of patients with pathogenic MYO15A variants is similar to that of patients with cochlear hearing loss, but may also exhibit features similar to ANSD, contrary to the expectations of stereocilia-related pathologies. Therefore, pathogenic MYO15A variants should be considered in the genetic evaluation of pediatric ANSD. Further studies are warranted to investigate hair cell function in individuals with stereocilia-related pathologies.

The Effect of (val)Ganciclovir on Hearing in Children With Symptomatic Congenital Cytomegalovirus Infection: A Prospective Controlled Trial

Elise De Cuyper*[1], Frederic Acke[1], Annelies Keymeulen[2], Els De Leenheer[1], Helen Van Hoecke[1], Elizaveta Padalko[2], An Boudewyns[3], Annick Gilles[3], Marie Muylle[4], Rudolf Kuhweide[4], Liesbeth Royackers[5], Christian Desloovere[5], Margriet Verstreken[6], Isabelle Schatteman[6], Ingeborg Dhooge[1]

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Category: Clinical Otolaryngology and Pathology

Background: Congenital cytomegalovirus (cCMV) infection is a major cause of neurocognitive disabilities, sensorineural hearing loss, and vestibular abnormalities during childhood worldwide. Treatment with oral valganciclovir is advised as first-line treatment in children with moderate to severe disease. The proportion of children treated with valganciclovir has considerably increased over the years; however, the effect on hearing outcome remains unknown. This study was performed to assess the effect of (val)ganciclovir on hearing outcome.

Methods: This prospective controlled trial presents multicentric data of the Flemish CMV registry (Belgium), collected between January 1, 2007, and February 7, 2022. Both treated and untreated cCMV-infected children with a minimal 4-year audiological follow-up were included. Primary outcome was hearing evolution (per ear analysis; described as hearing improvement, hearing deterioration, or late-onset hearing loss). To control for spontaneous hearing evolution, exact matching for risk factors of natural hearing evolution was performed between the treated and untreated group. The average absolute risk of hearing evolution and number needed to treat was calculated using a pooled regression analysis (g-computation, average marginal effects, cluster-robust standard errors).

Results: Of the 472 included children, 198 (41.9%) were symptomatic and 85 (18.0%) were treated. Of the 944 ears, 137 (14.5%) ears had congenital hearing loss of which profound congenital hearing loss was present in 72 ears (52.6%). The absolute chance of hearing improvement (i.e., in ears with congenital hearing loss) was 11.2% (SE, 0.05; n=91) in the untreated group and 10.9% (SE, 0.05; n=46) in the treated group. The absolute risk of hearing deterioration (i.e., in ears with non-profound congenital hearing loss) in the untreated group was 70.6% (SE, 0.08; n=34) compared to 61.3% (SE, 0.09; n=31) in the treated group. To prevent one ear from hearing deterioration, 11 ears need to be treated. The absolute risk of late-onset hearing loss was 7.8% (SE, 0.01; n=683) in the untreated group and 7.3% (SE, 0.02; n=124) in the treated group.

Conclusions: Based on this study, antiviral therapy seems to have limited beneficial effect on hearing outcome. These novel findings can aid clinicians and parents in their decision whether to initiate antiviral therapy. Future studies should focus on the antiviral effect on hearing outcome in asymptomatic children recently diagnosed with late-onset hearing loss.

GWAS of Sensorineural Hearing Loss by Clinical Diagnosis Elicits Multiple Cochlear-Related Hits

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Category: Genetics A: Genomics and Gene Regulation

Background: Hearing loss worldwide is predicted to increase 50% over the next 30 years. Although hearing assistive devices are helpful, the use of hearing aids is universally low. A cause-effect relationship for hearing assistive devices in delaying or preventing dementia has been supported, but not firmly established.
With a twin-study heritability of .36 to .67, previous genome-wide association studies (GWAS) for hearing loss elicited multiple loci and greater than 250 genetic variants. Large investigations have employed "minimal phenotyping": one-two self-reported items for case identification. This can lead to false positives and lower single-nucleotide heritabilities (h2). Ours is the first GWAS based solely on an ICD clinical diagnosis.

