FULL ABSTRACT
PODIUM PRESENTATION

Title: **A C. elegans model to study Pnpt1, a gene that causes hereditary hearing loss in humans**

Poster #: 1

Authors: Yuling Guo*, Can Wang, Jianfeng Liu, and X.Z. Shawn Xu

Affiliation: Life Science Institute, University of Michigan

A missense mutation in polynucleotide phosphorylase (PNPase or PNPT1), which mediates mitochondrial RNA import, causes hereditary non-syndromic hearing loss with no obvious additional symptoms in human patients. Pnpt1 is widely expressed across tissues and plays a general role in maintaining the proper function of mitochondria. However, it remains unclear how dysfunction of a “non-specific” gene, such as pnpt1, causes a rather specific disease in the auditory system. Here, we intend to develop a C. elegans model to study the role of Pnpt1 in auditory sensation. We recently reported that despite the lack of ears, C. elegans senses airborne sound through the mechanosensory neurons FLP/PVD and engages in phonotaxis behavior. In a genetic screen to identify genes required for auditory sensation, we isolated a missense mutation in the pnpt-1 gene, the worm homolog of human Pnpt1. We demonstrate that pnpt-1 plays a critical role in mediating auditory responses in FLP/PVD neurons using both behavioral assays and in vivo calcium imaging. Further analysis shows that our isolated missense mutation in pnpt-1 disrupts mitochondrial homeostasis specifically in sound-sensitive FLP/PVD neurons but not other sensory neurons, suggesting that a high demand for energy in sound-sensitive neurons may contribute to the observed auditory defect in the mutant. Importantly, a similar phenomenon was observed when introducing the disease-causing mutation from human Pnpt1 into the worm pnpt-1 gene by CRISPR/Cas9-based genome editing, suggesting functional conservation from invertebrates to vertebrates. Taken together, our findings establish C. elegans as a model to study how Pnpt1 specifically regulates mitochondrial homeostasis in the auditory system.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **A new conclusion on nonlinear cochlear signal processing: It is linear!**

Poster #: 2

Authors: Jont Allen*

Affiliation: Department of Electrical and Computer Engineering, University of Illinois

I will review my view of previously published experimental neural data on cochlear function, including the tympanic membrane, middle ear, basilar and tectorial membranes, inner and outer hair cells and auditory nerve.
Conclusions: My analysis of neural tuning curve data from 1985, using nonlinear distortion product generation has revealed a new and remarkably clear understanding of cochlear function. The most important and surprising result is that the cochlea is linear in its filtering properties for "low-side" suppressors below 65 [dB-SPL].

The experimental result were published in 1985. However this new and highly unexpected conclusion comes as a total surprise. The ramifications of this observation are likely important, for example in accurately diagnosing cochlear hearing loss, and for fitting hearing aids and cochlear implants.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **Active cochlear models: modeling and results**

Poster #: 3

Authors: Vipin Agarwal*(1), Karl Grosh (2)

Affiliation: 1. Department of Mechanical Engineering; 2. Department of Biomedical Engineering, University of Michigan

The mammalian cochlea is responsible for converting incoming acoustic energy to neural signals. Over the last decade, researchers have continuously improved models to include the anatomy, comprehensive representations of the electromechanical processes, and mechanics within the cochlea. The goal of these models is to predict the response of the healthy cochlea to simple and complex sounds. Such a model may allow us to predict how the cochlea might fail, say in response to loud sound or age, and guide the development of protective approaches. A complete spatial representation of the differences in peripheral speech processing between a healthy and compromised cochlea might direct future cochlear implant or hearing aid stimulation paradigms. In addition, a predictive mathematical model will enable the development of new noninvasive tests to better interrogate the health of one's hearing and may enable better speech recognition software.

Efficient modeling of the cochlear response is extremely challenging, because the length scales, which vary from the sub-micron to centimeters, and time scales, which vary from microseconds to seconds, that must be resolved increase compute times and constrain the choice of algorithms. Furthermore, the fundamental physics of the cochlea involves propagation speeds ranging from 1500 m/s (the speed of sound in water) to 2 m/s (the speed of the traveling wave near its peak response location) as well as viscous boundary layers associated with the fluid dynamics likewise challenge even the most efficient and stable computational methods.

While modern-day computational scientific computing software for modeling structural dynamics is powerful, this software is not configured to perform these computations in an efficient manner nor do they possess specialized algorithms to model cochlear function. Such computationally efficiency is needed to test various hypotheses and study different experimental paradigms. In the current work, we address these computational challenges and develop methods for computing in an efficient manner that address key scientific questions in cochlear biophysics, thereby seeking to move the field forward from both the scientific and numerical modeling perspectives. We have developed a hybrid analytic numeric software code called "CSound". The coding structure itself is very modular, since it is based on standard finite element coding structures. In addition, CSound is written using MATLAB, which is readily available. We illustrate our computational algorithms considering taper of the cochlear scalae, and a careful study of the spatial response. Furthermore, we compare the numerical results with the experiments. Lastly, we will outline our early efforts to make CSound easier to use and directly accessible to the community by creating a GitHub-based opensource resource.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **Auditory deficits following concussive traumatic brain injury**

Poster #: 4

Authors: Meredith C. Ziliak*(1,2), Joseph M. Fernandez(3,4), Riyi Shi(3,4), Edward L. Bartlett(1,3)

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Background

Each year, over three million people suffer from traumatic brain injury (TBI). More than 80% of cases are classified as mild.1 Mild TBI typically lack acute symptoms, leading to undiagnosed cases. Without proper diagnosis and treatment, a mTBI can progress into chronic, permanent disruption in cognition including worsened memory, learning ability, coordination, and communication. Furthermore, mTBI has been linked to the development and progression of neurodegenerative diseases such as Alzheimer's Disease and Parkinson's Disease, which cause accelerated cognitive decline.2

Given the consequences of undiagnosed mTBI, sensitive and specific biomarkers are needed to facilitate diagnosis and monitoring of mTBI. Auditory dysfunction, particularly for central auditory processing (CAP), may serve as a biomarker for subcortical and cortical damage due to mTBI. Acute CAP damage has been identified following acute mTBI, such as blast exposure, implicating changes in CAP as diagnostic markers for mTBI.3 Unlike fMRI, CAP alterations can be observed using high-throughput, non-invasive, and cost-effective methods, further supporting the development of CAP as a diagnostic marker for mTBI.

This study aimed to identify the neurophysiological, neuroanatomical, and neuroinflammatory effects of impact-induced mTBI on CAP. We are testing the hypothesis that CAP measures will be altered by mTBI even when the mTBI does not occur in temporal cortex. By recording changes in auditory evoked potentials (AEP) such as auditory brain stem response (ABR), envelope following response (EFR), and middle latency response (MLR), we aim to parse temporal and spatial differences in audiological observations in the impacted brain. We also explored the correlation between AEP recording results and anatomical changes post impact.

Methods

We used male Sprague-Dawley rats (3-4 months, 350-450g) that met parameters for maturity. The rats were impacted to induce mTBI and their ABRs were recorded to observe changes, followed by immunohistochemistry of extracted brain samples.

For the impact, rats were anesthetized with ketamine and xylazine (80mg/kg, 10mg/kg, respectively) according to IUACUC specifications. The rat was secured at the base of an inclined weight-drop apparatus and its head supported, but not fixed, to allow post-impact free head motion. Each rat was impacted once using a 495g weight, producing a velocity of 4.643m/s and initial energy of 4.948J. Rats were then assessed for injury severity and recovered on a heating pad.

Two channel AEP of all animals were recorded at day 0 (baseline), 1 day, 4 days, 7 days, 14 days, and 30 days post-exposure.

Similar to previous studies, two recording channels were used to record the AEP, each emphasizing different neural generators. Channel 1 was placed along the nasal ridge midline and channel 2 along the bregma interaurally. Hearing threshold was estimated using ABR threshold of click and 8kHz stimuli. Sustained AEP responses were elicited by amplitude modulated sounds in quiet and noise as well as speech-like vowel contours to drive subcortical and cortical activity.

Results/Conclusions

Preliminary results suggest mTBI, even without auditory stimulation, includes chronic, long-term changes to the CAP system's functioning and anatomy. Thus, CAP changes may serve as a sensitive biomarker for streamlining treatments and enhancing patient outcomes.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **Auditory lipidomics, an approach to identify unique molecular effects of noise trauma**

Poster #: 5

Authors: Gunseli Wallace*(1,2,3), Lingchao Ji, MBBS, PhD (2), Costas Lyssiotis, PhD (5,6,7), Gabriel Corfas, PhD (1,2,4)

Affiliation: 1.Cellular and Molecular Biology Graduate Program; 2.Kresge Hearing Research Institute; 3.Medical Scientist Training Program; 4.Neuroscience Graduate Program; 5.Department of Molecular and Integrative Physiology; 6.Department of Internal Medicine, Division of Gastroenterology; 7.Rogel Cancer Center, University of Michigan

Noise induced hearing loss is a problem of epidemic proportions, but we do not yet fully understand how noise damages the ear. It is well-established that synapses between inner hair cells (IHCs) and auditory neurons are the most labile part of the cochlea, and that their loss in response to mild noise exposure leads to a pathology called Hidden Hearing Loss. This synaptopathy is apparent immediately after a 2-hour exposure, suggesting it is caused by changes in metabolism rather than gene expression. To understand how noise damages the cochlea, our group previously compared the inner ear metabolome of exposed and control mice by measuring aqueous metabolites. To expand upon the metabolic changes induced by noise, we are now exploring the lipidome and the implications of its dysregulation.

Hearing and deaf mice were noise-exposed (2 hr, 98-100 dB @8-16 kHz) to distinguish between metabolic changes induced by IHC mechano-transduction or just mechanical stimulation. Immediately following exposure, the otic capsules were removed and untargeted lipidomics was performed on the tissues. Four hundred ninety-one unique species were detected, which were organized into 51 lipid classes. Unsaturated (monounsaturated and polyunsaturated) free fatty acids (FFA) were the only lipid type significantly altered ($p<0.05$) by noise, and this occurred only in hearing animals. Furthermore, primarily long-chain FFAs were altered with noise. Long-chain FFA are metabolized for energy primarily through beta-oxidation in the mitochondria, where they are brought in by the rate limiting enzyme CPT1A. To investigate if the inner ear depends on long-chain FFA metabolism for energy, we inhibited CPT1A globally by treating 8-week-old mice with 35 mg/kg Etomoxir daily. Etomoxir treatment did not alter hearing as measured by acoustic brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE) on days 1, 3, 7 and 14 of treatment. On day 16 of treatment, mice were noise exposed (2 hr, 100 dB @8-16 kHz) and Etomoxir treatment was ceased. There was no difference in the recovery from noise between Etomoxir and saline treated mice as measured by ABR and DPOAE several weeks post-noise.

Though preliminary, to our knowledge this is the first lipidomic study following noise exposure. Furthermore, our results indicate that function of the inner ear does not seem to be dependent on long chain FFA beta-oxidation in the mitochondria. In future experiments we will validate the decrease in the lipids reported with a larger cohort, as well as examine the dependence of metabolite levels on noise intensity. Furthermore, as starvation causes increases in FFAs in the blood, we will investigate the role of nutrient deprivation on normal hearing and the noise response.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **Calcium and integrin-binding protein 2 (CIB2) is essential for fast adaptation of mechanotransduction current in mammalian auditory hair cells**

Poster #: 6

Authors: Isabel Aristizábal-Ramírez*(1), Arnaud P.J. Giese(2), Abigail K. Dragich(1), K. Sofia Zuluaga-Osorio(1), Saima Riazuddin2, Zubair M. Ahmed(2), Gregory I. Frolenkov(1)

Affiliation: 1.Department of Physiology, University of Kentucky; 2.Department of Otorhinolaryngology Head & Neck Surgery, University of Maryland

The mechano-electrical transduction (MET) in mammalian auditory hair cells is characterized by a two-phase decline of the MET current in response to a step-like hair bundle deflection. This phenomenon is known as fast and slow adaptation. While slow adaptation is generally attributed to the operation of myosin motors, exact mechanisms of the fast adaptation are not clear. Although recent data argue that fast adaptation in mammalian auditory hair cells is Ca2+-independent, number of previous reports demonstrated that there are active mechanical processes within the hair bundle that are driven by Ca2+ influx through the MET channels. Calcium and Integrin-Binding protein 2 (CIB2) interacts with presumable pore-forming subunits of the MET channels, TMC1/2 and is essential for mechanotransduction (Giese et al., 2017; Liang et al., 2021). Additionally, CIB2 binds to whirlin that is located at the tips of stereocilia and tethered to the actin core of stereocilia. Therefore, CIB2 could serve as a Ca2+-dependent mechanical link modulating the tension within the MET complex. Therefore, we recorded MET responses in the cochlear outer hair cells of mice carrying the p.R186W Cib2 variant. Although this variant causes deafness in humans and mice, it does not disrupt CIB2-TMC1/2 interaction. Auditory hair cells of Cib2R186W/R186W mice have decreased but measurable MET currents. We found that MET responses evoked by fast (10-30 us) deflections of hair bundles in Cib2R186W/R186W outer hair cells exhibited only slow component of adaptation but not the fast one. Since Ca2+-driven adaptation is likely to be proportional to the single-channel conductance of the MET channels, we estimated this conductance by non-stationary fluctuation analysis and found no differences in single MET channel conductance between Cib2R186W/R186W and Cib2+/R186W outer hair cells. We concluded that CIB2 is essential for fast adaptation in the cochlear outer hair cells. Interestingly, deflections of hair bundles with fluid-jet showed increased resting MET current in Cib2R186W/R186W hair cells, especially at positive intracellular potentials that prevent Ca2+ influx through the MET channels. The latter result confirmed that the mechanisms responsible for fast adaptation and resting tension in the MET machinery may be fundamentally different.

Supported by NIDCD/NIH (R01 DC012564 to Z.A. and G.F.)

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**CHD7, the causative gene for charge syndrome, represses the transcription factor LHX1 to promote inner ear development**
Poster #:	7

Authors: Jennifer M. Skidmore*, Anna M. Graf, Jelka Cimerman, Donna M. Martin

Affiliation: Departments of Pediatrics and Human Genetics, University of Michigan

Background: Loss of the chromatin remodeler CHD7 results in CHARGE syndrome, a multiple congenital anomaly disorder. Hallmark features of CHARGE syndrome include ocular coloboma, heart defects, atresia of the choanae, retardation of growth and development, genital abnormalities, and ear abnormalities including external ear malformations, deafness, and vestibular dysfunction. Mice that are heterozygous for Chd7 null alleles are an excellent model for CHARGE syndrome, as many of the same tissues are affected, including the ear. Chd7Gt/+ mice exhibit highly penetrant malformations of the lateral and posterior semicircular canals that can be detected as early as embryonic day 12.5. The mechanisms by which CHD7 promotes normal inner ear development are not well understood.

Objective: Our goal is to understand the unique contributions of CHD7 to inner ear development.
Design/Methods: Using a mouse model for CHARGE Syndrome (Chd7Gt/+), we utilized genetic crosses to identify specific contributions of Chd7 and Lhx1 to inner ear development. To identify targets of Chd7, we compared RNA-sequencing data from otocysts microdissected from Chd7+/+ and Chd7Gt/+ E10.5 embryos. We also performed immunohistochemistry and paint-fills on wild type and mutant embryonic inner ears.

Results: We identified 164 downregulated genes and 43 upregulated genes in E10.5 Chd7Gt/+ otocysts compared to wild type controls. The gene Lhx1, encoding a LIM homeodomain transcription factor, was upregulated 4-fold in Chd7gt/+ E10.5 otocysts. Immunostaining showed LHX1 in the caudal E10.5 wild type otocyst and ectopic LHX1 in the Chd7Gt/Gt ventromedial otocyst. We next examined inner ears from E14.5 Chd7Gt/+;Lhx1+/TLZ embryos by paint-fill and found that Chd7Gt/+;Lhx1+/TLZ ears exhibited lateral semicircular canal defects similar to those in Chd7Gt/+ mice.