Methods: Cases included at least one diagnosis of bilateral sensorineural hearing loss with no evidence of tinnitus. Controls had no evidence of hearing loss and no tinnitus ICD or self-report. After quality control, GWAS was performed for three ancestry groups (EA, AA, and LA) separately in PLINK 2.0, then combined in meta-analysis. Functional annotation of GWAS results was performed with FUMA. Loci were subsequently fine mapped with per-SNP heritabilities as priors. Phenome-wide association study (PheWAS) information was obtained for EA ancestry SNPs through the GWAS Atlas (https://atlas.ctglab.nl/). Putative protein-protein interactions were explored within STRING.
Results: The cohort consisted of 143,861 cases and 226,680 controls (prevalence = 38.8%). GWAS in EA ancestry identified 23 significant loci and trans-ancestry analysis added seven, for a total of 30 loci, 16 of which are novel. Fine mapping reduced the implicated area to 26.7% of the original loci. Of 53 genes within the fine-mapped area or identified by MAGMA as significant, 11 are known hearing loss genes, 15 are related to ear functions, three are involved in stereociliary processes, 19 are engaged in neuronal networks, 9 in neurodevelopmental abnormalities, and 4 are specific for calcium level control. In addition, APOE was identified within a significant locus for the first time.
Conclusions: This study is unique in that phenotype selection was more objective than previous GWAS, while removing tinnitus from cases and controls. By utilizing an objective phenotype, we feel we have identified loci with greater specificity to sensorineural hearing loss. To that end, eleven hits are associated with congenital hearing loss genes, several of which are novel to this study, i.e., WHRN, SETBP1, RREB1, and RBPMS. PheWAS demonstrates significant association with multiple Alzheimer's Dementia phenotypes. Hearing loss connection to AD has been extensively researched, including dosages of the APOE□ allele, which has been noted to increase the risk of hearing loss by 2.0-fold. Future directions of study include the role of APOE in dementia and hearing loss, in addition to GWAS using audiogram data.

Predominance of Auditory but Not Vestibular Deficits in the Mouse Model of Congenital Cytomegalovirus Infection

Chi-Chieh Chang[1], Ying-Chang Lu[1], Pei-Hsuan Lin[1], Tien-Chen Liu[1], Po-Nien Tsao[1], Chun-Yi Lu[1], Chen-Chi Wu*[1]

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Category: Clinical Otolaryngology and Pathology

Background: Inner ear involvement resulting in sensorineural hearing impairment (SNHI) and vestibular dysfunction is a common and permanent consequence of congenital cytomegalovirus (cCMV) infection. In contrast to the extensive research on SNHI, there has been relatively little research on the vestibular effects of cCMV infection. In this study, we aimed to comprehensively assess both hearing and vestibular function in a cCMV mouse model that closely resembles clinical phenotypes.
Methods: We established a congenital murine CMV (MCMV) infection model by intraperitoneal inoculation in C57BL/6 mice. We performed a comprehensive phenotypic characterization of this model, including

auditory, vestibular, and histological assessments. Auditory characteristics were assessed using auditory brainstem response and analyzed for SNHI severity and laterality. Vestibular function was assessed using open field, swim, and rotarod tests. In addition, histopathologic studies were performed to investigate the underlying mechanisms associated with these deficits.
Results: Our study demonstrated that intraperitoneal MCMV infection in C57BL/6 mice successfully recapitulated the diverse auditory features seen in humans with cCMV infection. These auditory deficits ranged in severity and laterality, reflecting the clinical spectrum. In contrast, vestibular function, as assessed by various behavioral tests, remained less affected and showed no significant correlation with SNHI. Histopathological examination revealed that swollen cytopathic effects were predominantly observed in the spiral ganglion neurons of MCMV-infected mice with SNHI, whereas the vestibular organs remained relatively intact.
Conclusions: We have optimized an intraperitoneal MCMV infection model in C57BL/6 mice that closely mimics the inner ear manifestations of cCMV infection. Our results provide phenotypic and histopathologic evidence highlighting the predominance of auditory over vestibular deficits in cCMV infection. This platform serves as a valuable resource for testing therapeutic interventions targeting the inner ear sequelae of cCMV infection.