Conclusions: Lhx1 is expressed in the caudal otocyst and is upregulated ventromedially with loss of Chd7. Despite this increased Lhx1 expression, loss of a single allele of Lhx1 is not sufficient to prevent the inner ear malformations in Chd7Gt/+ mice. Future experiments will examine whether two copy loss of Lhx1 in the inner ear is sufficient to rescue Chd7Gt/+ semicircular canal defects. These studies help establish molecular genetic regulatory networks critical for inner ear development in normal and disease states.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **Modulatory Effects of TNF-alpha on Blast-induced Hearing Loss and Tinnitus**

Poster #: 8

Authors: Hao Luo, Bin Liu, Edward Pace, Shaowen Bao, and Jinsheng Zhang

Affiliation: Wayne State University

Cochlear electrical stimulation (CES) is known to generate promising results in managing patients' tinnitus. However, the underlying mechanisms of CES-induced tinnitus suppression remains unclear. We set out to implant the rat cochlea via the round window or the modiolus. An operant behavioral paradigm of Conditioned Licking Suppression was used to test for tinnitus following noise exposure and CES. Electrophysiological recordings were conducted in the left auditory nerve (AN) and right auditory cortex (AC) simultaneously. In the acute experiments, we found that noise exposure (8-16 kHz, 110 dB SPL, 1 hour) resulted in a decreased spontaneous firing rate in the AN and an increased activity rate in the AC. In the chronic experiments, we found that after noise exposure to induce tinnitus, rats with behavioral evidence of tinnitus manifested with a decreased spontaneous firing rate in the AN and increased activity rate in the AC. We also found that CES yielded suppression of behavioral evidence of noise-induced tinnitus. The behavioral result was accompanied by the reversal of activity changes in the AN and AC. The results have demonstrated that noise-induced tinnitus may result from decreased spontaneous activity in the AN and increased spontaneous activity in the AC and that CES-induced tinnitus suppression may result from CES-induced reversal of the noise-induced decrease in the AN activity and the increase in AC activity rates.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **Comparing tinnitus questionnaire scores, self-reported loudness ratings, and psychoacoustic loudness measurements**

Poster #: 9

Authors: Travis Riffle*, Gerilyn Jones, David Martel, Kara Schwartz-Leyzac, Greg Basura, Emily Stucken, Jackie Souter, Susan Shore

Affiliation: Kresge Hearing Research Institute, University of Michigan

Background: Tinnitus, the phantom perception of sound, affects approximately 15% of the adult population. Tinnitus is commonly assessed using self-report questionnaires as well as pitch and loudness matching tasks. The questionnaires are designed to assess the degree of distress caused by tinnitus, and the psychoacoustic tasks provide a measurement of the tinnitus loudness. The purpose of this study is to investigate the relationship between tinnitus questionnaire responses and psychoacoustic measurements of tinnitus loudness.

Methods: 99 adults (aged 22-70) were recruited as part of a clinical trial investigating a novel tinnitus treatment. At their screening appointment, subjects completed the Tinnitus Functional Index questionnaire (TFI), and at their baseline appointment they completed Tinntester loudness matching software (Roberts et al, J. Assoc. Res. Otolaryngol., 2008). The TFI is a 25-question survey comprised of multiple subsections that provides an overall measure of tinnitus distress as well as individual subsection scores. Question #2 (Q2) in the TFI asks participants to rate how strong or loud their tinnitus was over the past week. Tinntester software was used to obtain loudness matching and minimum masking level (MML) measurements. In addition, the software included a visual analog scale (VAS) that required participants to rate tinnitus loudness on a scale from 0-100. For loudness measures, auditory stimuli comprised of pure tones and narrowband noise ranging from 500 Hz – 12 kHz were presented through headphones and participants were instructed to adjust the level of the sound until it matched the loudness of their tinnitus. Each frequency was measured twice and measurements were averaged to obtain an overall loudness value. For the MML, participants determined the level at which a 2kHz high-pass noise masked their tinnitus. Two runs were completed and averaged to obtain an MML value. Correlations were performed between all questionnaire scores, subjective ratings, and loudness measurements to evaluate the relationship between the measures.
Results: Both psychoacoustic loudness measures (Tinntester Loudness and MML) were significantly correlated with one another, and the questionnaire measures (TFI, TFI Q2, and Tinntester VAS) were all significantly correlated with each other. While there was a significant positive correlation between the Auditory subsection of the TFI and the MML, none of the other questionnaire measures correlated with either loudness measure.

Conclusions: The findings of this study showed that participants' subjective survey responses and VAS tinnitus scores were related to tinnitus distress. The psychoacoustic measurements were consistent with each other but were not correlated to the subjective responses. This finding suggests that overall tinnitus loudness measures are not systematically related to perceived tinnitus distress.

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**Deficits in vocalization -in-noise categorization after noise-induced temporary threshold shifts in guinea pigs**
Poster #:	10

Authors: Marianny Pernia, Manaswini Kar, Kayla Williams, Srivatsun Sadagopan

Affiliation: University of Pittsburgh

Exposure to moderate-to-intense sounds can produce temporary threshold shifts (TTS) in the audiogram. Such TTS is hypothesized to contribute to lasting speech perception deficits in noisy listening conditions but not in clean conditions. In normal hearing animals, cortical neurons show selectivity for specific sound features. Preliminary data from our lab suggest that A1 layer 2/3 neurons retain their selectivity for acoustic features across different listening conditions, and that independent cortical mechanisms may underlie selectivity for acoustic features and invariance to noisy conditions. Because TTS may be associated with speech perception deficits in noise and since high-threshold auditory nerve fibers required for encoding sounds at loud levels are affected in TTS, we hypothesized that at loud sound levels, only the invariance circuitry will be affected by TTS. To address this hypothesis, we are developing a guinea pig (GP) model to probe complex sound-in-noise categorization deficits. We induced TTS in two groups of GPs, using 2–8 kHz noise (noise-exposure group), or 4 – 8 kHz noise (categorization-control group) at 106 to 109 dB SPL for 2 hours. An elevated hearing threshold by about 10 dB SPL which recovered to baseline values, and a decreased wave I amplitude was observed in all animals using ABRs. We then estimated the thresholds for call categorization at varying noise levels at both low and high overall sound intensities before and after TTS using pupillometry. We measured the call-in-noise categorization performance of the noise-exposure group using calls containing spectral energy within the noise exposure band, and of the categorization-control group using calls containing spectral energy at frequencies lower than the noise exposure band. Consistent with the loss of high threshold auditory nerve fibers, we found that call-in-noise categorization was affected at only loud sound levels, and only for calls with frequency content within or above the noise range used for TTS induction. These data demonstrate that TTS and its sequelae can indeed result in lasting deficits in complex sound perception. In ongoing experiments, we are characterizing neural representations of calls before and after TTS in different laminae of A1 using local field potential and single-unit recordings combined with decoding models.

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**Dendritic non-linearities drive sensorimotor activity in auditory cortico-collicular neurons of behaving mice**
Poster #:	11

Authors: *Alexander N. Ford, Pierre F. Apostolides

Affiliation: Kresge Hearing Research Institute, University of Michigan

Thick tufted layer 5 pyramidal neurons send “corticofugal” projections to sub-cortical circuits, and are thus the major pathway via which the neocortex controls behavior. However, the cellular mechanisms driving layer 5 neuron activity in behaving animals are poorly understood. In recent years, several studies have suggested that dendritic Ca2+ spikes are important drivers of layer 5 activity in the awake state. These long lasting, regenerative potentials initiate in apical dendrites and trigger high-frequency action potential bursts at the soma. Given that the neocortex is generally assumed to transmit a rate code (London et al., 2010), dendritic Ca2+ spikes likely initiate a uniquely salient cortical efferent signal. However, little is known about the behavioral conditions that generate dendritic Ca2+ spikes, owing to the difficulty of quantifying dendritic activity using standard in vivo electrophysiology.
We address this knowledge gap using sub-cellular 2-photon imaging. To this end, we sparsely expressed the genetically encoded Ca2+ indicator GCaMP6f in auditory cortex layer 5 pyramidal neurons by injecting a retrograde AAV in the inferior colliculus, an important auditory midbrain hub and major recipient of descending corticofugal signals. We then imaged Ca2+ activity in layer 5 dendrites as head-fixed mice performed a discriminative auditory GO/NO-GO task to receive sugar water rewards. Prior in vitro data showed that Ca2+ signals in apical trunk dendrites are specifically due to dendritic Ca2+ spikes rather than back-propagating action potentials from the axosomatic compartment (Ranganathan et al., 2018; Beaulieu-Laroche et al., 2019).

We imaged Ca2+ signals in 1554 auditory cortico-collicular apical trunks across 78 sessions in 14 behaving mice. 56.4% (n=880) dendritic trunks showed task-related activity. Surprisingly, only 11.9% were significantly active during sound presentation (n=105) and most sound modulated dendrites (48%; n=429) were inhibited during the sound. Instead, the majority of Ca2+ spikes occurred following sound offset (43%; n=382) when mice reported their perceptual choice by licking a water spout. Trial-by-trial analyses of neural activity showed that dendritic spikes primarily reflected goal-directed motor activity, independent of reward-consumption.
Thus, dendritic spikes in auditory cortico-collicular neurons are triggered by the motor consequences of salient sounds. A recent study suggested that dendritic spikes in somatosensory cortical layer 5 neurons are necessary for behaviorally relevant sensory adaptation (Ranganathan et al., 2018). In addition, classic work (Bajo et al., 2010) showed that the auditory cortico-collicular neurons studied here are necessary for re-learning a sound localization task following monaural hearing loss. In tandem with our work, these results suggest that motor-related dendritic spikes of auditory cortico-collicular neurons may act as a ‘teaching signal’ for plasticity in hierarchically lower circuits, thereby promoting sensory learning. This hypothesis is tested in a related study presented at this event (Czarny et al.).

FULL ABSTRACT
PODIUM PRESENTATION

Title: **Development of LC-MS method for detection and quantification of gentamicin**

Poster #: 12

Authors: Shreshtha Dash, Peter S. Steyger

Affiliation: Creighton University

Purpose: Quantifying drugs in the inner ear is challenging due to its small size and relative inaccessibility. The ototoxic drug, gentamicin, is used to treat severe bacterial infections and vestibular disorders such as Meniere's disease. It consists of 4 major C-subtypes - C1, C1a, C2 and C2a. Simultaneous detection of these is difficult because gentamicin lacks UV-absorbing chromophores. Liquid chromatography coupled to mass spectrometry can quantify gentamicin due to its high sensitivity and separation of components in biological samples. We present a novel protocol for simultaneous detection of multiple gentamicin components.
Methods: We developed an UHPLC-MS method to detect and quantify gentamicin using a Q ExactiveTM hybrid Quadrupole-OrbitrapTM mass spectrometer with ESI interface (Thermo Scientific) coupled to a VanquishTM Flex Binary UHPLC System using an Acquity UPLC BEH C18 column (130 Å, 1.7 µm, 2.1 mm X 50 mm with guard column) and Xcalibur software. Chromatography separation was achieved using a gradient mobile phase of NFPA in water (A), and NFPA in acetonitrile (B) for a total runtime of 15 minutes at a flow rate of 0.3 ml/min with column maintained at 35°C. A range of gentamicin standards (0.1-50 µg/ml) were prepared, with amikacin (1 µg/ml) as an internal standard. MS/MS analysis was performed where gentamicin and amikacin were fragmented in ESI probe in positive ionization mode and collision energy was maintained at 10 ev.

Results: Gentamicin could be detected at 0.3 µg/ml, and the standard curve obtained after plotting concentrations of gentamicin vs ratios of area under UHPLC-MS chromatogram peaks specific to gentamicin and internal standard, amikacin was linear with $R2 > 0.99$. Fragment ions were seen for each major components of gentamicin and amikacin, with retention times of 2.85- 2.90 min or 2.12 min for gentamicin and amikacin, respectively. This method will be validated for its intra-interday precision and accuracy.
Conclusions: A novel UHPLC-MS method was developed to detect and quantify low levels of ototoxic gentamicin in standards and, ultimately, in inner ear tissues.

Acknowledgement: Funded by Office of Naval Research and a Bellucci Predoctoral Research Award.

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**Developmental expression of nicotinic acetylcholine receptors at a central auditory synapse**
Poster #:	13

Authors: Mackenna Wollet* and Jun Hee Kim, PhD

Affiliation: Department of Cellular and Integrative Physiology, UT Health San Antonio,

In the central auditory system, acetylcholine signaling modulates activity across brain regions from the cochlear nucleus to the auditory cortex. Within the auditory brainstem, recent studies suggest cholinergic inputs to the medial nucleus of the trapezoid body (MNTB) aid in signal-in-noise detection mediated in part by nicotinic receptors. Nicotinic acetylcholine receptors (nAChR) are essential for proper development of glutamatergic terminals in the cortex and hippocampus. nAChRs are highly expressed in the MNTB during early postnatal development demonstrated by RNA and protein quantification. However, the actions of nicotinic receptors on the development and activity of the calyx of Held synapse are unknown.

Using patch-clamp recordings in acute brainstem slices, we interrogated nicotinic receptor-mediated effects on the calyx synapse in mice during the second postnatal week. In the presence of atropine, spontaneous events were recorded in MNTB principal neurons before and after acute acetylcholine application (1mM, 1s) to evaluate effects of nicotinic receptor activation on presynaptic neurotransmitter release. nAChR-mediated inward currents and depolarization generated from acute acetylcholine application were also measured to evaluate postsynaptic nAChR activities. When examining presynaptic and postsynaptic effects of nicotinic receptor activation, contribution of the α7 nAChR was calculated with subtraction utilizing bath perfusion of methyllycaconitine citrate. Our data show the presence of nicotinic receptor activity on the presynaptic and postsynaptic membranes of the Calyx of Held synapse. nAChR-mediated postsynaptic currents significantly decline from postnatal day 8 to 12. When currents were normalized to cell capacitance, tonotopic location of MNTB neurons significantly contributed to the amplitude of nAChR-mediated currents. Our data suggest nicotinic acetylcholine receptors are present during the critical period of auditory brainstem development and may play a critical role in Calyx synapse maturation and activity.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **Does impedance reflect intrascalar tissue in the implanted cochlea?**

Poster #: 14

Authors: Deborah J. Colesa*, Katie L. Colesa, Yuki Low, Donald L. Swiderski, Yehoash Raphael, Bryan E. Pfingst

Affiliation: Kresge Hearing Research Institute, University of Michigan

Introduction

The development of intrascalar tissue (e.g., fibrous tissue and new bone) following cochlear implantation is a potential threat to the long-term preservation of both electrical and acoustical hearing in implanted patients. Monitoring this tissue non-invasively and developing ways to control it is desired. Impedance measures, which are a common non-invasive test of implant integrity, might enable monitoring of changes in the cochlear environment. Impedance, in a general sense, is a measure of how much a system resists the flow of electrical current; therefore, increases in impedance might reflect growth of intrascalar tissue. Here, we monitored impedances over time and assessed their relationship to intrascalar tissue, evaluated histologically at endpoints after long-term cochlear implantation.

Methods

Forty-six adult male guinea pigs were chronically implanted with a banded cochlear implant in either a hearing ear, an ear treated with neomycin and inoculated with a neurotrophin, or an ear treated with only neomycin. Bipolar and monopolar impedances for a 1 kHz, 1 µA rms sinusoid were measured several times a week for 4 to 21 months. Then, histology (mid-modiolar sections) was performed near the primary measurement electrode. The intrascalar tissue at this location was evaluated in two ways: 1. Tissue between the implant footprint (if one was present) and the spiral ganglion neurons was ranked as: low, medium, or high following the classification procedure reported by Swiderski et al., JARO, 2020, and 2. Tissue located in the entire scala tympani area of this location was quantified in terms of percent filled with bone, fibrous tissue, and both combined. Impedance trends over time were assessed, and tissue rankings or percentages, were compared to final impedance levels (average of the final 10 measures).

Results

Histology revealed a wide range of intrascalar tissue types, distributions and amounts in both assessed scala tympani regions. Individual impedance trends over time were variable, and not specific to an amount or type of intrascalar tissue. Bipolar and Monopolar impedances obtained near the time of histological assessment were highly correlated with each other although bipolar impedance was greater than the monopolar impedance in ears with higher intrascalar tissue. High impedances were significantly correlated with larger amounts of new-bone (bipolar $r2 = 0.53$, $p<0.0001$; monopolar $r2 = 0.63$, $p<0.0001$) but not with amounts of fibrous tissue or combined fibrous tissue and bone in the scala tympani. However, only about 50% of the variance in impedance was accounted for by the amount of bone formation.

Conclusions

While impedances were higher on average in ears with the most intrascalar tissue and new bone, the data did not show consistent relationship between impedance and intrascalar tissue in all cases. The presence of bone in the scala tympani had the greatest impact on the impedance but still only explained about 50% of the variance. Impedance as a measure of the intra-cochlear environment seems to be complex and is possibly dependent on multiple factors, including the electrode-tissue interface, that require further analysis. Measures of individual components of complex impedance might be helpful in assessing this relationship.