How Low Can You Go? Feasibility of Dose Reduction in Preoperative Imaging With the New Photon Counting Detector CT and AI Improved Images

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Category: Clinical Otolaryngology and Pathology
Background: Preoperative imaging is an important part of the preoperative work-up for otologic surgery, especially in cochlear implant cases. With the trend towards individualized and atraumatic surgery, high-quality preoperative imaging is essential to yield the best results. Different imaging modalities have been employed over time with high-resolution computed tomography being widespread. Besides the imaging quality and resolution, radiation-saving modalities and protocols are a major concern to protect patients against adverse radiation effects, e.g., radiation cataracts and even malignant processes. A newly introduced detector technology, namely the photon counting detector computed tomography, yields superior image resolution and quality. Besides advantages in image properties, previous studies have shown a reduction in the administered radiation dose. This study aimed to evaluate the feasibility maximal of dose reduction in preoperative imaging.
Methods: Cadavers were scanned with the standard clinical protocol for temporal bone imaging and further dose-reduced protocols. The obtained datasets were processed by an artificial intelligence (AI)-based algorithm to improve usability. The results were compared to the standard of care and the original, unprocessed images. Special focus was paid to the implications for cochlear implant surgery with measurements of cochlear dimensions and 3D cochlear and vestibular segmentations. Implications for CI electrode selection were evaluated.
Results: Images could be obtained down to 5% of the original radiation dose. All scans were successfully processed by the AI tool. AI processing improved the overall image quality and usability. Automated 3D segmentation of the cochlea yielded better results with the dose-reduced protocol compared to the vestibulum, especially for the original, unprocessed images.
Conclusions: With the newly developed photon counting detector computed tomography high-detail images could be obtained, even with scan protocols that only administer a fraction of the original radiation dose. AI processing is a promising tool to further improve the obtained images.

The Transcription Factor Prdm1a is at the Core of the Hair Cell Evolutionary and Developmental Gene Regulatory Network

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Select a Category Genetics A: Genomics and Gene Regulation

Background: A major cause of hearing loss in mammals is the lack of regeneration in the cochlea following damage to mechanosensory hair cells. The zebrafish Danio rerio has hair cells in the lateral line and inner ear, which share genetic, functional, and structural similarity with mammalian ear hair cells. A key difference is that zebrafish hair cells readily regenerate following death to restore full function. The lateral line undergoes proliferative regeneration, while ear support cells undergo non-proliferative differentiation into hair cells. Investigating the gene regulatory networks (GRNs) that drive hair cell development and regeneration provides a powerful tool to identify key differences between regenerating and non-regenerating species. The transcription factor prdm1a is expressed in hair cells of the zebrafish lateral line, but not in hair cells of zebrafish or mammalian ears. Mammalian Prdm1 controls fate switches in various cell types, and prdm1a promotes proliferation in zebrafish. Thus, we investigated prdm1a for its central role in the hair cell development and regeneration GRN.

Methods: We mutated prdm1a in zebrafish by truncating the protein to eliminate the zinc finger domains. We assayed hair cell development and regeneration, including the number of developing or regenerated hair and support cells, and cell proliferation. We performed scRNA-seq on lateral line cells from prdm1a mutants and siblings, and bioinformatically clustered hair cells and performed a differential gene expression analysis. We identified enhancers and promoters of prdm1a targets and investigated their ability to drive expression of a GFP reporter in vivo. To build the GRN of different hair cell types, we integrated ear scRNA-seq and compared the hair cell transcriptomes.

Results: In prdm1a mutants, there is a drastic reduction of hair cells and cell proliferation during development and regeneration. Specifically, there is a proliferation defect in the support cells that divide and mature into regenerated hair cells. scRNA-seq revealed a fate switch between lateral line and inner ear hair cells, with a multitude of inner ear hair cell-specific genes ectopically expressed in mutant lateral line hair cells. Physical changes and comparison with zebrafish ear scRNA-seq identified vestibular striolar hair cells as the altered identity of mutant hair cells. Motif searches revealed that Prdm1 binding sites are highly enriched in the promoters and enhancers of ectopically expressed genes. These enhancers drive GFP expression in prdm1a mutant hair cells, but not in siblings. Finally, expressing prdm1a in the inner ear hair cells was sufficient to repress inner ear hair cell and prdm1a target genes.

Conclusions: These findings show that prdm1a is central in the zebrafish hair cell GRN. It determines hair cell fate, with consequences for development and regeneration. As prdm1a is not expressed in mammalian hair cells, it is an ideal gene for investigating differences between regenerating and non-regenerating hair cells.

Podium Session 25 - Exploring the Auditory Nerve: Spontaneous Firing, Efferent Modulation, and Advanced Imaging

10:15 a.m. - 12:15 p.m.

Grand Ballroom Salon E

Multiscale Three-Dimensional Imaging of Neural Projection From Cochlea to Cochlear Nucleus

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[1]*Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine*

Category: Auditory Nerve

Background: The cochlear nucleus (CN) is the target for Auditory Brainstem Implantation (ABI), a treatment for restoring auditory function in individuals for whom cochlear implants are not effective. However, speech recognition outcomes following ABI fall short of expectations, due to poor frequency dependent targeting of the stimulus site in ABI approach. This study seeks to follow the neural projections from cochlea to CN, with 3D mapping at multiscale levels that allow visualization of neural distributions and projections in CN.

Methods: PEGASOS tissue clearing technique was used to render the entire brain tissue with bilateral cochlea transparent in Thy1-YFP16 mice, three-dimensional mesoscopic structure of neural projections was achieved using confocal microscopy with 4x and 10x objective lenses. To further trace the projections of individual spiral ganglion neurons (SGNs) to CN, we utilized the innovative Transparent Embedding Solvent System (TESOS) technique in conjunction with transgenic mice. Additionally, we combined TESOS with anterograde or retrograde adeno-associated virus-based tracer methods. Polymerization of cleared tissues and a cutting-stitching approach was applied to overcome the limitations imposed by the working distance of the 63x/1.4 NA and 40x/1.3 NA objective lenses. Images were analyzed using Imaris and Vaa3D software.
Results: The 3D structure of the cochlea to the brain has been successfully mapped at a mesoscopic resolution, illustrating the overall architecture and relationship between the cochlea, vestibule, cochlear nucleus, facial nerves and brain. Three efficient label strategies were developed to reveal the neural projections from the SGNs to the CN. First approach used the retrograde injection of AAV2/R-hSyn-Cre-WPRE-hGH-pA into reporter mice alone or in combination with rAAV-VAG-DIO-EYFP-WPREs in the CN of C57BL/6 mice. This approach enabled tracing of the neural projection from the peripheral to the central nervous system. To follow the targets of the neural projection, two transsynaptic labeling methods were employed: injecting 1) AAV1-hSyn-Cre into the cochlea of reporter mice or 2) rabies virus in the CN after AAV2/9-hSyn-Cre and helper-AAV injections. In addition to the classical projection pattern of auditory nerves, where the ascending branches bifurcate at the AVCN and form descending branches innervating the PVCN and DCN; We observed that the projections in AVCN are not always tonotopically matched those in PVCN, and some bifurcated projections predominately innervate VCN only. We also identified auditory nerve fibers that are non-bifurcate in all frequency regions investigated. Intriguingly, we revealed for the first time single auditory nerve could innervate 4~5 target neurons of distinct morphologies in the CN.
Conclusions: Enhanced tissue clearing methods and versatile viral labeling approaches enabled the reconstruction of three-dimensional images of cochlea-SGN-CN. Projections at single-axon resolution demonstrated three projection pathways of single auditory nerve fiber.
Projection of single auditory nerve fiber's target neurons diverse in morphology and location in the CN.

An MRI Protocol to Assess the Effects of Noise Exposure on the Peripheral and Central Auditory Pathway

Rebecca Dewey*[1], Hannah Guest[2], Garreth Prendergast[2], Rebecca Millman[2], Christopher Plack[3], Susan Francis[4]

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Category: Auditory Nerve
Background: Findings from animal studies suggest that noise exposure can substantially damage the auditory nerve without noticeable hair-cell damage or audiometric threshold elevation. This damage may lead to changes in the peripheral (auditory nerve) and central (auditory brainstem and cortex) auditory system, and exacerbate the effects of aging. Here, we present a magnetic resonance imaging (MRI) protocol to determine (1) the contributions of age and noise exposure to peripheral and central auditory damage, and (2) the degree of association between structural MRI measures of the auditory system and electrophysiological/behavioural measures of auditory function.
Methods: Participants: We aim to recruit 200 participants across four groups (G1-4; each n=50): G1: 18-19 yrs, low noise exposure, normal hearing; G2: 30-50 yrs; low noise exposure, normal hearing; G3: 30-50 yrs,

high noise exposure, normal hearing; G4: 30-50 yrs, high noise exposure, suspected noise-induced hearing loss. Recruitment opened in May 2022 and will close in April 2025.
Audiology: Participants will undergo pure-tone audiometry (0.5-8 kHz) and the Noise Exposure Structured Interview (NESI) to determine eligibility. Eligible participants will complete extended high-frequency audiometry, distortion product otoacoustic emissions, middle ear muscle reflexes, auditory brainstem responses, and speech-in-noise testing.