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FULL ABSTRACT
PODIUM PRESENTATION

Title: **DPOAE probe placement variability & reliability in anesthetized mice**

Poster #: 15

Authors: Tamara Iccaoui*, Mark Chertoff, Lani Martin

Affiliation: University of Kansas Medical Center

When using DPOAEs for monitoring hearing status over time, it's important to have a reliable and repeatable technique. DPOAE responses rely greatly on the signal-to-noise (SNR) ratio. Therefore environmental noise or even slight movement could impact the SNR ratio and lead to a reduced or absent response. This environmental noise could be significantly reduced or enlarged based on the probe placement. Aim: This study aims to measure the reliability of probe re-insertion on the DPOAE response on anesthetized mice. Methods: DPOAEs were performed on 4 anaesthetized Bhlhe22 Cre-ERT2 mice in response to a primary tone frequency of 8,16, and 24 kHz. Data was collected twice each session weekly, for 5-6 weeks at 80 to 30 dB SPL. To determine the variability of the responses within-session, the standard deviations (SD) of DPOAE amplitudes from all 4 mice over the 5-6 week period were averaged at each frequency for all intensity levels. An Intraclass Correlation Coefficients (ICC) analysis was also done on R studio to determine the reliability of the response across all session. Results: The average SD within session across all intensity levels was 4.759 dB SPL at 8 kHz, 5.859 dB SPL at 16 kHz, and 4.942 at 24 kHz. The ICC analysis across sessions showed a mean ICC of 0.902 at 8 kHz, 0.845 at 16 kHz, and 0.886 at 24 kHz. Conclusion: These values indicate an excellent reliability and variability within and across sessions. The expected variance from probe placement is around 4.19 dB SPL.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **Effects of cochlear synaptopathy on tone-in-noise coding in the cochlear nucleus**

Poster #: 16

Authors: A. Hockley*, L.R. Cassinotti, M. Selesko, G. Corfas, S.E. Shore

Affiliation: Kresge Hearing Research Institute, University of Michigan

Hearing impairment in the absence of threshold shifts is characterized by permanent damage to synapses between inner hair cells and high-threshold auditory nerve fibers (ANFs). Cochlear synaptopathy is a potential major health issue in humans, as many listeners with clinically normal audiograms still have difficulty hearing in noisy settings and aged human temporal bones demonstrate widespread synapse loss despite no explicit otopathology. Previous behavioral studies have shown no effect of synaptopathy on tone-in-noise detection thresholds, but this has not been confirmed by neural recordings, nor has the effect of synaptopathy been shown on suprathreshold neural responses in noise. Here, we examine the effect of synaptopathy on tone-in-noise coding on the direct recipients of ANFs – cells in the cochlear nucleus.

Guinea pigs received a unilateral sound overexposure to the left ears (7 kHz centered, third-octave noise at 102 dB SPL for 2 h), producing unilateral temporary threshold shifts. At 4 weeks post-exposure, loss of auditory nerve synapses and reduced ABR wave 1 amplitudes were observed specifically on the left side. A separate group of animals received sham noise exposures. Single unit responses were recorded from several cell types in the cochlear nucleus. Puretone stimuli (2-24kHz; 0-90 dB SPL) were used to generate receptive fields and rate-level functions in the presence of either 0, 40 or 60 dB SPL continuous broadband noise.

The synaptopathy-inducing noise exposure did not affect mean single-unit tone-in-noise threshold , nor the lowest tone-in-noise thresholds in each animal, demonstrating equivalent tone-in-noise detection thresholds compared to sham animals. However, synaptopathy reduced neural responses in response to suprathreshold tones in the presence of background noise.

These results demonstrate that despite not altering tone-in-noise thresholds, synaptopathy results in reduced activity in the cochlear nucleus in response to suprathreshold tones in the presence of background noise. Overall, these data have implications for hearing in the presence of background noise and offer a new method to objectively test for synaptopathy.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **Effects of working memory on frequency modulation detection threshold**

Poster #: 17

Authors: Benjamin C. Kohlmeier*, Adam K. Bosen, Sara E. Harris, Stephen T. Neely, and Aryn M. Kamerer

Affiliation: Boys Town National Research Hospital

Temporal processing allows us to discriminate between features of sound, and is therefore an essential component of speech perception. Individual temporal processing ability is commonly assessed using measures of temporal resolution, often through behavioral detection or discrimination tasks. Though studies employing temporal processing tasks often seek to assess auditory physiology, cognitive abilities may influence performance on these tasks. For example, an alternative forced choice task may require creation of a target stimulus template which must then be maintained, retrieved, and compared to each interval, processes of working memory. If this true, then increases in task difficulty, such as with more alternate choices or near threshold, will increase the demand on working memory.

To examine if there may be a relationship between temporal processing and working memory, we predict individual performance on a frequency-modulation detection task using serial recall of auditory digits and free recall of visual words as measures of working memory. Furthermore, we compare frequency-modulation detection threshold and influence of working memory in a three-interval versus one-interval forced choice task. We expect to see a meaningful range of performance on frequency-modulation detection and working memory tasks in participants ages 19-40 with hearing thresholds □ 25 dB HL, while minimizing confounding effects of age-related cognitive and auditory changes or hearing loss. We hypothesize that there will be a relationship between individual working memory capacity and frequency-modulation detection threshold, and that the number of alternative choices given in the task may influence how much working memory predicts frequency-modulation detection threshold.

Observing effects, or lack thereof, of working memory capacity on frequency-modulation detection will provide evidence for or against the consideration of cognitive effects on testing methods for temporal processing.

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**Electrophysiological and morphological properties of inhibitory and excitatory principal neurons of mouse lateral superior olive**
Poster #:	18

Authors: Hariprakash Haragopal*, Bradley D. Winters

Affiliation: Northeast Ohio Medical University, Hearing Research Group, Department of Anatomy and Neurobiology

The lateral superior olive (LSO) nucleus in the brainstem is critical for horizontal sound localization. LSO principal neurons (PNs) compare excitatory inputs driven by the ipsilateral ear with inhibitory inputs driven by the contralateral ear. The textbook version of LSO function is encoding ongoing interaural level differences. However, recent findings and deductions based on the extreme timing fidelity provided by the calyx of Held suggest that a major function of the LSO lies in extraction of interaural timing differences particularly for transient broadband sounds. These functional roles place disparate demands on the cellular properties of LSO neurons. There is also cellular diversity in the LSO that is not well understood that may underlie specialization for certain roles. Here we examine inhibitory and excitatory LSO PNs in mouse brain slices using whole-cell patch-clamp and two-photon imaging. We find that they differ in electrophysiological and morphological properties that would impact integrative processes involved in sound localization.

To target specific cell types, we used knock-in reporter mice that co-express the fluorescent protein tdTomato with vesicular glutamate transporter 2 (vGlut2). PNs were selected based on size and electrophysiology. Glutamatergic PNs had prominent somatic fluorescence while other PNs exhibited a distinct lack of fluorescence in their soma, rendering putative inhibitory cells “black holes.” Immunostaining for glycine and in situ hybridization suggest no overlap between glutamatergic and glycinergic LSO neurons.

Compared to excitatory PNs (n=48), inhibitory PNs (n=41) had 5mV lower resting membrane potential (I:-69.81, E:-64.91mV) and 47% higher input resistance (I:56.55, E:30.24MΩ). Inhibitory PNs also had correspondingly slower membrane time constants and lower rheobase (I:325.49, E:490.21pA). These findings suggest that despite their more hyperpolarized resting membrane potential, inhibitory LSO PNs are more excitable. Threshold was not different between the groups, however, inhibitory PNs had lower AP peaks (I:-3.88, E:7.24mV) suggesting differences in voltage gated sodium channels. AP half-width was wider in inhibitory PNs (I:0.216, E:181ms). Excitatory PNs exhibited larger sag potentials in response to hyperpolarizing current injections (I:12.83, E:17.57mV) suggesting higher HCN channel density.

In both LSO PN types, we observed two action potential firing modes. Onset-burst responses were more common and had 3 to 5 spikes at higher current injection levels. Multi-spiking neurons capable of sustained firing rates up to 550Hz were found throughout the body of the LSO. Neither group exhibited tonotopic bias. Multi-spiking was less common in inhibitory neurons (I:27%, E:50%).

Inhibitory and excitatory PNs had similar maximum dendritic extension, soma volume, and primary dendrite diameter, however, excitatory PNs had larger total dendritic length (I:426.90, E:603.70µm), number of dendritic branch points (I:2.41, E:3.83), and number of primary dendrites (I:3.52, E:4.41). These data suggest excitatory PNs have more complicated dendritic arbors which may favor more integrative sound localization functions.

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**Excitatory commissural synapses on VIP neurons in the inferior colliculus activate NR2D-containing NMDA receptors at resting membrane potential**
Poster #:	19

Authors: Audrey Drotos*, Michael Roberts

Affiliation: Kresge Hearing Research Institute, University of Michigan

The inferior colliculus (IC) is a hub of integration for ascending auditory information and plays an important role in sound processing. VIP neurons are glutamatergic stellate neurons found throughout the IC, and they receive both excitatory and inhibitory inputs from the contralateral IC through the commissure. Prior research has shown that excitatory commissural inputs to VIP neurons can elicit EPSPs that include an NMDA receptor (NMDAR) component at resting membrane potential, even though most NMDARs require depolarization to relieve Mg2+ block before they can be activated. We therefore hypothesized that VIP neurons express NMDARs containing NR2C/D or NR3A subunits, which are less susceptible to Mg2+ block than the more common NR2A/B-containing receptors. To test this hypothesis, we targeted whole-cell patch-clamp recordings to VIP neurons in VIP-IRES-Cre x Ai14 mice and used electric and optogenetic stimulation to activate excitatory commissural inputs. The NMDAR-mediated component of EPSPs was isolated using the AMPA receptor antagonist NBQX. We then applied PPDA, an NR2C/D subunit selective antagonist, followed by CIQ, an NR2C/D selective positive allosteric modulator, to determine whether the NMDAR component was mediated by receptors containing these subunits. Using these methods, we found that commissurally evoked EPSPs in VIP neurons were often only partially blocked by the AMPA receptor antagonist NBQX, even though VIP neurons were held near their resting membrane potential. The remaining EPSP component was partly sensitive to PPDA and CIQ, suggesting that VIP neurons express NMDARs containing NR2C/D subunits. PPDA and CIQ also alter current-voltage relationships in VIP neurons, specifically at hyperpolarized potentials where Mg2+ block is prevalent, suggesting that these neurons express NR2C/D subunit-containing receptors. Additionally, we performed single molecule fluorescent in situ hybridization (RNAscope) to determine whether NR2C or NR2D subunit mRNA is present in VIP neurons. Our RNAscope experiments show that 92% of VIP neurons express NR2D mRNA while only 8% express NR2C mRNA. Our results demonstrate that VIP neurons express NMDARs that contain NR2D subunits, which allows these receptors to activate at resting membrane potential. This mechanism could expand the time window for synaptic integration in VIP neurons given the longer temporal dynamics of NMDA receptors, suggesting that VIP neurons may play a computational role that involves integrating auditory information over periods of tens of milliseconds.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **Head orientation and head stability in rats is altered after exposure to intense noise or intratympanic injection of sodium arsanilate**

Poster #: 20

Authors: Mamiko Niwa*, Marie Anderson, Hannah N. Beck, David Bauer, W. Michael King

Affiliation: Kresge Hearing Research Institute, University of Michigan

Exposure to intense noise is known to induce damages in the cochlea, leading to temporary or permanent hearing loss. Recently, exposure to 6-hour, 120dB noise has been shown to reduce the vestibular short-latency evoked potential (VsEP), suggesting that noise exposure also affects vestibular parts of the inner ear. The attenuated VsEP responses are also associated with reduced calretinin staining in the calyx-only afferents of saccule (Stewart et al. 2020). Here, we study if intense noise exposure alters vestibular behavior, specifically animals' head stability and head orientation with respect to gravity.

In our study, a motion sensor (Yost labs, 3-space LX Embedded) was attached to a rat's head to monitor head angular velocity and linear acceleration. The animal was secured in a whole-body restraint device that allowed it to move its head freely. The animal was placed on a servo-controlled, horizontal turntable, and subjected to abrupt whole-body rotations about an earth vertical axis. We recorded animals' head movements before and after a 4-hour, 120 dB noise exposure. Additionally, we recorded head movements in three other animals that were treated by intra-tympanic injection of sodium arsanilate, which is known to damage hair cells and synapses in the vestibular end organs. These animals are considered as a positive control of vestibular damage in the study. Here, we focus our report on head orientation and stability during the inter-trial-intervals of the behavioral experiments. We report preliminary results from 8 rats (5 males and 3 females) exposed to intense noise and 3 male rats exposed to sodium arsanilate.

All 3 animals treated with sodium arsanilate showed severe vestibular deficits. The distribution of resting head positions with respect to gravity showed a significant broadening, suggesting the animals' sense of gravity was severely affected by the lesion. The stability of resting head position was also significantly reduced, suggesting that these animals had severe deficits in their ability to control head position. Animals exposed to noise displayed more subtle, but significant changes in head orientation and stability. Five out of 8 animals showed significant increases in the variability of resting head position in either pitch or roll. In addition, 7 out of 8 animals showed significantly reduced pitch angles (chins closer to chest) in their resting head positions after noise exposure. This suggests their sense of gravity was altered by noise exposure, although the effect size was smaller compared to the animals exposed to arsanilate. Interestingly, some changes seen in the noise-treated animals were opposite in direction to the changes seen with the arsanilate-treated animals. For example, some noise-treated animals showed a small, but significant increase in head stability as opposed to the decreased head stability seen with arsanilate exposure. Further analyses will be needed to provide possible explanations behind this finding.

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**Hearing recovery induced by DNA demethylation in a chemically deafened adult mouse mode**
Poster #:	21
Authors:	Xin Deng*(1) and Zhengqing Hu(1,2)
Affiliation:	1.Department of Otolaryngology-HNS, Wayne State University School of Medicine; 2.John D Dingell VA Medical Center

Functional hair cell regeneration in the adult mammalian inner ear remains challenging. This study aimed to investigate the function of new hair cells induced by a DNA demethylating agent 5-azacytidine. Adult mice were deafened chemically, followed by injection of 5-azacytidine or vehicle into the inner ear. Functionality of regenerated hair cells was evaluated by expression of hair cell proteins, auditory brainstem response (ABR) and distortion-product otoacoustic emission (DPOAE) tests for 6 weeks. In the vehicle-treated group, no cells expressed the hair cell-specific protein Myosin VIIa in the cochlea, whereas numerous Myosin VIIa-expressing cells were found in the 5-azacitidine-treated cochlea, suggesting the regeneration of auditory hair cells. Moreover, regenerated hair cells were co-labeled with functional proteins Espin and Prestin. Expression of ribbon synapse proteins suggested synapse formation between new hair cells and neurons. In hearing tests, progressive improvements in ABR (5-30 dB SPL) and DPOAE (5-20 dB) thresholds were observed in 5-azacytidine-treated mice. In vehicle-treated mice, there were <5 dB threshold changes in hearing tests. This study demonstrated the ability of 5-azacytidine to promote the functional regeneration of auditory hair cells in a mature mouse model via DNA demethylation, which may provide insights into hearing regeneration using an epigenetic approach.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **Hyperacusis correlates in noise-overexposed guinea pigs**

Poster #: 22

Authors: David T. Martel* and Susan E. Shore

Affiliation: Kresge Hearing Research Institute; Biomedical Engineering; Molecular and Integrative Physiology, University of Michigan

Background: Hyperacusis is characterized by steepened loudness growth, collapsed sound intensity tolerance, and faster reaction times to sounds (for review, see Tyler et al, Am. J. Audiol. 2014). Tinnitus, phantom sound perception, is frequently co-morbid with hyperacusis, as both commonly arise following cochlear damage. Following noise-overexposure, ventral cochlear nucleus (VCN) bushy cells exhibit firing patterns consistent with psychophysical characteristics of hyperacusis (Martel and Shore, Sci. Rep., 2020). Since bushy cells contribute to the auditory brainstem response (ABR), hyperacusis-related increases in bushy-cell excitability may underlie increased ABR wave amplitudes. Furthermore, increased ABR wave amplitudes correlated with hyperacusis and tinnitus behavioral measures in mice following ototoxicity (Longnecker et al, Front. Neurosci, 2020). Here, we further elucidate the bushy-cells role in hyperacusis and tinnitus in guinea pigs, using a novel hyperacusis behavioral test.

Methods: Acoustic startle (pinna) reflexes (ASR) were measured to assess hyperacusis and tinnitus. Hyperacusis was assessed by measuring changes in startle amplitude and reaction-time latency post-noise-overexposure (Chen et al, JARO, 2013). Broadband noise pulses (BBN; 2ms duration), clicks (100us/phase) and upsweep chirps (100Hz-30kHz; 2.1ms duration) were presented over a range of intensities (60-100 dB SPL; 10 dB steps; 15-20s intertrial interval). One testing session consisted of five repetitions of each unique intensity-sound combination.

Tinnitus was assessed using gap/prepulse-inhibition of the ASR (Berger et al, J. Neurosci. Methods, 2013). For each session, background carrier bands (3-6,8-16kHz) were presented at 65 dB, while the startle reflex was activated using a BBN pulse (2-20kHz; 20ms; 0.1ms rise/fall). Prepulse stimuli were a gap or sound-pulse inserted into the background carrier (50ms; 5ms rise/fall; 50ms delay re startle). Twenty trials were presented for each carrier band.