MRI protocol: The protocol has been developed and optimised on a Philips 3.0 T Ingenia MR scanner (Philips Healthcare, Best, Netherlands) using a 32-element SENSE head coil. High-resolution T2-DRIVE and Diffusion Tensor Imaging (DTI) scans are collected to characterise auditory nerve structural integrity. The DTI scan was optimised (for image readout and choice and number of b-values from 0-700 smm-2, with a TSE readout, two b=300 smm-2 and six b=700 smm-2 selected) to measure the fractional anisotropy and apparent diffusion coefficient using DTIFIT (FSL). NOise reduction with DIstribution Corrected (NORDIC) significantly improves the image SNR (p less than 0.0001). A whole-brain DTI UK Biobank protocol will assess central auditory structural connectivity. Whole-brain morphometric measures (volume, thickness, curvature) are estimated from an MPRAGE scan, while whole-brain T1 mapping is used to assess myelination. High-resolution resting-state functional connectivity is measured within the cortical and sub-cortical auditory network. The peripheral and central auditory MRI measures will be compared to audiological measures.

Results: To date, 140 participants have been recruited, G1:30, G2:44, G3:49, and G4:17, with mean (±standard deviation) ages of 19(±1), 40(±6), 40(±6), and 42(±7) years, and NESI scores of 1(±2), 4(±4), 72(±68), and 158(±217) units, respectively.
Conclusions: An MRI protocol to study the auditory nerve, brainstem, and central auditory brain is being applied across noise exposure and hearing loss groups. Future aims include determining if MRI measures can be useful diagnostically (for guiding treatments), and for validating other measures of synaptopathy. This could then provide personalised healthcare, regarding, for example, reducing further noise exposure, and provide evidence for noise exposure regulations and policy.

Evidence of a Non-Linear Relationship Between Cochlear Deafferentation and Speech Perception in Noise

Naomi Bramhall*[1], Elizabeth Flores[2], Garnett McMillan[3]

[1]*VA NCRAR,* [2]*University of Tennessee Health Science Center,* [3]*VA RR and D NCRAR*

Category: Auditory Nerve
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 models. However, the impacts of cochlear synaptopathy and other forms of cochlear deafferentation on complex speech perception remain unclear due to mixed results in previous studies. Differences across studies in terms of the specific physiological and perceptual measures used may help explain the mixed results. Recent findings suggest that the magnitude of the envelope following response (EFR) may be a better correlate of cochlear deafferentation than auditory brainstem response (ABR) wave I amplitude or the magnitude of the middle ear muscle reflex (MEMR). In particular, the EFR for a rectangular amplitude modulated (RAM) stimulus may be a superior measure of cochlear deafferentation because the sharp onset of the stimulus promotes neural synchrony, resulting in a larger response than the more traditional sinusoidally amplitude modulated (SAM) stimulus.
Methods: In this study, we sampled from a population of adults with normal to near normal hearing thresholds who were expected to exhibit a broad range of degrees of age- and noise exposure-related cochlear synaptopathy – military Veterans and non-Veterans aged 18-59 years. In this sample, we measured EFR magnitude for a RAM stimulus, performance on the Words in Noise (WIN) test, and distortion product otoacoustic emissions (DPOAEs).
Results: Study results suggested little impact of DPOAE level on WIN performance in a population with normal to near normal hearing thresholds. In contrast, EFR magnitude appeared to have a non-linear relationship with WIN performance. For EFR magnitudes on the lower end of the range observed in the sample, WIN performance improved as EFR magnitude increased. However, this relationship plateaued at

higher EFR magnitudes and further increases in EFR magnitude were not associated with better WIN scores. On average, study participants performed poorer on WIN list 2 than on WIN list 1, potentially due to order effects because WIN list 1 was completed first. The non-linear relationship between EFR magnitude and WIN performance was more apparent for WIN list 1 than for WIN list 2.
Conclusions: One possible explanation for the observed non-linear relationship between EFR magnitude and WIN performance is that due to the high degree of redundancy in auditory neural coding, the impacts of cochlear deafferentation on speech perception in noise only become noticeable when a certain threshold level of cochlear deafferentation is reached. This could explain the mixed results across previous studies because only studies that sampled from a population at high risk for synaptopathy due to age or military/occupational noise exposure would be expected to have sufficient deafferentation in the sample to observe a relationship with speech perception performance.

Frequency Selectivity in Monkey Auditory Nerve Studied With Suprathreshold Multicomponent Stimuli

Philip Joris*[1], Eric Verschooten[1], Myles Mc Laughlin[1], Corstiaen Versteegh[2], Marcel Van Der Heijden[2]

[1]*KU Leuven,* [2]*Erasmus MC*

Category: Auditory Nerve
Background: Data of very different kinds (psychophysics, acoustic emissions, mass potentials, single fiber data) suggest that frequency tuning is sharper in humans than in commonly used animal models, but several criticisms have been raised against this interpretation. Most of the diverse methods that have been used in this debate are indirect. Data from single auditory nerve (AN) fibers occupy a pivotal position, being the direct output of the cochlea and input to the CNS. A previous study reported higher Q-values in AN fiber of macaque relative to the cat. A limitation of these and other AN-fiber data brought into this debate is that frequency selectivity was measured with tonal threshold tuning curves, which do not directly assess spectral filtering because their shape is strongly affected by cochlear nonlinearity. Our aim was to measure spectral filtering with wideband suprathreshold stimuli.
Methods: We measured tuning curves in single AN fibers of anesthetized macaque monkeys using the "zwuis" stimulus. This is a suprathreshold, wideband, multicomponent stimulus which was designed to allow characterization of spectral filtering at any cochlear locus (van der Heijden and Joris, 2006, 2003). Traditional tonal threshold tuning curves were also obtained from which characteristic frequency (CF) and spontaneous rate were determined. We compare data obtained in monkey with data obtained in cat using the same techniques.
Results: Comparison of Q10 values of the zwuis-based filters in cat and macaque shows a trend of higher values in the latter for CFs above a few kHz. The general trend is qualitatively similar to that observed with threshold tuning curves (Joris et al., 2011) and forward-masked evoked potentials (Verschooten et al., 2018), in that the Q-values are similar at low CFs and then increasingly diverge with CF. However, quantitatively the differences between the two species are smaller than in these previous studies. We also examined differences in group delay between regions at a fiber's CF and its low-frequency tail, expecting larger differences to be associated with sharper tuning. The phase data are consistent with the interpretation of sharper frequency tuning in monkey.
Conclusions: We conclude that use of suprathreshold, wide-band stimuli supports the interpretation of sharper frequency selectivity in macaque AN fibers relative to the cat, although the difference is less marked than apparent from the assessment with threshold-based data.

Efferent Effects on Low-Frequency Auditory Nerve Neurophonic in Chinchilla

Eric Verschooten*[1], Philip Joris[1]

[1]*KU Leuven*

Category: Auditory Nerve
Background: The medial olivocochlear (MOC) system is a part of the auditory system that provides neural feedback to outer hair cells in the cochlea. Activation of medial olivocochlear efferent fibers modulates the

input of the afferent pathway, reducing neural responses. In previous work, we found that efferent effects in humans are low-frequency biased. In that work, we gauged efferent activity with the auditory nerve neurophonic - the temporal ongoing neural phase-locked synchronized potential – because the compound action potential (CAP) is poorly synchronized in humans at low frequencies. Here, we investigated efferent effects on the neurophonic in chinchilla, using electrical efferent activation.
Methods: MOC fibers were electrical stimulated along the midline of the floor of the fourth ventricle in the region of the internal genu of the facial nerve, where MOC fibers run close to the surface. Electrical pulses were delivered differentially by a galvanically separated current source through a stimulation probe consisting of two 330 μm spaced concentric stimulation electrodes. Bipolar current pulses (150 μs of both polarities, with a silent period of 50 μs in between) were delivered at an optimal rate of 300 Hz. To disentangle the neurophonic from the cochlear microphonic, a stimulus paradigm was used that consisted of 16 different combinations of tonal maskers, probes, opposite polarities, and electrical stimulation that was alternately turned on and off at each condition to avoid drift effects. At both sides, effects of the middle ear muscles were eliminated by cutting the tendon of the tensor tympany and removing the facial nerve branch innervating the stapedius muscle. The current amplitude of the electrical pulses was chosen to be below the activation threshold of other muscles.
Results: Efferent effects on CAPs were present in most chinchillas, but with large strength variations between individuals. In sensitive animals, CAP effects were present at all frequencies, but were systematically larger at lower frequencies and lower acoustic intensities. Effects on the neurophonic to tones in the phase-locking range were similar to those on CAPs, and consisted not only of a significant decrease in amplitude but often also a small phase delay. In addition, a concomitant increase in cochlear microphonic was present. The combination of these two effects is a hallmark of efferent activation in outer hair cells.
Conclusions: We conclude that the neural phase-locked potential, the neurophonic, is a valid alternative to the CAP for assessing the effects of activation of the MOC system at low frequencies, and that it provides opportunities for efferent research in humans.