ABRs were measured using tone-pips (8-20 kHz, 4kHz-steps; 5ms; 0.5ms rise/fall), clicks (100us/phase) and upsweep exponential chirps (100Hz-30kHz; 2.1ms duration). ABRs were measured at baseline to establish normal hearing (N=7). Four weeks of baseline behavioral data were collected. Guinea pigs were anesthetized (ketamine/xylazine) then exposed to unilateral narrowband noise (103 dB SPL; 7kHz centered, quarter octave band) in a temporary-threshold shift paradigm. ABRs (every two weeks) and behavioral assessments (biweekly) continued for an additional twelve weeks.

Results: We found that a single noise-overexposure can induce both tinnitus and hyperacusis (N=3/7; hyperacusis-alone: N=2/7). Preliminary results in hyperacusis animals indicate that ABR amplitude-intensity functions are steeper-and-with greater amplitudes compared to animals with neither hyperacusis nor tinnitus.

Conclusions: Consistent with other studies, we found that hyperacusis and tinnitus are co-morbid following auditory damage. Moreover, ABR enhancements at suprathreshold intensities occurred in hyperacusis animals, suggesting that VCN bushy-cell firing patterns and hyperacusis behavior are linked. Future studies will investigate precisely how VCN bushy-cell firing patterns contribute to hyperacusis-related ABR enhancements.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **Hypermyelination of cranial nerve VIII in a mouse model of CHARGE syndrome**

Poster #: 23

Authors: K. Elaine Ritter*(1), Sloane M. Lynch(2), Ashley M. Gorris(2), Lisa A. Beyer(3), Lisa L. Kabara(3), Yehoash Raphael(3), Donna M. Martin(1,4)

Affiliation: 1.Department of Pediatrics; 2.College of Literature, Science and Art; 3.Department of Otolaryngology, Kresge Hearing Research Institute; 4.Department of Human Genetics, University of Michigan

Background & Objectives: CHARGE syndrome is a multiple anomaly developmental disorder characterized by a variety of sensory deficits, including sensorineural hearing loss. The majority of cases of CHARGE are caused by pathogenic variants in Chromodomain DNA-binding Protein 7 (CHD7), a chromatin remodeler important for the development of neurons and glial cells. The structural substrate for hearing loss is not well understood. Using the Chd7Gt/+ mouse model of CHARGE syndrome, we sought to determine how Chd7 haploinsufficiency affects mature neurons, myelinating Schwann cells, and inner hair cell innervation in the cochlea.
Methods: Auditory Brainstem Responses (ABRs) were recorded in adult Chd7+/+ and Chd7Gt/+ animals. Cochleae were sub-dissected and processed for transmission electron microscopy (TEM). TEM images were captured in the apical and basal regions of the spiral ganglion. Myelin thickness and axon diameter were measured using ImageJ. Semi-thin sections of spiral ganglia were imaged for further histological analysis, cell counting, and nerve density quantification. Immunohistochemical staining for hair cell synaptic markers CtBP2 and GluA2 was conducted on 4-week old wild-type and Chd7Gt/+ cochleae, which were imaged by confocal microscopy.

Results: Analysis of ABR recordings in Chd7Gt/+ adult animals show elevated ABR thresholds at 4 kHz and 16 kHz, but not at 32 kHz. ABR Wave I peak latency and amplitude in Chd7Gt/+ mice are not significantly different from wild-type controls. Proportions of neurons and glial cells in the spiral ganglion are not significantly different, nor are densities of nerve projections from the spiral ganglion to the organ of Corti. Staining for CtBP2 and GluA2 showed no differences in hair cell synapse formation in Chd7Gt/+ mutant cochleae. However, G-ratio analysis of myelin thickness in peripheral spiral ganglion Type I neuronal projections indicates subtle but statistically significant hypermyelination in Chd7Gt/+ mice.

Conclusions: Collectively these results suggest that in the mouse, the inner ear is largely resilient to haploinsufficiency of CHD7, with the exception of myelin sheaths produced by Schwann cells encasing the peripheral aspect of the auditory nerve. Previous studies in Chd7Gt/+ embryos showed substantial neuronal loss in the developing spiral ganglion, and here we found that the inner ear is able to compensate for Chd7 loss by adulthood. There may be other peripheral or central auditory components contributing to sensorineural hearing loss in Chd7Gt/+ mice, and middle ear defects may be the primary source of increased ABR thresholds in this mouse model of CHARGE syndrome. Our work was supported by NIH Grants R01 DC014456 (DM, YR), R01 DC018404 (DM) and T32 DC000011 (KER).

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**Hypomyelination reduces parvalbumin-expressing interneuron density and auditory cortex inhibitory function**
Poster #:	24

Authors: Beatriz de Carvalho Borges*(1), Xiangying Meng(2, 3), Patrick Long(1), Patrick Oliver Kanold(2, 3), Gabriel Corfas(1)

Affiliation: 1.Kresge Hearing Research Institute, University of Michigan; 2. Department of Biomedical Engineering, Johns Hopkins University; 3. Department of Biology, University of Maryland

For a long time, myelin was thought to be restricted to excitatory neurons, and studies on dysmyelination focused primarily on excitatory cells. Recent evidence showed that axons of inhibitory neurons in the neocortex are also myelinated, but the role of myelin on inhibitory circuits remains unknown. It has been demonstrated that the trophic factor Neuregulin 1 (NRG1) plays an important role in myelination. NRG1 is expressed by neurons and activates transmembrane tyrosine kinase ErbB receptors (ErbBRs) in oligodendrocytes, leading to myelin gene expression and increased myelin thickness. Here we studied the impact of mild hypomyelination on both excitatory and inhibitory connectivity in the primary auditory cortex (A1) with well-characterized mouse models of hypomyelination due to loss of oligodendrocyte NRG1/ErbB receptor signaling. We used CNP-DN-ErbB4 mice, a transgenic line that expresses a dominant-negative ErbB4 receptor in myelinating cells. Any cell expressing DN-ErbB4 receptors becomes unresponsive to NRG1. CNP-DN-ErbB4 mice presented reduced MBP mRNA and protein expression, and reduced MBP+ axonal area in A1, evidencing a mild central hypomyelination. Using laser-scanning photostimulation, we found that CNP-DN-ErbB4 mice have reduced functional inhibitory connections to A1 L2/3 neurons without changes in excitatory connections, resulting in altered excitatory/inhibitory balance. Molecular analysis of multiple synaptic markers demonstrated that these effects are not associated with altered expression of GABAergic and glutamatergic synaptic components. No differences in mRNA expression of markers of inhibitory interneurons somatostatin and VIP were found. Remarkably, the parvalbumin (PV) mRNA expression and the density of PV+ neurons was reduced in the A1 of CNP-DN-ErbB4. While immunostaining shows that hypomyelination occurs in both PV+ and PV- axons, there is a strong correlation between MBP and PV expression suggesting that myelination influences PV expression. Corroborating these findings, we used another mouse model to promote loss of oligodendrocyte ErbB signaling, the PLP1-creERT: ErbB3 flox mice, and found mild hypomyelination and reduced PV expression and PV+ neuronal density in A1. These results show that subtle defects in myelination can lead to large changes in gene expression and function of PV interneurons, which result in large-scale changes in network function in the neocortex. Together, the results demonstrate that mild hypomyelination impacts A1 neuronal networks, reducing inhibitory activity, and shifting networks towards excitation.

FULL ABSTRACT
PODIUM PRESENTATION

MIDWEST AUDITORY RESEARCH CONFERENCE
presented by the Kresge Hearing Research Institute

Title:	**Inducing tinnitus in guinea pigs through long-term potentiation of fusiform cells in the dorsal cochlear nucleus**
Poster #:	25

Authors: Michael Selesko, Calvin Wu, Adam Hockley, David Martel, Susan E. Shore

Affiliation: Kresge Hearing Research Institute, University of Michigan

Introduction: The principal output neurons of the dorsal cochlear nucleus, fusiform cells, integrate somatosensory input on their apical dendrites and auditory input on their basal dendrites. The synapses at the apical dendrites exhibit stimulus-timing-dependent plasticity (STDP), which can be elicited through paired somatosensory-sound stimulation. Long-term potentiation (LTP) or long-term depression (LTD) of the synapses is produced depending on the order and timing of the paired stimuli (Koehler and Shore, J Neurosci 2013). Fusiform cells in noise-exposed guinea pigs with behavioral evidence of tinnitus show increases in spontaneous firing rate and synchrony. Using a paired somatosensory-sound stimuli to induce LTD in guinea pigs with tinnitus leads to a reduction the behavioral and physiological signs of tinnitus (Marks et al., Sci Transl Med 2018). The aim of the present study was to test whether inducing LTP in unexposed guinea pigs would induce tinnitus.

Methods: Normal-hearing guinea pigs were divided into four treatment groups: LTP-inducing bimodal stimulation, unimodal somatosensory stimulation (which also induces LTP), unimodal auditory stimulation, or a sham group, in which no treatment was delivered. For the somatosensory stimulation, transcutaneous electrodes on the surface of the neck were used to stimulate the dorsal column pathway. Calibrated earphones were used for the sound stimulation in the left ear. Treatments were applied with animals under sedation for 40 min/day, 5 days a week for 4 weeks. Sham animals were sedated but did not received either stimulus. Following treatments, animals were anesthetized and single-unit recordings from fusiform cells were performed.

Results: More than 50% of guinea pigs that received the LTP-inducing bimodal and somatosensory stimulation, which is also expected to induce LTP, showed behavioral evidence of tinnitus. Fusiform cells from animals that developed tinnitus showed BF-restricted increases in spontaneous firing rates and bursting, physiological correlates of tinnitus.

Conclusions: These results show that tinnitus can be induced through LTP induction of DCN fusiform cells, even in the absence of noise exposure and cochlear damage.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **Innovative approaches to studying and diagnosing endolymphatic hydrops**

Poster #: 26

Authors: Jeffery T. Lichtenhan, John J. Guinan Jr., Victoria A. Sanchez, and Shawn S. Goodman

Affiliation: University of South Florida, Eaton-Peabody Laboratories, University of South Florida, University of Iowa

Ménière's disease symptoms are some of the most debilitating of inner-ear disorders and include vertigo, tinnitus, sensations of aural fullness, and low-frequency sensorineural hearing loss. Endolymphatic hydrops is identified by Reissner's membrane bulging into scala vestibuli, but the relationships between endolymphatic hydrops and Ménière's-disease symptomology is not well understood. For example, histology from Ménière's-diseased human temporal bones seldom shows sensory cell loss in low-frequency regions so the origin of the low-frequency loss is a mystery. Most clinical treatments for Ménière's disease that help preserve function do little to alleviate the symptoms long term. One reason may be that treatment often doesn't begin until the ear is permanently damaged. If the condition could be identified early, some of the treatments that should work in theory, but do not in clinical practice, may help alleviate symptoms. Since humans present to the clinic with permanent, not chronic, conditions, we developed a robust chronic guinea pig model of endolymphatic hydrops (Valenzuela et al. 2020). We induced small volumes of artificial endolymph into the high-frequency base of the guinea pig cochlea and found that measurements of low-frequency hearing by the Auditory Nerve Overlapped Waveform [ANOW] changed substantially while traditional objective measurements of mid- to high-frequency hearing were unaffected (Lichtenhan et al. 2017). This suggested that excess endolymph collects in the more distensible cochlear apex, and that the ANOW could be used for early detection of endolymphatic hydrops. In later work, we found that, indeed, ANOW can detect low-frequency dysfunction before endolymphatic hydrops developed to an extent that could be measured with the classic gold-standard histological assessment of scala media cross sectional area (Lee et al. 2020). Moreover, when endolymphatic hydrops is histologically measurable in the later stages of the condition, the severity of hearing loss correlated with the degree of endolymphatic hydrops. Our body of work on endolymphatic hydrops has moved us closer to understanding the origin of symptoms associated with Ménière's disease.

Our research on endolymphatic hydrops in animals was enabled by several innovations: our development of a drug perfusion technique into the cochlear apex, an objective measure of low-frequency hearing, and a refined model of endolymphatic hydrops. Our findings in animals provide the foundation for translational research to humans, and include 1) shifts in the cochlear frequency place map that likely underly functional human diplacusis, 2) cochlear responses consistent with objective assessment of perceptual loudness recruitment, 3) low-frequency hearing loss, and 4) a joint otoacoustic emission (OAE) profile showing that endolymphatic hydrops has differing effects on reflection-source and distortion-source OAEs (Guinan et al. 2021; Lefler et al. 2021). Applying these functional measurements to humans can overcome limitations of emerging, and important, approaches for imaging endolymphatic hydrops: ears with endolymphatic hydrops that are measurable imaging techniques don't always have Ménière's disease. Our findings suggest that the process of differential diagnosis may be improved by using more advanced functional assessments. In particular, we hypothesize that translating our animal-developed measurements to humans will help with early identification of Ménière's and provide objective assessment of treatment monitoring.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **Integration of sound and locomotion information by auditory cortical neuronal ensembles**

Poster #: 27

Authors: Carlos Arturo Vivaldo, Joonyeup Lee, MaryClaire Shorkey, Ajay Keerthy, Gideon Rothschild

Affiliation: Department of Psychology, NGP; Kresge Hearing Research Institute, University of Michigan

The ability to process and act upon incoming sounds during locomotion is critical for survival. Intriguingly, sound responses of auditory cortical neurons are on average weaker during locomotion as compared to immobility and these results have been suggested to reflect a computational resource allocation shift from auditory to visual processing. However, the evolutionary benefit of this hypothesis remains unclear. In particular, whether weaker sound-evoked responses during locomotion indeed reflect a reduced involvement of the auditory cortex, or whether they result from an alternative neural computation in this state remains unresolved. To address this question, we first used neural inactivation in behaving mice and found that the auditory cortex plays a critical role in sound-guided behavior during locomotion. To investigate the nature of this processing, we used two-photon calcium imaging of local excitatory auditory cortical neural populations in awake mice. We found that underlying a net inhibitory effect of locomotion on sound-evoked response magnitude, spatially intermingled neuronal subpopulations were differentially influenced by locomotion. Further, the net inhibitory effect of locomotion on sound-evoked responses was strongly shaped by elevated ongoing activity. Importantly, rather than reflecting enhanced “noise”, this ongoing activity reliably encoded the animal’s locomotion speed. Prediction analyses revealed that sound, locomotive state and their integration are strongly encoded by auditory cortical ensemble activity. Finally, we found consistent patterns of locomotion-sound integration in electrophysiologically recorded activity in freely moving rats. Together, our data suggest that auditory cortical ensembles are not simply suppressed by locomotion but rather encode it alongside sound information to support sound perception during locomotion.

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**Interaction between stimulus and hair bundle rate constants and a kinetic model of stereocilia with higher fidelity kinematic relations**
Poster #:	28

Authors: Varun Goyal* (1), Karl Grosh (2)

Affiliation: 1.Department of Mechanical Engineering, University of Michigan; 2.Department of Biomedical Engineering, University of Michigan; 3.Kresge Hearing Research Institute; University of Michigan

The current and displacement responses associated with the mechanical stimulation of the mammalian outer hair cell (OHC) hair bundle (HB) depend on viscous effects and adaptation of the channel gating mechanism. Further, the rise-time of the applied force governs the bundle responses. To study these effects, we simulated a two-degree-of-freedom model of isolated HB developed by Tinevez et al. (Biophys. J., 93(11), 4053-4067 (2007)), called the TMJ model. We applied an external force having an exponential rise (τ_F) to a constant value to solve the nonlinear system. We determined the apparent operating range (OR) and its dependence on τ_F. Additionally, we developed a linearized model by applying a static, biasing load to compute approximate closed-form solutions for accessing the dependence of the responses on τ_F and adaptation time constants. Finally, we included geometric nonlinearities in a new HB kinetic model to determine if they influenced model predictions of the bundle responses. Hence, we derived more precise, geometrical relations between the two rows of stereocilia to establish coupled kinematic relations in the model and solved the nonlinear system for the same external force applied to the TMJ model.

From the TMJ model, for resting open probabilities between 5% and 40%, the bundle displacement took longer to saturate to a steady value as we increased τ_F. The current response peak declined and shifted rightwards with increasing τ_F as it approached 0.45 ms, after which there was no peak observed. Correspondingly, the OR increased with τ_F and remained constant (> 300 nm) once τ_F crossed 0.45 ms. The linearized model revealed two adaptation time constants- fast (τ_{FA}) and slow (τ_{SA}), in addition to the rise-time of the force (τ_F) for the closed-form solutions. The external force controlled the current peak when τ_F lay between fast and slow adaptation time constants. We found a smaller range for the OR (30 - 50 nm) when τ_F was small. However, it was overestimated by a factor of 10 when τ_F exceeded the slow adaptation time constant (0.3 ms from the linear model for a resting open probability of 40%).