Lateral Olivocochlear Efferent Inputs Contribute to Setting Auditory Nerve Fiber Spontaneous Firing Rates in Vitro

Philippe Vincent*[1], Elisabeth Glowatzki[1]

[1]*Johns Hopkins School of Medicine*

Category: Auditory Nerve
Background: The lateral olivocochlear (LOC) efferent fibers originate from the lateral superior olive in the brainstem. LOC fibers project towards the cochlea to make axo-dendritic synapses onto the unmyelinated endings of type I auditory nerve fibers (ANFs), close to where they contact the inner hair cells (IHCs) to form the first afferent synapse in the auditory pathway. LOC fibers are known to release multiple neurotransmitters, including acetylcholine, dopamine, GABA, and neuropeptides like Neuropeptide Y, Substance P and CGRP (Puel, 1995). In vivo intracochlear perfusion of acetylcholine (Felix and Ehrenberger, 1992) or dopamine (Ruel et al., 2001) during single unit recordings from ANFs in vivo have shown changes in ANF firing rates. The LOC efferent system is thought to operate as a feedback loop, dynamically changing ANF activity in response to sound exposure (Wu et al., 2020; Frank et al., 2023), and possibly protecting the IHC afferent synapse from noise damage (Maison et al., 2012). However, it is unclear how the LOC efferent system precisely regulates the IHC/ANF encoding properties, and which underlying mechanisms contribute to this regulation.
Methods: Channelrhodopsin was expressed in LOC fibers using the Cre-dependent choline acetyltransferase (ChAT) promoter to activate LOC fibers. ANF firing rates were recorded in acutely excised apical cochlear coils from 4-week-old C57BL/6J mice using extracellular loose patch recordings from their bouton endings (Wu et al. 2016), while stimulating LOC fibers optogenetically. At room temperature, ANFs with spontaneous rates (SRs) less than 10 spikes/s were defined as ‘low SR’ (typically located modiolar on the IHC), and with SRs greater than 10 spikes/s as ‘high SR’ (typically pillar).
Results: In response to light activation of LOC fibers, the firing rate of low SR fibers increased from a median value of 1.74 to 14.8 spikes/s, turning low SR into high SR fibers. This effect was blocked by the perfusion of scopolamine, a non-selective metabotropic acetylcholine receptor (mAChR) blocker. Similar recordings

performed from high SR fibers did not change their firing rate in response to LOC fiber activation. However, the perfusion of scopolamine during recordings from high SR fibers, in the absence of LOC stimulation, decreased high SR fiber firing rate from a median value of 29.64 to 5.82 spikes/s, suggesting that tonic release of ACh by the LOC fibers contacting high SR fibers is setting their firing rate in the in vitro experimental setting.
Conclusions: Here we demonstrate that cholinergic LOC efferent input can dramatically change ANF firing rates, turning low SR fibers into high SR fibers, when LOCs are activated, and turning high SR fibers into low SR fibers, when a mAChR blocker is used. These results make it likely that lateral olivocochlear cholinergic efferent inputs contribute to setting ANF SR rates in vivo.