The inclusion of higher fidelity geometric and kinematic constraints in the model predicted about 8% and 47% lower bundle displacements and adaptation motor displacements, respectively, compared to the TMJ model. We introduced two geometric and one constitutive nonlinearity in the model. The geometric nonlinearities arise from the moment-arm and a vectorial component of the gating force. Currently, we are working to determine optimal parameters for this model with the help of available experimental data in the literature to conclude how significant the geometric nonlinearities are and discuss the accuracy of the predicted responses.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **Loss of afferent synapses in the cochlear nucleus following cochlear synaptopathy**

Poster #: 29

Authors: J. Haely *, A. Hockley, L.R. Cassinotti LR, G. Corfas, S.E. Shore

Affiliation: Kresge Hearing Research Institute, Department of Otolaryngology, University of Michigan

Hearing impairment in the absence of threshold shifts (hidden hearing loss) is characterized by permanent damage to synapses between inner hair cells and high-threshold auditory nerve fibers (HT-ANFs). Cell types of the cochlear nucleus receive different proportions of inputs from HT-ANFs, with small cells receiving their sole afferent input from HT-ANFs. Therefore, this study focusses on how afferent input from HT-ANFs to the cochlear nucleus are altered following synaptopathy, and whether there is a greater loss of afferent synapses in the SCC than other CN regions.

Guinea pigs received a unilateral sound overexposure to the left ears (7 kHz centered, third-octave noise at 102 dB SPL for 2 h), producing unilateral temporary threshold shifts. A separate group of animals received a sham exposure. At 4 weeks post-exposure, cochlear synaptopathy and reduced ABR wave 1 amplitudes were restricted to the left side. Brains were fixed and sectioned at 40µm followed by immuno-staining for vGluT1. Straining was carried out in parallel on all sections and all images were captured on a confocal microscope using equal settings.

Cochlear synaptopathy resulted in a decreased vGluT1 staining across the cochlear nucleus, with the largest loss in the VCN. The small cell cap did not show a greater loss of ANF synapses, despite receiving specific input from HT-ANFs.
These data show reductions in ANF synapses in the ipsilateral CN resulting from mild cochlear synaptopathy in the presence of temporary threshold shifts after noise exposure. Central changes may contribute to difficulty hearing in noisy settings, which characterizes hidden hearing loss, which is a major health issue in humans.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **Loss of strial phenotype in genetically recovered lpr (Lupus) mouse model**

Poster #: 30

Authors: Kevin K. Ohlemiller (1), Jaclynn M. Lett (1), Mackenzie L. Mills *(2), Robert H. Withnell (2)

Affiliation: 1.Washington University School of Medicine; 2.Indiana University

Background

The MRL-Faslpr mouse develops an autoimmune disease resembling lupus with a phenotype that has been reported to include hearing loss caused by strial pathology and a reduced endocochlear potential. This mouse model putatively provides for the study of cochlear mechanics in the presence of a low EP with no outer hair cell pathology. Previous literature has indicated exclusively strial pathology in this model by 20 weeks of age, with significantly elevated auditory brainstem response thresholds and reduced EP in comparison to wild types. We evaluated currently-sold MRL/MpJ-Faslpr stocks and have found that the current phenotype does not reflect previously published observations. Genetic drift in stocks maintained at the Jackson Laboratory (JAX) may have reduced the utility of this model for auditory research.

Methods

Homozygous mutant (MRL/MpJ-Faslpr) and wild type (MRL/MpJ) breeding pairs were obtained from JAX and bred at Washington University School of Medicine. Genotypes were identified using quantitative polymerase chain reaction. Initial sample sizes included 25 homozygous mutants (8 female, 17 male), 30 heterozygous mutants (20 female, 10 male), and 10 wild types (6 female, 4 male). Auditory function was assessed by collecting auditory brainstem response (ABR) thresholds in response to tone-burst stimuli at 6, 12, 18, 24, 28.3, 40, and 56.6 kHz. Endocochlear potential (EP) was measured at 7 or 40 weeks and ABRs were measured at 7, 18, and 40 weeks.

Results

Baseline EP was established in heterozygote controls with an average of 101 mV at 7 weeks (n=10). Heterozygotes showed no decrease in average EP by 40 weeks (102 mV, n=15). Homozygous mutants and wild type controls showed little decrease in average EP by 40 weeks (93 mV, n=6 and 78 mV, n=4, respectively) compared to heterozygotes. Overall, ABR thresholds were relatively stable, particularly in the mid-frequencies, showing sparse evidence of an age-related threshold shift in any group by 40 weeks. Age-related shifts were only observed in 3 out of the 26 mutants tested and, of those 3 animals, 2 exhibited normal EP values.

Conclusions

The overall stability of thresholds and EPs in the mutants by 40 weeks of age appears to contradict previous findings in this model. Our observations suggest that the auditory profile of this strain, as sold by JAX, has changed, perhaps due to loss of modifying alleles on the MRL/MpJ background. Further investigation, including histopathology of stria vascularis tissue, may confirm the loss of strial pathology and explain the change in this strain's auditory function. Our current observations on the MRL-Faslpr mouse model pose caveats for researchers considering the use of this mouse as a model for presbycusis or autoimmune hearing loss.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **Mechanisms of secondary injury and auditory deficits following mild blast induced trauma**

Poster #: 31

Authors: Joseph Fernandez*(1,3), Emily Han(2), Edward Bartlett(1,2), Riyi Shi(1,3)

Affiliation: 1.Weldon School of Biomedical Engineering, Purdue University; 2.Department of Biological Sciences, Purdue University; 3.Department of Basic Medical Sciences, Purdue University

Blast-induced hearing difficulties affect thousands of veterans and civilians each year. The long-term impact of blast exposure on the central auditory system (CAS) can last months, even years, without major external injury, and is hypothesized to contribute to many behavioral complaints associated with mild blast traumatic brain injury (bTBI). However, the mechanisms that underlie these long-term impairments are still poorly understood. Although initial mechanical injury likely plays a role in central auditory damage, a secondary molecular mechanism of damage likely results in the chronic auditory deficits following mild bTBI. Oxidative stress, along with inflammation, have been suggested as key players in secondary molecular damage in other models of CNS injury, including other TBIs, and may underlie functional auditory deficits in mild bTBI as well.

Here, we recorded the changes in a variety of auditory evoked potential (AEPs) in blast-exposed and noise-controlled rats over the course of two months to understand regionally and temporal specific deficits. We compared these results to molecular and anatomical changes observed in immunohistochemistry (IHC) staining. We examined changes in markers for cellular membrane damage and acrolein protein adducts for oxidative stress. Additionally, we examined GAD65/67 for changes in cellular inhibition/excitation patterns. These markers were assessed along the auditory pathway, namely, within the superior olivary complex (SOC), inferior colliculus (IC), and auditory thalamus (AT).

Increased TMR and acrolein protein adduct staining in blast animals indicate initial mechanical injury within the SOC and ventral axon streams in the brainstem at the 2-day post injury time point. 7-day and 14-day time points show increased TMR staining within the SOC of blast animals. Staining for GAD65/67 shows initial increase in 2-day IC, but decrease in 2-day SOC and AT. Over the course of 14 days, this pattern reverses, indicating potential temporally specific changes in inhibition/excitation within these auditory systems. Comparisons of ABR wave amplitudes/ratios and middle latency response, suggest functional changes correlated with these anatomical results.

Taken together, our results suggest that an acute cascade of (axonal) membrane damage and oxidative stress results in a temporally dependent inhibition/excitation imbalance over the course of two weeks that is correlated with slight changes in auditory processing.

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**Modeling top-down and bottom-up mechanisms for robust auditory categorization in noise and in reverberation**
Poster #:	32

Authors: Satyabrata Parida*,(1), Shi Tong Liu, (2) Srivatsun Sadagopan, (1,2,3)

Affiliation: 1.Department of Neurobiology, 2.Department of Bioengineering, 3.Department of Communication Science and Disorders, University of Pittsburgh

For robust vocal communication in the real-world, the auditory system must generalize over production variability in vocalizations (trial-to-trial and subject-to-subject variability) and environmental variability (e.g., masking noise, reverberation). These computations are likely mediated by a hierarchical processing strategy, which, broadly speaking, can be modeled as a dense spectrotemporal representational stage followed by a sparse feature detection stage and a feature combination stage. We previously implemented this architecture in a computational model, and showed that optimal performance in call-categorization tasks can be achieved by detecting a handful of intermediate-complexity features that generalize over within-category production variability while maximally contrasting between call categories. Here, we extend the model to generalize over environmental variability.

To improve model performance in noise and in reverberation, we explored three biologically-feasible possibilities: (1) training the model in a range of noisy and reverberant conditions, (2) implementing bottom-up adaptation to sound statistics in the spectrotemporal representational stage (STadapt), and (3) implementing top-down sensitivity adjustment in the feature detection stage (FDadjust). Training the model over a range of conditions improved model performance in noise and reverberation, but this improvement did not derive from fundamental differences in the features learned by the model. STadapt, which is a form of contrast-gain control, improved model performance both in noise and in reverberation, likely by restoring the dynamic range of informative spectrotemporal call features. FDadjust, which captures aspects of top-down attentional modulation, also led to significant benefits in both noise and reverberation. Surprisingly, the strength of top-down modulation scaled with SNR in noisy conditions, but not with the reverberation time constant. These results are strikingly consistent with psychoacoustic results in that listening effort scales with noise but not with reverberation strength. Results were largely similar for models trained to categorize marmoset vocalizations or guinea pig vocalizations. In ongoing experiments, we are evaluating how model performance due to these proposed mechanisms compares with guinea pig behavioral call-categorization performance in noisy conditions.

Overall, these results highlight the differential contributions of bottom-up and top-down mechanisms at the spectrotemporal and feature detection stages to achieve perceptual invariance to environmental variability in auditory categorization tasks.

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**Multiple sources and overlapping terminations of cholinergic innervation of auditory brainstem nuclei**
Poster #:	33

Authors: Brett R Schofield*, Nichole L. Beebe, William A. Noftz

Affiliation: Northeast Ohio Medical University

Acetylcholine (ACh) is associated with many aspects of hearing. Early work suggested that different cholinergic cell groups modulate different levels of the auditory pathway. Cholinergic cells of the basal forebrain modulate auditory processing in the auditory cortex. Projections from the pontomesencephalic tegmentum (PMT) modulate the auditory thalamus and the inferior colliculus. Finally, projections from the superior olivary complex modulate auditory function in the cochlea and cochlear nucleus (CN). Recent work identified additional cholinergic projections from the lateral paragigantocellular nucleus (LPGi, Stornetta et al. '13) and also more extensive projections from the SOC (Beebe et al. '21). Experimental manipulation of the cholinergic pathways is complicated because multiple sources of cholinergic axons can terminate in a single target; e.g. cholinergic cells in the PMT and in the LPGi project to the inferior colliculus. We used retrograde tracing and chemically-selective anterograde tracing with adeno-associated viruses to examine the projections from different cholinergic cell groups to a variety of auditory brainstem nuclei in normal-hearing ChAT-Cre transgenic mice.

Retrograde tracing identified innervation of the cochlear nucleus from cholinergic cells in the PMT, SOC and LPGi. Viral tracing showed that cholinergic cells from each of the three sources terminate more densely in the granule cell area and the dorsal CN, less in the ventral CN and minimally in the octopus cell area. The viral tracing revealed numerous additional areas of overlapping cholinergic projections. The dorsal, intermediate and ventral nuclei of the lateral lemniscus receive cholinergic projections from the PMT, SOC and LPGi. More rostrally, the nucleus of the brachium of the IC, a key relay for auditory information to reach both the thalamus and the superior colliculus, receives cholinergic innervation from the PMT and the LPGi. The cholinergic axons from different sources appear to overlap in each of the auditory nuclei.

We conclude that subcortical auditory nuclei typically receive overlapping cholinergic projections from multiple sources. We propose that the overlapping projections are not redundant but instead serve different functions. The PPT and LDT are associated with attention, reward, sleep-wake cycle and cortically-driven plasticity. Projections from the SOC are likely to provide modulation that is more narrowly-tuned to specific auditory stimuli. These projections might provide frequency-specific modulation of neuronal sensitivity or gain. The LPGi is a small nucleus of the reticular formation. The response properties of LPGi neurons are unknown, but their connections with the CN, IC and auditory cortex provide opportunities for acoustically-driven top-down and bottom-up modulation. The wide distribution of the cholinergic projections indicate modulation at virtually all levels of the subcortical auditory pathway. The multitude of sources support the many claims of diverse cholinergic functions in hearing.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **Myosin 15 is dispensable for the activity-driven plasticity of the auditory stereocilia cytoskeleton**

Poster #: 34

Authors: Ana I. López-Porras*, Desislava A. Marinkova, Anna K. Miller, A. Catalina Vélez-Ortega

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Modified microvilli in the inner ear detect mechanical sound waves through the opening of mechano-electrical transduction (MET) channels at their tips. Even at rest, there is a MET channel current that results in a calcium influx into the cell. It has been previously demonstrated that this entry of calcium ions at rest is essential for the stability of actin cytoskeleton of these modified microvilli, known as stereocilia (Velez-Ortega, et al. Elife, 2017). Yet, the molecular mechanism involved in this maintenance phenomenon is still unknown. Given that the non-conventional myosin 15 is required for the normal elongation and maintenance of the stereocilia bundle (Probst et al., Science, 1998; Belyantseva et al., Nat Cell Biol, 2005; Fang et al., Elife, 2015), we wondered whether myosin 15 is necessary to deliver the molecular machinery involved in the calcium-dependent stability of the stereocilia cytoskeleton. We used scanning electron microscopy to determine whether any of the two previously reported isoforms of myosin 15 are essential for MET-dependent stereocilia remodeling. Shaker-2 mice have a missense mutation in the motor domain which prevents all myosin 15 isoforms from reaching the stereocilia. Auditory hair cells in shaker-2 mice have abnormally short stereocilia but still exhibit MET currents during early postnatal development (Stepanyan et al., J Physiol, 2006). We found that the blockage of the MET channels in shaker-2 cochlear explants leads to the thinning and shortening of stereocilia, with more prominent changes in inner than in outer hair cells. Myo15ΔN/ΔN mice lack the long isoform of myosin 15, they develop stereocilia bundles of normal heights and staircase arrangements with normal MET currents, but exhibit progressive hearing loss (Fang et al., Elife, 2015). However, after MET channel blockage, auditory hair cells from Myo15ΔN/ΔN mice exhibit greater stereocilia shortening than heterozygous or wild-type controls. Interestingly, while heterozygous mice develop normal hearing, the remodeling of their stereocilia cytoskeleton after MET channel blockage was greater than in wild type littermates. This indicates that the levels of myosin 15 long isoform could impact the degree of calcium-dependent stability of the stereocilia cytoskeleton. In conclusion, our data indicate that the molecular machinery involved in the calcium-dependent stability of the stereocilia cytoskeleton does not rely solely on myosin 15. However, individuals carrying “recessive” deafness-causing mutations affecting the long isoform of myosin XVA could have impaired stability of their auditory hair cell stereocilia bundles.

Supported by NIDCD/NIH (R21DC017247 to A.C.V.)

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**NIH Toolbox-cognition performance of older persons with normal hearing, cochlear implant candidates, and cochlear implant users**
Poster #:	35

Authors: Cameron K. Perrin*, Joseph M. Levin, Kara C. Schvartz-Leyzac, Gabrielle S. Watson, Bruno Giordani, and Bryan E. Pfingst

Affiliation: Michigan Medicine, Medical University of South Carolina, University of Michigan

Hearing loss has been associated with lower scores on cognitive tests, as well as with incident Mild Cognitive Impairment and Alzheimer's disease. In order to parse out hearing and cognitive effects in aging adults, we used a iPad-based, hearing-adapted NIH Toolbox Battery-Cognition (NIHTB-Ca) to assess older healthy controls (n=15) and older persons with two distinct approaches to hearing correction: individuals with cochlear implants (n=15) and individuals with hearing aids awaiting cochlear implants (n=14). As expected in groups with differences in hearing, we found that all groups performed significantly differently from each other on speech recognition ($p<0.001$), with the normal hearing group performing the best and the pre-implant group performing the worst. For NIHTB-CBa scores, the normal hearing group performed better than both the hearing aid and cochlear implant groups on the Picture Vocabulary ($p=0.003$) and List Sorting tasks ($p<0.001$), and the normal hearing group also performed better than the cochlear implant group on the Oral Reading task ($p=0.003$). Duration of self-reported hearing loss was not correlated with cognitive performance on any of the tasks among the hearing correction groups. Although, as expected, the three groups differed in terms of speech perception scores, differences between normal hearing and hearing correction groups were specifically evident on the two measures most reflective of educational attainment (Vocabulary, Reading) and on a measure of working memory (List Sorting). The role of working memory, as well as stage of education when significant hearing loss is first noted, bears further attention.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **nNos+ neurons of the shell inferior colliculus**

Poster #: 36

Authors: Pierre F. Apostolides*, Jordyn Czarny, Alexander N. Ford, Anokhi Pawar, Hannah M. Oberle, Michael T. Roberts

Affiliation: Kresge Hearing Research Institute, University of Michigan

The inferior colliculus (IC) is an evolutionarily ancient midbrain hub, and the first major bifurcation site for primary and higher-order auditory pathways. A primary pathway arises from tonotopically organized central IC neurons that mainly project to the primary auditory thalamus; this pathway is thought to faithfully encode physical attributes of sounds destined for the primary auditory cortex. In parallel, a "higher-order" pathway arises from dorsal and lateral shell IC neurons that preferentially project to higher-order thalamic nuclei, and thus provide signals to behaviorally relevant, second-order targets in the amygdala, striatum, and non-primary auditory cortex. Relative to central IC neurons, the physiology and function of shell IC neurons are poorly understood. Progress on this front has been frustrated by a lack of molecular markers to selectively target and subsequently manipulate specific populations of shell IC neurons.

Here we show that neuronal nitric oxide synthase (nNos) is a putative molecular marker for a sub-population of shell IC neurons. Crossing nNos-cre mice to Ai14 tdTomato reporter mice reveals that nNos+ neurons are primarily found in dorso-medial and lateral shell IC regions, in agreement with published nNos histology. By injecting a cre-dependent GFP virus into the IC of nNos-cre mice, we find that nNos+ neurons have local axonal projections restricted to the IC shell, as well as selective long-range projections to the higher-order auditory thalamus. In vitro patch-clamp recordings and biocytin reconstructions show that nNos+ neurons are spiny neurons with adapting firing patterns qualitatively similar to the shell IC neurons described by PH Smith (1992). Circuit mapping experiments using a soma-targeted excitatory opsin (st-Chrome) show that nNos+ neurons are glutamatergic and can drive feed-forward inhibition in the IC, indicating that their axons must synapse onto local GABA neurons with recurrent intra-collicular projections. We also conducted 2-photon Ca2+ imaging in the IC of nNos-cre mice performing an appetitive, auditory discrimination task. We find that nNos+ neurons respond not only to sounds, but also transmit non-auditory signals when mice perform operant actions during the outcome of behavioral trials.

Our data provide the first insight into the anatomical, biophysical, and behaviorally relevant characteristics of a neuronal sub-population in the shell IC. A wealth of studies indicate that auditory and trial outcome signals in the higher-order auditory thalamus and amygdala are necessary for establishing the learned valence of sounds. The existence of identical activity upstream in the shell IC thus suggests that some of this necessity instead reflects computations inherited from the auditory midbrain.

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**Non-auditory activity of auditory corticocollicular neurons supports discriminative auditory learning**
Poster #:	37

Authors: Jordyn E. Czarny*, Alexander N. Ford, Meike M. Rogalla, Gunnar L. Quass, Pierre F. Apostolides

Affiliation: Kresge Hearing Research Institute, University of Michigan

Layer 5 pyramidal neurons in auditory cortex send "corticofugal" projections to nearly all sub-cortical auditory circuits, thereby enabling top-down modulation of ascending information. Of particular interest is the corticofugal pathway to the inferior colliculus (IC), given the IC's role as an important midbrain hub for complex sound perception. Surprisingly, previous studies show that ablating auditory cortico-collicular neurons does not impair sound perception, but rather impairs perceptual re-learning sound guided behaviors during hearing loss (Bajo et al., 2010). Thus, corticofugal activity may promote learning-related plasticity in downstream targets, but the underlying mechanisms are poorly understood.

In mice performing an auditory GO/NO-GO discrimination task, imaging data from our lab showed that the majority of task-related activity in auditory cortico-collicular neurons occurred following sound offset and reflected mice's instrumental actions during reward consumption (see related poster at this conference, Ford et al.). Thus, the role of auditory cortico-collicular neurons in perceptual learning may be primarily motor related rather than strictly sensory. Indeed, we hypothesize that motor-related activity in layer 5 auditory cortico-collicular neurons may act as a top-down "teaching signal" to stamp in learned associations between sound cues and subsequent outcomes.

To test our hypothesis, we selectively expressed the inhibitory opsin GtACR1 in layer 5 auditory cortico-collicular neurons, thereby enabling temporally precise silencing of corticofugal activity during learning. Following habituation and handling, mice were head-fixed and trained daily on an operant, auditory GO/NO-GO discrimination task. In this task, mice were rewarded with a drop of sugar water reward if they licked a waterspout during a 2 s answer period following a "GO" sound (1 s duration, 4-16 kHz band-pass white noise). On NO-GO trials, the band-pass noise sound was presented with 100% sinusoidal amplitude modulation depth, and mice had to withhold their licking during the answer period. False alarms on NO-GO trials were punished with a 7 s increased inter-trial interval. On each trial, 625 nm LED light was delivered over the auditory cortex only during the answer period following sound offset, thereby selectively silencing the motor, but not acoustic responses of auditory cortico-collicular activity.

Control and GtACR1-expressing mice (n=15/group) required a similar number of sessions to associate the "GO" sound and sugar water availability, suggesting that answer period activity is not required for mice to learn a simple operant detection task. By contrast, silencing answer period activity caused a statistically significant impairment in mice's ability to discriminate GO and NO-GO sounds, with GtACR1-mice showing lower levels of across-session discrimination learning compared to controls. Our data show that motor-related activity promotes learned auditory discrimination. The results further suggest important constraints on the synaptic plasticity rules that mediate the auditory cortico-collicular pathway's proposed role in perceptual learning following hearing loss.

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**Investigations on a Possible Mechanism for High-frequency Electromechanical Conversion of Outer Hair Cells**
Poster #:	38

Authors: Wen Cai*, Karl Grosh

Affiliation: University of Michigan

The outer hair cell (OHC) of the mammalian cochlea is the nexus of the active processes giving rise to the nonlinear, biologically vulnerable, acoustic response. We present a model for the behavior of the OHC in view of its mechanical and electrical properties, and the external loading of the cell. Because of the low-pass electrical membrane impedance and rate dependent processes, there is a continuing debate on the mechanism of the amplification process at high frequencies. We will focus on the electrical-to-mechanical energy conversion at the cellular level, and show how we must consider the external mechanical loading of the cell to interpret the power transfer. In addition, we show that simple models can be used to fit in vitro data from experiments, but subtle model changes in the parameters change the predictions of power deposition by the OHCs.

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**On the emerging fields of perceptual and cognitive 1H-magnetic resonance spectroscopy (MRS) in blast-induced hearing loss and tinnitus**
Poster #:	39

Authors: Anthony T. Cacace*, John L. Woodard

Affiliation: Wayne State University, Departments of Communication Sciences & Disorders, and Department of Psychology

Introduction and Background.
The collective exploration of relationships, technologies, and actions of various types of molecules that constitute an organism's cellular composition encompasses the field of metabolomics. In the current context, improvised-explosive devices represent those factors responsible for the majority of blast-related injuries to the human body and central nervous system. When such actions affect the ear and brain, it is hypothesized that biochemical alterations underlie this traumatic cascade of events.
Because perceptual, cognitive, and metabolic relationships have not been examined with veracity in humans, interrogating the functional and molecular response of tissue damage with MRI takes on a high degree of significance.

Methods:
Participants included 22 adults (20 males, two females; 26-73 years of age). Auditory perceptual variables included pure-tone audiometry, monosyllabic word recognition in quiet and in noise, tinnitus loudness measures, and self-perceived assessment of tinnitus handicap; cognitive factors included variables associated with memory, attention, and processing speed derived from the Automated Neuropsychological Assessment Metrics (ANAM). Biochemical assays of brain tissue were derived from auditory cortical areas of the left and right temporal lobes. They included N-acetyl aspartate (NAA), choline (Cho), creatine (Cr), myoinositol (MI), and mixed signals of glutamate, glutamine, and GABA.
MRI data were collected on a 3 Tesla Siemens Verio scanner using the Stimulated Echo Acquisition Mode (STEAM) pulse sequence to assess spectroscopy data from a single voxel. A Bayesian correlation analysis estimated relations among perceptual, cognitive, and metabolic variables, where the correlation coefficient (r) reflects the Pearson statistic. The Bayesian Factor10 (BF10) supersedes the p-value by representing the relationship between the alternative and null hypotheses. A higher BF10 represents greater evidence in favor of the alternative hypothesis.

Results:
ANAM – Spectroscopy showed a correlation between code substitution throughput (CDS; processing speed) with NAA localized to the auditory cortex in the right temporal lobe ($r = 0.52$, BF10 = 4.3). Age related effects were also observed. Auditory – Spectroscopy Choline concentration in the left hemisphere was sensitive to the Monsell threshold Indexes from the left and right ears ($r = 0.57$, BF10 = 8.1 and $r = 0.52$, BF10 = 4.3, respectively), and WIN test performance for the right ear ($r = 0.65$, BF10 = 19.9). MI concentration from the left hemisphere was inversely associated with tinnitus loudness ($r = -0.58$, BF10 = 9.4).
ANAMs – Auditory perceptual effects showed CDS throughput (processing speed) to be associated with 6 kHz threshold from the left ear ($r = 0.59$, BF10 = 12.7) and right ear ($r = 0.5$, BF10 = 3.85), CDS Mean reaction time also correlated with 6 kHz threshold for the left ear ($r = 0.57$, BF10 = 9.7).

Discussion:
Select MRS metabolites (NAA, Cho, and MI) were sensitive to neuropsychological and perceptual variables, demonstrating these factors' importance.

Conclusion:
The emerging fields of perceptual and cognitive MRS represent a dynamic "platform for discovery" that will advance the areas of blast-induced hearing loss, tinnitus, and other sensory and cognitive areas of research.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **Origins and termination patterns of cholinergic axons in the mouse inferior colliculus**

Poster #: 40

Authors: William A Noftz*, Pooyan Mirjalili, Nichole L. Beebe, Brett R. Schofield

Affiliation: Department of Anatomy and Neurobiology, Hearing Research Group, Northeast Ohio Medical University, Rootstown, OH

Receptor binding studies indicate that the inferior colliculus (IC) is densely innervated by cholinergic axons across mammalian species. Physiological studies demonstrated that a majority of IC neurons are affected by acetylcholine. Our previous work in guinea pigs and rats demonstrated that a majority of cholinergic inputs to the IC originate from the pontomesencephalic tegmentum, including the pedunculopontine tegmental nucleus (PPT) and the laterodorsal tegmental nucleus (LDT), which project bilaterally to the IC with an ipsilateral dominance. A brief report suggested that the lateral paragigantocellular nucleus (LPGi), a small nucleus in the reticular formation just caudal to the superior olivary complex, sends cholinergic projections to the medial part of the IC (Stornetta et a., '13 Brain Struct Func 218:455) but few details are available. Mice have become especially useful as a model for analyzing neuronal circuitry but information on cholinergic innervation of the mouse IC is quite limited.

We used traditional retrograde tracers, immunochemical markers of cholinergic cells, and chemically-selective viral anterograde tracers in transgenic animals to characterize cholinergic inputs to the IC in normal-hearing adult mice. The retrograde tracers showed that the PPT contained nearly half of the cholinergic cells that project to the IC, the LDT contained about one third, and the LPGI the remainder. In the PPT and LDT the projecting cells were more numerous on the ipsilateral side, whereas the LPGi contained similar cell numbers bilaterally. Anterograde tracing of cholinergic axons confirmed bilateral projections from all three areas. Moreover, axons from all three areas terminated throughout the IC.

The PPT and LDT are associated with attention, reward, sleep-wake cycle and cortically-driven plasticity, suggesting a multitude of functions for these cholinergic projections to the IC. The LPGi is a small nucleus of the reticular formation with an unknown role in auditory processing. Its connections with many auditory structures, including the cochlear nucleus, IC and auditory cortex, provide opportunities for both top-down and bottom-up modulation.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **Physiological and anatomical properties of utricular hair cells and afferents in Gpr156del/del mice lacking a mirror-image hair cell organization**

Poster #: 41

Authors: Kazuya Ono*(1), Omar López Ramírez(1), Basile Tarchini(2), Ruth Anne Eatock(1)

Affiliation: 1. Department of Neurobiology, University of Chicago; 2. The Jackson Laboratory, Bar Harbor, Maine

In otolith organs, hair bundles of hair cells have varying orientations that reverse along the line of polarity reversal (LPR) located within or at the edge of the central striolar zone. This striking anatomical feature creates simultaneous excitatory and inhibitory responses to translational stimuli, but the significance for vestibular function is not clear. Recent work has shown that the reversal is controlled by the transcription factor Emx2, localized on one side of the LPR, acting via G protein-coupled receptor GPR156, which is uniformly expressed in vestibular hair cells (Kindt et al., Nat Commun 12:2861, 2021). Gpr156del/del otolith organs lose the LPR without clear macroscopic anatomical defects. In this study, we examined whether loss of LPR affects physiological and anatomical properties of individual hair cells and primary afferent neurons.

Whole-cell patch clamp recordings were made from hair cells or afferent calyceal endings in excised utricles from post-natal (P12-100) Gpr156del/+ and Gpr156del/del mice. Responses of hair cells and afferent terminals were recorded to steps of hair bundle deflection, applied by a rigid probe, or of membrane voltage or current, applied by the whole-cell recording electrode. The receptive fields of recorded afferents in the utricular nerve were visualized by diffusion of Alexa594 dye included in the KCl-based pipette solution. Most data were collected from the lateral extrastriola (LES), where hair bundles are misoriented by ~180° by Gpr156 deletion.
Gpr156 deletion had no significant effect on mechanoelectrical transduction by hair cells or on the physiological differentiation between type I and type II hair cells, as indicated by expression of type-specific voltage-gated potassium conductances (n = 9-29 for each combination of cell type (I and II) and genotype).

We also investigated whether loss of LPR, which normally occurs at the LES/striolar boundary, affects differences in the normal afferent firing patterns of extrastriolar and striolar zones of the utricle: depolarizing current steps to wildtype afferent neurons tend to evoke transient firing in the striola and sustained firing in the extrastriola, a property that correlates with spike timing regularity. We found that current steps applied to LES calyces of both Gpr156del/+ and Gpr156del/del utricles elicited multiple spikes (sustained responses), suggesting no effect of Gpr156 loss on afferent firing patterns. We hypothesize based on this result that zonal differences in spike timing regularity are similarly unaffected.

In WT utricles, each afferent innervates hair cells on one side of the LPR. We investigate whether this strict innervation pattern is altered in utricles of Gpr156del/del mice. Although the LPR is missing, the location of the striolar/LES boundary can be detected by the normal zonal differences in oncomodulin and calbindin immunoreactivity. Our preliminary results have found no aberrant dendritic arbors that innervate hair cells across the striolar/LES boundary.

In summary, we find that the deletion of Gpr156 and resulting loss of bundle orientation reversal in the utricular LES does not disrupt key properties of individual hair cells and afferent terminals.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **Population coding of temporally modulated sounds in the non-lemniscal inferior colliculus**

Poster #: 42

Authors: Kaiwen Shi*, Gunnar Lennart Quass, Pierre François Apostolides

Affiliation: Kresge Hearing Research Institute, University of Michigan Medicine

The inferior colliculus (IC) is an evolutionarily ancient midbrain circuit important for perceiving amplitude modulated (AM) sounds such as conspecific vocalizations and human speech. The IC is comprised of several sub-regions: A primary central region receives ascending auditory inputs from the brainstem and projects to primary auditory thalamus, while non-primary "shell" regions integrate intra-collicular inputs and project to behaviorally relevant, higher-order thalamic regions interfacing with the amygdala and striatum. Decades of studies on AM coding have focused on central IC neurons.(Joris et al., 2004; Rees & Palmer, 1989; Rode et al., 2013) By contrast, little is known about whether and how shell IC neurons represent AM sounds, owing to the difficulty of recording from these neurons located near the tectal surface. Given the importance of vocalizations in learned and innate behaviors (Wöhr et al., 2016), understanding how shell IC neurons respond to AM sounds might provide mechanistic insight into how the brain transforms time-varying, acoustic signals into behaviorally relevant commands.

Here, we used 2-photon Ca2+ imaging to study how shell IC neurons of awake, head-fixed mice respond to AM sounds. The calcium-indicator GCaMP6f was expressed in shell IC neurons of 5-8 weeks old C57/Bl6J- or VGAT-ires-cre/Ai14 mice; 2-photon microscopy was used to record neural activity in the shell IC as the mice were passively listening to AM sound stimuli with varying modulation depths and -rates. We analyzed responses from 691 neurons recorded from eight mice, and constructed a Convolutional Neural Network classifier to decode stimulus identity from shell IC population data using the recorded fluorescence traces. For VGAT-Cre mice, we separately analyzed GABAergic and non-GABAergic cells to identify any group-level differences between inhibitory- and excitatory neurons. We hypothesized that population activity in the shell IC generates a continuous representation of rate and depth, rather than the individual combinations. To this end, we trained the classifier to predict the joint coding of modulation rate and depth, modulation depth under a given rate, and modulation rate under a given depth.