The Link Between Auditory Nerve Dysfunction and Cochlear Macrophage Activation to Endocochlear Potential Declines

Cindy Wang[1], Jeffrey Rumschlag[1], Tyreek Jenkins[1], Kelly Harris[1], Hainan Lang*[1]

[1]*Medical University of South Carolina*

Category: Auditory Nerve
Background: Macrophages are present within the stria vascularis (SV) and auditory nerve (AN); cochlear inflammation (resulting from macrophage activation) is associated with hearing loss. Cochlear inflammation results in increased drug–induced hearing loss and endocochlear potential (EP) decline. The EP is generated by the SV, a stratified epithelium enriched with capillaries. The SV is a key element of the blood-labyrinth barrier, which may be regulated by macrophages. The role of macrophage activation in AN dysfunction is largely unknown. About 95% of AN axons and spiral ganglion neurons (SGNs) are myelinated. Myelin defects as a result of ototoxicity, noise exposure, or with aging, may contribute to AN dysfunction. Using a new mouse model of cochlear inflammation, we examine the link between macrophage activation, EP decline, and myelin defects, and determine the extent to which they disrupt AN function.
Methods: Lysophosphatidylcholine (LPC) was used as a myelin toxin for generating a model of demyelination. Increasing evidence supports that LPC also plays a role in tissue damage via dysregulation of macrophage activity. Application of LPC was conducted by (1) topical application to the round window niche (RW group), and (2) intracochlear perfusion in young adult CBA/CaJ mice. The effects of LPC treatment on AN function were examined by assessing ABR wave I suprathresholds and phase-locking values (PLV; also termed inter-trial phase coherence). EP was measured before the cochleas were collected for the examination of cochlear pathology.
Results: Both methods result in elevated ABR wave I thresholds 7 days post LPC application. The RW group had mild threshold shifts (~20 dB) while the intracochlear perfusion group showed moderate to severe hearing loss (~50 dB). AN functional deficits were present in both models, but more pronounced in the intracochlear perfusion group than in the RW group. A significant EP reduction (~56 mV) was identified in intracochlear perfusion mice. Increased macrophage activation of the SV and other strial pathology were observed in both groups. Intracochlear perfusion mice displayed much higher macrophage density than that of the RW group. A loss of strial intermediate and basal cells was seen in intracochlear perfusion mice while only enlarged extracellular space around intermediate cells was identified in the RW group. In the AN, disruption of myelin structures was seen in most SGNs of the intracochlear but not the RW group. Myelin abnormality was seen in the glia-glia transition zone in both groups.
Conclusions: Both LPC application methods result in macrophage activation in adult mice. Macrophage activation in the SV, contributes to strial degeneration and AN myelin defects, specifically in SGNs. This degeneration contributed to increased thresholds, EP reduction, and AN dysfunction. LPC intracochlear perfusion in mice provides a new animal model to study the role of cochlear inflammation and EP decline on AN dysfunction.

Investigating Single-Unit Auditory Nerve Fiber Responses in the Budgerigar

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Category: Auditory Nerve

Background: Budgerigars (a parakeet species) have been used extensively in previous behavioral auditory research because they can hear lower frequencies that humans rely on for speech comprehension. Furthermore, they demonstrate 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. However, neurophysiological investigations are limited, with no prior studies of auditory-nerve fiber (ANF) response properties in this species. Hence, the current experiment characterized basic ANF response properties, including frequency tuning curves and post-stimulus time histograms, in the budgerigar.

Methods: The auditory nerve was accessed through a craniotomy above the anterior, vertical semicircular canal. Then a part of the cerebellum was aspirated over the auditory nerve and brainstem. A glass electrode with 40 to 80 MΩ impedance was used to record single-fiber responses.

Results: ANF recordings were successfully made in the budgerigar. As hypothesized, tuning curves of the budgerigar were similar to other avians and mammals. Tuning curves of ANFs were V-shaped, with characteristic frequencies within the range of the budgerigar's behavioral sensitivity (i.e., up to 5-6 kHz). The most sensitive thresholds were consistent with behavioral audiometric thresholds, in the range of 10 to 30 dB SPL. Sharpness of tuning (Q10) was similar to that expected from psychophysical tuning curves from published behavioral studies. From our rate-level functions, we found ANFs to have an increasing firing rate as sound levels increase as in other animals, with a diversity of saturation profiles. In response to tones, these ANFs showed a strong onset response followed by adaptation towards a steady state rate and strong synchronization to fine structure.

Conclusions: Results demonstrate ANFs in the budgerigar have similar characteristics as other avian and mammalian ANFs. Future studies will characterize changes in ANF response properties in budgerigars with experimentally induced cochlear synaptopathy. New research in this species can provide important insight into the neural bases of normal and impaired hearing.

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