We found that that all major AM rate filters previously described in the central IC, including low-pass, high-pass, band-pass, and band-reject filters, were also well represented in excitatory and inhibitory neurons of the shell IC. Overwhelmingly, increasing amplitude modulation depth of a carrier sound enhanced shell IC neuron responses to all modulation rates, indicating a linear and monotonic encoding of modulation depth. The decoder trained on the shell IC neural data achieves high decoding accuracy comparable to a cortical spike train decoder (Downer et al., 2021), peaking around 80% decoding accuracy in most categories for 100% modulation depth. In addition, classification accuracy for modulation rate decreased monotonically as a function of difference between training- and testing modulation depth, further corroborating a linear representation of modulation depth. Our data reveal a substantial population level representation of temporally modulated sounds in the shell IC, comparable to established results in the central IC. These results have important implications for understanding how ascending auditory pathways funnel behaviorally relevant signals towards the limbic system.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **Postsynaptic responses of IC neurons in unanesthetized mice reveals the importance of temporal integration of excitatory and inhibitory inputs for sound processing**

Poster #: 43

Authors: Chun-Jen Hsiao* and Alexander V. Galazyuk

Affiliation: Northeast Ohio Medical University, Department of Anatomy and Neurobiology

Inferior colliculus (IC) is a major integrative center of the central auditory system. It receives and integrates ascending as well as descending information from many auditory as well as from non-auditory brain structures. Deep knowledge about temporal integration of excitatory and inhibitory inputs is critical for our understanding of information processing in IC neurons. Previous in-vivo IC studies mainly utilized extracellular recording techniques often in anesthetized animals. The goal of the present study was to examine postsynaptic responses in IC neurons to pure tones in unanesthetized animals to determine how temporal integration of excitatory and inhibitory inputs contribute to sound-evoked firing.

Intracellular recordings were conducted with quartz micropipettes filled with 1 M potassium acetate having impedance around 250 MΩ in unanesthetized mice. Electrodes were inserted into the IC via a small opening (≈100 µm) in the skull. Spontaneous and sound evoked activity to pure tones presented at different sound frequencies and at three sound levels (30, 40, and 55 dB SPL) were recorded. The resting membrane potential (RMP), spontaneous firing rate (SFR), characteristic frequency (CF), and timing of both the postsynaptic potentials and sound evoked spikes contributed to our data analysis.

RMPs and SFRs of IC neurons ranged from -35.07 mV to -79.58 mV (-48.53 mV, mean) and from 0 Hz to 77.4 Hz (11.03 Hz, mean), respectively. We found no correlation between RMPs and SFRs. Three different response types to pure tones were observed at neurons' characteristic frequency: onset (n=17, 50%), sustained (n=14, 41.18%), offset (n=3, 8.82%). These response types had characteristic underlying synaptic mechanisms. Onset response type usually exhibited EPSP/spike. Sustained responses typically showed EPSPs lasting during the sound duration. The offset type showed a long lasting IPSPs followed by a postinhibitory rebound. The onset and sustained response types often showed a complex temporal integration between excitatory and inhibitory inputs on IC neurons.

Our research suggests that a complex temporal integration of excitatory and inhibitory inputs underlines different response types of IC neurons in their responses to pure tones.

Supported by NIH R01 DC016918 from the National Institute on Deafness and Other Communication Disorders of the U.S. Public Health Service.

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**Quantitative profiling of cochlear synaptosomal proteins in cisplatin-induced synaptic dysfunction**
Poster #:	44

Authors: Monazza Shahab, Rita Rosati, Paul Stemmer, Samson Jamesdaniel

Affiliation: Wayne State University

Background: The disruption of ribbon synapses in cochlear synaptopathy impairs the transmission of auditory signals from the cochlear sensory receptor cells to the auditory cortex. Although cisplatin-induced loss of ribbon synapses is well documented, the associated otopathology as well as the underlying mechanisms are yet to be fully understood. We reported that cisplatin treatment induces nitrative stress resulting in the nitration of cochlear proteins and selective inhibition of nitrative stress attenuates cisplatin-induced ototoxicity. Others have reported that synaptic proteins are susceptible to nitration, which can alter protein function and abundance. Therefore, we hypothesized that cisplatin treatment alters the abundance of synaptosomal proteins in the cochlea and selective inhibition of cochlear nitrative stress attenuates these changes and prevents associated synaptic dysfunction.
Methods: To test our hypothesis we employed 5-week-old male CBA/J mice and treated them with cisplatin (3mg/kg/day) for 5 days. To selectively inhibit nitrative stress, we co-treated the mice with MnTBAP (10mg/kg/day) for 7 days. Auditory brainstem responses (ABR) were recorded before and after treatment and the amplitude and latency of wave1 was measured to assess synaptic dysfunction. The animals were sacrificed on the 8th day, cochlear synaptosomal proteins were extracted then profiled using multiplexed tandem mass spectrometry. The enrichment of synaptosomal proteins was validated by immunoblotting with anti-CtBP2 and anti-SNAP25.

Results and Discussion: ABRs indicated that the hearing threshold levels in mice co-treated with MnTBAP were 2-10 dB lower than those observed in cisplatin treated mice (n=6), indicating an oto-protective effect of MnTBAP. Moreover, MnTBAP co-treatment reversed the cisplatin-induced decrease in the amplitude of wave1 (24 and 32KHz frequencies, $p<0.01$ or $p<0.05$, n=6) suggesting that cisplatin-induced decrease in the firing of cochlear neurons was attenuated by MnTBAP. Additionally, MnTBAP co-treatment significantly attenuated cisplatin-induced increase in wave1 latency (24 and 32KHz, $p<0.01$ and $p<0.05$, n=6) suggesting that cisplatin-induced decrease in the speed of signal transmission is attenuated by MnTBAP co-treatment. Mass spectrometry analysis of the cochlear synaptosomes showed that cisplatin treatment decreased the abundance of 104 proteins including syntax-binding protein 2, proteolipid protein 2, SNAP 25 and CtBP2, while increasing the abundance of 253 proteins, including Rab34, synapsin-3, synaptojanin-1 and dynamin-3. The MnTBAP co-treatment attenuated cisplatin-induced changes in the abundance of 35 proteins including proteolipid protein 2, syntaxin-binding protein 2, dynamin-3, synaptojanin-1, and synapsin 3, suggesting a potential role of oxidative/nitrative stress in cisplatin-induced cochlear synaptic dysfunction.

Conclusion: These results suggest that cisplatin-induced synaptic dysfunction is accompanied by changes in the abundance of at least 358 cochlear synaptic proteins. Inhibiting the nitrative stress with MnTBAP prevents the cisplatin-induced changes in abundance for only 35 of those proteins while still attenuating the cisplatin-induced cochlear synaptic dysfunction. These findings provide a set of candidate biomarkers for cochlear synaptopathy and suggest that selective inhibition of nitrative stress is a promising strategy for preventing cisplatin-induced hidden hearing loss.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **Recording high-frequency transient evoked otoacoustic emissions in humans**

Poster #: 45

Authors: Sebnem Dundar*, Jonathan H. Siegel

Affiliation: Northwestern University, Hugh Knowles Center, Roxelyn and Richard Pepper Department of Communication Sciences and Disorders

Background:
Reports of otoacoustic emissions (OAEs) at frequencies above 4 kHz in response to transient acoustic stimuli (high-frequency TEOAEs) have been rare in humans (Goodman, Fitzpatrick, Ellison, Jesteadt, & Keefe, 2009; Keefe et al., 2019), and these reports demonstrate that high-frequency TEOAEs are present. We have explored whether we could record the high-frequency TEOAEs in humans using a method known to be successful for the same purpose in chinchillas (Charaziak & Siegel, 2015).

Methods:
We compared TEOAEs evoked by tone pips, stimulus frequency otoacoustic emissions (SFOAEs), and behavioral hearing thresholds measured with high resolution at matching frequencies in normal-hearing young adults. TEOAEs were measured in a series of conditions involving the combinations of different probe frequencies and probe levels. TEOAEs were evoked using tone pips (1 ms duration with 0.5 ms rise/fall time) centered at 4, 6, and 9 kHz frequencies with probe levels set to 40, 50, and 60 dB FPL (forward pressure level), similar to the method described by Charaziak & Siegel (2015). OAEs were separated from the stimulus pressure using both the compression and suppression paradigms. The reference level was fixed to 80 dB FPL for the compression method. The suppressor level was fixed to 70 dB FPL with a frequency set to 0.2 kHz higher than the probe frequency. SFOAEs were evoked using pure tones, with frequency changing from ~3 kHz to ~12 kHz in ~100 Hz steps and level fixed to 37 dB FPL. OAE responses were in emitted pressure level (EPL) units (Charaziak & Shera, 2017). The level, gain, phase, and group delay characteristics of the TEOAEs and SFOAEs were analyzed and compared within and between subjects.

Results:
Results obtained from 6 subjects (5 F, 1 M, aged 23-28 yrs) indicate that the tone pips could be used to evoke measurable OAE responses at high frequencies up to ~12 kHz in humans when it is used in combination with either the compression or the suppression paradigm.

Conclusion:
The method that is shown to be successful in recording high-frequency TEOAEs in chinchillas is successful for the same purpose in humans. Tone pips could be used, as an alternative to filtered clicks and chirps (Keefe et al., 2019), to measure high-frequency TEOAEs in humans. Characteristics of the high-frequency TEOAEs reported so far indicate that the variations in otoacoustic emission measurement methodology appear to yield valid measurements of high-frequency TEOAEs in humans.

FULL ABSTRACT
PODIUM PRESENTATION

MIDWEST AUDITORY RESEARCH CONFERENCE
presented by the Kresge Hearing Research Institute

Title:	**Recurrent circuits amplify corticofugal signals and drive feed-forward inhibition in the inferior colliculus**
Poster #:	46

Authors: Hannah M. Oberle*, Alexander N. Ford, Pierre F. Apostolides

Affiliation: Kresge Hearing Research Institute, Department of Otolaryngology - Head & Neck Surgery, Neuroscience Graduate Program, and Molecular and Integrative Physiology, University of Michigan

The inferior colliculus (IC), a midbrain auditory region, integrates ascending brainstem projections and descending auditory cortex projections. Surprisingly, while the descending projections are predominately glutamatergic, auditory cortex activity often lead inhibits the IC. Anatomy data show that auditory cortical projections more often synapse upon glutamate neurons, with fewer direct synapses onto local IC GABAergic neurons. How does auditory cortex inhibit the IC? We addressed this question using electrophysiology, optogenetics, and pharmacology in acute brain slices from adult mice.

In vivo recordings in the IC confirmed that auditory cortex stimulation reliably evokes inhibition, similar to previous studies. Interestingly, optogenetic stimulation of cortico-collicular axons with single light flashes in vitro generated larger excitatory post-synaptic potentials (EPSPs) in IC glutamate compared to IC GABA neurons, confirming anatomical predictions. However, optogenetic stimulation with light trains (25 flashes at 50 Hz) drove to large, asynchronous EPSPs in GABA neurons, with the variation in onset latency suggesting polysynaptic activity. In comparison, glutamate neurons had shorter and less varied onset latencies, supporting a monosynaptic connection from descending auditory cortex projections. These data suggest that descending axons drive action potentials in IC glutamate neurons which subsequently synapse onto local GABA neurons and generate large recurrent excitation. Indeed, recording from IC GABA neurons while optogenetically stimulating local glutamate neurons revealed strong unitary glutamatergic inputs from the local circuit, with multiple presynaptic glutamate neurons often converging on a single GABA neurons. Thus, descending activation of IC GABA neurons does not solely rely on strong corticofugal synapses. Rather, a local excitatory circuit amplifies descending signals.

Functionally, cortico-collicular axon stimulation generated local GABA-A receptor mediated inhibition which controlled the temporal integration of descending signals. Altogether, we identify a circuit mechanism that supports auditory cortex driven inhibition in the IC despite apparently weak descending connectivity onto IC GABA neurons.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **Regulation of synaptic transmission by mGluR5 in mouse LSO neurons**

Poster #: 47

Authors: Tasmuna T. Tanmy *, Huimei Wang, Yong Lu

Affiliation: Northeast Ohio Medical University

In the lateral superior olive (LSO), activation of metabotropic glutamate receptors (mGluRs) with generic (non-subtype-specific) agonists suppresses synaptic excitation (Wu & Fu, 1998, Hear Res) as well as synaptic inhibition (Nishimaki et al., 2007, Eur J Neurosci). It is unknown whether mGluR5 is involved in any of these modulatory functions. Here, we used whole-cell patch recording to examine the modulatory effects of mGluR5 on both synaptic inhibition and synaptic excitation in LSO neurons. mGluR5 is one of the two members of group I mGluRs (the other member is mGluR1). There is no specific agonist for mGluR5. To activate mGluR5, we bath-applied a group I mGluR agonist 3,5-DHPG (200 µM) in the presence of mGluR1 antagonist JNJ16259685 (10 nM). Cell morphology was revealed with fluorescence dye Alexa488 during recordings, and/or biocytin staining after physiology recordings. The cell morphology was used to identify the cell types based on dendritic arborization (bipolar principal cells vs non-principal cells). In some cells, the cell type was also identified based on neurotransmitter (glutamatergic vs non-glutamatergic neurons) by crossing vesicular glutamate transporter 2-Cre (VGluT2-Cre) mice to a reporter mouse line (ROSA26-tdTomato). Pharmacological activation of mGluR5 enhanced spontaneous inhibitory transmission and depolarized LSO neurons, regardless of cell types. Spontaneous excitatory transmission, in contrast, was not changed by mGluR5 activation. Interestingly, modulation of evoked synaptic transmission was highly variable in a majority of neurons. These results revealed differential modulation of synaptic transmission in the LSO, likely in a transmitter and release mode dependent and cell type independent fashion.

Supported by NIH/NIDCD R01DC016054 (YL).

FULL ABSTRACT
PODIUM PRESENTATION

Title: **Representation of auditory space in the shell of the inferior colliculus**

Poster #: 48

Authors: Meike M. Rogalla*, Gunnar L. Quass, Deepak Dileepkumar, Gunseli Wallace, Harry V. Yardley, Alexander F. Ford, Pierre F. Apostolides

Affiliation: Kresge Hearing Research Institute, University of Michigan

Spatial hearing enables humans and animals to localize sounds in their vicinity, which contributes to survival. Unlike vision or touch, the peripheral auditory system lacks a spatial map at the sensory receptor level; sound source location is therefore derived centrally from binaural (timing and intensity) and monaural (spectral) cues. In the case of profound unilateral hearing loss, binaural cues are no longer available, thereby limiting spatial hearing. However, monaurally occluded humans and other animals can regain sound localization sensitivity following perceptual learning exercises. It is assumed that the observable re-learning of sound localization relies on the context-dependent re-calibration of auditory space by monaural cues. Thus, central experience-dependent auditory plasticity mechanisms must exist to re-calibrate sound localization circuits after monaural hearing loss, but these mechanisms are unknown.
In avians, the external cortex of the inferior colliculus (ICX) is a major central site of experience-dependent spatial plasticity. Circumstantial evidence similarly implicates the mammalian analogue of the ICX, the “shell” nuclei of the inferior colliculus (shell IC), as plasticity loci for sound localization cues. However, the neural population coding of spatial information in the mammalian shell IC remains poorly understood: Shell IC neurons are located at the IC surface and have sparse firing rates, thereby complicating study via classical electrophysiology approaches. Understanding the physiological representation of auditory space in the mammalian shell IC is a necessary first step to identifying underlying mechanisms of spatial cue representation and its plasticity in mammals.

We addressed this knowledge gap by developing a novel movable acoustic delivery system that consists of a servo motor equipped with an arm, carrying a speaker. This approach enabled us to present acoustic stimuli from distinct spatial positions within the horizontal frontal field while performing cellular resolution 2-photon Ca2+-imaging in the left shell IC of awake, head-fixed mice (n=4). Broadband noise stimuli were presented from -90° to 90° in steps of 30° within the horizontal plane.

We could reveal spatial tuning in the left shell region of the auditory midbrain: neurons displayed sharp onset and/or sustained responses, whereas other neurons had spatially tuned sound offset responses. In contrast to the central IC, where spatial tuning shows a contralateral dominance, we found both contra- and ipsi-lateral selective neurons, such that a single hemisphere contained a representation of the entire horizontal field. Although previous data suggested a monotonic code for spatial representations in the mammalian auditory system, many shell IC neurons were tuned to discrete contra- and ipsi-lateral positions. Tuning required binaural integration and was impervious to representational drift: spatial tuning of IC shell neurons broadened or shifted towards the contralateral hemifield after inserting an ear plug into the left ear. These broad tuning curves remained stable for several days of sustained ear plugging, and pre-plugging tuning recovered immediately following plug removal.

To our knowledge, these results are the first insight into spatial population codes of the mammalian shell IC. Future studies will test the hypothesis that active engagement in a localization task is required for plasticity of spatial tuning during monaural hearing loss.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **Resurgent and persistent sodium currents enhance spiking excitability in mouse vestibular ganglion neurons**

Poster #: 49

Authors: Selina Baeza-Loya*, Ruth Anne Eatock

Affiliation: Dept of Neurobiology, University of Chicago

The vestibular inner ear transmits information to the brain via two populations of primary vestibular ganglion neurons (VGNs) which differ in the regularity of action potential (AP) timing. Studies of isolated VGNs have shown that irregular neurons are less excitable than regular neurons, producing transient and sustained spiking in response to current steps, respectively. Although voltage-gated sodium (NaV) currents drive the rising phase of APs, their contributions to excitability and regularity differences are not fully understood. NaV currents in VGNs are dominated by transient (inactivating) currents but can include persistent (noninactivating) and/or resurgent currents (which flow after relief from inactivation block). Here we consider the impact of different NaV current modes on patterns of AP firing.

Whole-cell recordings were taken from mouse VGNs (postnatal days, (P)3-25) isolated and cultured overnight. In a sample of 100 VGNs, all had large transient NaV currents blocked by 1 µM TTX; 62 had persistent current (P3-25), 6 of 81 had resurgent current (P11-20), and 4 (P11-20) had both persistent and resurgent currents. Application of NaV1.6 blocker 4,9-anhydro-tetrodotoxin (4,9-ah-TTX) partially blocked all three current modes, indicating that a substantial portion of each is carried through NaV1.6 channels. In current clamp, application of 4,9-ah-TTX decreased neuronal excitability: increasing current threshold for spiking in all VGNs and decreasing AP rate in sustained (regular) VGNs. NaV channel agonist increased transient and persistent current, and enhanced spike excitability and regularity in response to injected EPSC trains.

We lack ways to experimentally isolate the effects of transient, persistent, and resurgent currents on spiking because they flow through the same channel subunits. Therefore, we adapted a conductance-based VGN model with transient NaV current (Hight and Kalluri, J Neurophysiol 116:503, 2016), adding persistent and resurgent currents (Venugopal et al., PLoS Comput Bio 15(6): e1007154, 2019) and adjusting expressions for properties measured in our VGNs. Adding persistent and resurgent components had a negligible effect on firing by the model transient (irregular) VGN, where the effects of KLV channels are over-riding. In contrast, for the model sustained (regular) VGN, adding resurgent currents decreased first-spike latency (delay relative to EPSC onset), reduced interspike intervals (increased rate), and hyperpolarized voltage threshold, and reduced spike accommodation.

These results suggest that increasing NaV channel availability in the after-spike interval with persistent and resurgent currents may enhance spiking excitability characteristic of regular VGNs. The difference in regularity may represent distinct encoding strategies, with regular afferents using a rate code and irregular afferent using a temporal code.
Supported by NIDCD R01 DC012347 and HHMI Gilliam.

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**Retinoic acid regulates the efficiency and the anterior-posterior fate of inner ear organoids**
Poster #:	50

Authors: Liqian Liu, Moe Moyer, R. Keith Duncan*

Affiliation: Department of Otolaryngology-Head & Neck Surgery, University of Michigan

The early development of inner ear organoids—from germ layer to otocyst formation—are highly controlled with timed chemical cues that recapitulate major signals in vivo. In contrast, later stages of differentiation—from otic vesicle (OV) to organoid formation—are self-guided with little outside influence, even though these stages are guided by several key morphogens in vivo. In prior work, we found that late-stage cultures were sensitive to retinoic acid (RA) signaling, an essential morphogen in normal inner ear development. In this study, we sought to gain greater control over RA signaling during OV formation in organoid cultures. We generated mouse embryonic stem cells carrying a lacZ transgene under the control of RA response elements and adapted these cells to the organoid culture paradigm. Exogenous control over RA signaling was achieved by inhibiting endogenous RA synthesis combined with exogenous application of all-trans RA (atRA). RA level was modulated from culture day (D) 8 to D12, which corresponded to OV formation but was prior to organoid development. LacZ transgene expression was monitored by X-gal staining. LacZ expression was asymmetrically distributed around the OVs suggesting that RA signaling was polarized, with a greater response in the portion facing the outer epithelium of the spheroids that contain these vesicles. The intensity of the lacZ staining varied widely between individual OVs; the highest levels were equivalent to those from cultures treated with 500 nM atRA on D8 to D12, a treatment that completely inhibited organoid-genesis. When endogenous RA synthesis was inhibited and atRA was added at increasing doses from 0.5 to 500 nM, the expression of anterior markers (Lfng, Sox2) decreased and a posterior marker (Lmx1a) increased, consistent with a shift in sensory to nonsensory fate specification. Notably, moderate levels of atRA (~5 nM) increased the efficiency of organoid production compared to control cultures. This study demonstrated that late-stage organoid development is regulated by RA, a key morphogen guiding organogenesis in vivo. Observations of extremely high RA signaling in some cultures—levels similar to conditions that inhibit organoid-genesis—suggested that variations in endogenous RA levels could negatively impact organoid culture efficiency. By gaining greater control over RA signaling, we improved the efficiency of organoid production and could shift the expression of sensory and nonsensory biomarkers. Further study is needed to determine whether modulation of RA level can increase organoid hair cell production and whether this platform can help catalog the RA-responsive genes driving organogenesis and cell fate specification.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **Role of neuron-oligodendrocyte interaction in temporal fidelity of action potential at the nerve terminal in the auditory brainstem**

Poster #: 51

Authors: Wan-Chen Wu*, Kaila Nip, Han-Gyu Bae, and Jun Hee Kim

Affiliation: University of Texas Health San Antonio

Auditory processing abnormalities are common and prominent features of neurodevelopmental disorders such as autism spectrum disorder (ASD). However, the causes and mechanisms have not been sufficiently explored. Temporal precision of signal transduction is necessary for proper auditory processing in the mammalian brain. Our previous study showed that altered myelination critically impaired the temporal fidelity of auditory transmission and neural connectivity in rodents. We recently characterized a subpopulation of oligodendrocyte lineage cells (OLs), the myelin-producing glial cells that express the voltage-gated Na+ channel 1.2 (Nav1.2) and display spikes. The gene, SCN2A, encoding the alpha subunit of Nav1.2, is highly linked to ASD. In this study, we investigated how the loss of oligodendroglial SCN2A impacts axon-OL interaction, axonal conduction, and presynaptic excitability in the auditory brainstem using an OL-specific SCN2A knockout mouse model (SCN2A cKO). Analysis of action potential (AP) waveforms at presynaptic terminal showed that there was no significant difference in threshold, amplitude, and width of AP. Loss of OL SCN2A did not alter intrinsic properties of spiking at the nerve terminal. However, SCN2A cKO mice displayed a slower conduction and increased AP failures during a high-frequency train stimulation, which may be associated with structural changes in myelin segments (internodes) and altered axon-OL interaction. Our results suggest that Nav1.2-mediated OL excitability is important for neuron-OL communication, constructing internodes, and proper neurotransmission in the developing auditory brainstem.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **Somatic maneuvers and their effect on tinnitus**

Poster #: 52

Authors: Gerilyn Jones*; Travis Riffle, David T. Martel, Emily Stucken, Greg J. Basura, Jacqueline Souter, Susan E. Shore

Affiliation: Kresge Hearing Research Institute, University of Michigan

Title: Somatic Maneuvers and their effect on Tinnitus

Background: Tinnitus is the perception of sound in the absence of external auditory input. The most common factor associated with tinnitus is hearing loss. Up to 80% of tinnitus patients can modulate their tinnitus by movements of the head or neck – a phenomenon termed "somatic or somatosensory tinnitus (Levine, Abel; CRANIO, 2004)." Herein, we examine the relationships between hearing threshold, somatic tinnitus, and perceived tinnitus severity.

Methods: Data from 207 participants was used for this analysis. Modulation assessments were performed either in-person in a standard audiological clinical sound booth, or remotely while participants were in a quiet room. Standard pure tone audiometry was performed, and the Tinnitus Functional Index (TFI; Meikle et al, 2012) questionnaire was utilized to assess reactions to tinnitus. To quantify somatic-induced changes, participants were instructed to report increases or decreases in tinnitus loudness on a 0-4 scale (0 = no change; 4 = greatest change) (Roberts reference/Levine reference). Modulation scores were grouped by cranial nerve (CN) innervation: CN III, IV, VI for eye maneuvers, CN V for jaw, CN VII for cheek maneuvers, CN XI and dorsal column spinal nerves for neck maneuvers, and CN XII for tongue maneuvers. CN XI and dorsal column spinal nerves are also separated by a subset of maneuvers: Passive, Active, and Active with Resistance.

Results: The mean number of modulations that altered tinnitus loudness per subject was 16.14 . Further, more than half of somatic maneuvers increased tinnitus loudness. Somatic maneuvers involving CN V, XI or spinal nerves were the most likely to alter tinnitus loudness (counts/proportions). For CN XI and spinal nerve maneuvers, Active with Resistance maneuvers consistently elicited a change in tinnitus loudness, while Active maneuvers elicited more increases than Passive movements. TFI scores positively correlated with the sum of effective modulations ($r=0.216$; $p=0.004$), suggesting that subjects with more effective modulations had more bothersome tinnitus. Moreover, pure tone average (PTA; 500Hz, 1kHz, 2kHz, 4kHz) and the sum of effective modulations were inversely correlated ($r=-0.118$; $p=0.011$).

Conclusions: The negative correlation between PTA and effective somatic maneuvers suggest that better hearing thresholds were associated with more effective somatic modulations. Furthermore, greater tinnitus severity was associated with an increased ability to modulate tinnitus with a somatic maneuver. Interestingly, jaw and neck movements were associated with more effective modulations, supporting previous work showing that projections from these regions activate the cochlear nucleus neurons linked to tinnitus generation.

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**SOX11 and CHD7 act in the same gene regulatory network to promote inner ear development**
Poster #:	53

Authors: Jelka Cimerman(1)*, Ethan D. Sperry(4,5), Ronus Hojjati(5), Donald L. Swiderski(3), Yehoash Raphael(3), and Donna M. Martin(1,2,3)

Affiliation: 1.Departments of Pediatrics, 2.Human Genetics, 3.Otolaryngology – Head and Neck Surgery and 4. Medical Scientist Training Program , 5.College of Literature, Science, and the Arts , University of Michigan

Background:
Development of the inner ear depends on a precise spatiotemporal orchestration of gene expression via transcriptional networks in the cell nucleus. SOX11, a SoxC transcription factor of the SRY-related high-mobility-group (HMG) box family, has been implicated in inner ear morphogenesis. In neural stem cells, SOX11 is a genetic target of CHD7, an ATP-dependent chromatin remodeler in which regulates nucleosomes and transcription. Haploinsufficiency of CHD7 results in CHARGE syndrome which presents with hearing loss and balance impairment due to inner ear dysplasia. The specific mechanisms by which loss of CHD7 results in inner ear malformation are not well understood. Since pathogenic variants in either CHD7 or SOX11 have been shown to cause phenotypically similar developmental malformations, we asked whether SOX11 and CHD7 function together in a common genetic regulatory network in the developing otocyst.

Objective:
We aimed to explore potential molecular genetic pathways of Sox11 and Chd7 in the developing mouse inner ear.

Design/Methods:
We analyzed Sox11+/+ and Sox11-/- mice for (1) morphological abnormalities using histological studies and paintfill of inner ears from embryonic ages E11.5-E14.5 (2) changes in inner ear gene expression using immunofluorescence in situ hybridization assays and qRT-PCR assay and (3) cell proliferation and apoptosis using BrdU incorporation and anti-Caspase3.

Results:
We observed abnormalities in the lateral and posterior semicircular canals in Sox11-/- mice. Interestingly, cell proliferation was increased in the canal fusion plate in Sox11 mutants. Expression of Sox11 was reduced in the otocyst with loss of Chd7, and Bmp4 levels in the presumptive lateral crista ampullaris were reduced with Sox11 loss.

Conclusions:
Our results indicate a Chd7-Sox11 gene regulatory network that is critical for mouse vestibular system development. Ongoing studies are designed to identify cis-regulatory elements that mediate Sox11 and Chd7 transcriptional regulation in the developing otocyst, with the long-term goal of designing interventions for vestibular dysfunction in humans.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **SOX2 and CHD7 cooperate to regulate development of the inner ear**

Poster #: 54

Authors: Jingxia Gao*, Jelka Cimerman, K. Elaine Ritter, Jennifer M. Skidmore, Donna M. Martin

Affiliation: Departments of Pediatrics and Human Genetics, University of Michigan

Background: CHD7, encoding Chromodomain-containing Helicase DNA binding protein 7, is highly expressed in the developing ear. Pathogenic variants in CHD7 cause CHARGE (Coloboma, Heart defects, Atresia Choanae, Growth retardation, Genital abnormalities, and Ear abnormalities) syndrome, which features ear malformations. SOX2 (Sex determining region Y-box 2) is one of the earliest genes expressed in neurosensory progenitors within the otic epithelium and continues to be expressed through postnatal stages. SOX2 regulates both sensory and non-sensory development of the inner ear. SOX2 and CHD7 have been shown to physically interact in neural stem cells, but whether Chd7 and Sox2 genes interact functionally, and the consequences of this interaction, remain elusive. Objective: Our goal is to examine inner ear phenotypes in mice with heterozygous deletion of both Sox2 and Chd7 and to determine the underlying mechanisms leading to these phenotypes.

Design/Methods: We examined postnatal survival of doubly heterozygous Sox2CreER/+;Chd7Gt/+ mice in comparison to wild type (Sox2+/+;Chd7+/+) and single heterozygous (Sox2CreER/+ or Chd7Gt/+) littermates. To characterize inner ear phenotypes, we measured cochlear length, paint-filled the inner ear labyrinth structure and stained whole-mounted cochlear sensory epithelia with Phalloidin (Actin) or antibodies to MYO7A, Neurofilament, or Prox1. To identify downstream genetic targets of Chd7 and Sox2, we performed bulk RNA sequencing on otocysts microdissected from Sox2+/+;Chd7+/+ , Chd7Gt/+ , Sox2CreER/+ and Sox2CreER/+;Chd7Gt/+ E10.5 embryos.

Results: Sox2CreER/+;Chd7Gt/+ mice exhibited earlier postnatal death compared to wild type or single heterozygous mutant littermates. The vestibular system of Sox2CreER/+;Chd7Gt/+ mice showed severe anterior, posterior, and lateral semicircular canal dysplasia along with malformed endolymphatic duct and absent ampullae and utricles. Cochleae from Sox2CreER/+;Chd7Gt/+ mice were significantly shortened and exhibited supernumerary outer hair cells with corresponding supporting cells. Sox2CreER/+ mice also exhibited supernumerary inner hair cells but normal cochlear length. Neither hair cell stereociliary bundle orientation nor cochlear innervation were affected by single or double heterozygous deletion of Sox2 and Chd7. Differential gene expression analysis via RNA sequencing revealed additive effects of CHD7 and SOX2 on gene expression in the E10.5 otocyst. Notably, Dlx1 and Dlx2 expression was significantly down-regulated in doubly heterozygous (Sox2CreER/+;Chd7Gt/+) otocysts.

Conclusions: SOX2 and CHD7 functionally interact to regulate development of the inner ear, likely through changes in target gene expression. Ongoing experiments are aimed at determining whether changes in convergent extension, cell proliferation, cell death, or a combination of these mechanisms contribute to the cochlear defects observed with heterozygous deletion of both Sox2 and Chd7.

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**Synapse-specific enhancement of AMPA receptor function by synaptically released zinc in mouse auditory cortex**
Poster #:	55

Authors: Philip T.R. Bender*, Mason McCollum, Benjamin Z. Mendelson, Charles T. Anderson

Affiliation: West Virginia University School of Medicine, Department of Neuroscience, Rockefeller Neuroscience Institute

The primary auditory cortex is crucial for the perception of sounds. This cortical area is highly enriched in synaptically released zinc which acts as a potent modulator of cortical function and sound processing. Zinc (as Zn2+) is loaded into synaptic vesicles by the zinc transporter protein ZnT3 where it is coreleased with glutamate during synaptic transmission. Synaptically released zinc shapes multiple aspects of synaptic signaling and can modulate AMPA and NMDA glutamate receptor function.

The primary auditory cortex contains highly organized networks of neurons that form precise synaptic microcircuits to process auditory information. Despite the importance of synaptic microcircuit organization of the cortex and the different functional properties of these synapses for auditory function, synaptic- and circuit-level mechanisms that support the diversity and specificity of these connections are less-well understood. Mounting evidence strongly suggests that synaptic zinc is released from a subset of glutamatergic cortical neurons and in a cortical layer-specific manner. Since the principles governing cortical microcircuit organization relate to both cortical layer and neuronal type, this suggests that synaptic zinc may contribute to the specificity and diversity of intracortical synaptic microcircuits.

Here, to understand the role of synaptic zinc signaling at specific cortical synapses, we made acute brain slices of the auditory cortex, and performed whole-cell patch-clamp recordings from identified layer 5 corticocollicular neurons and layer 5 corticocallosal neurons. We optogenetically stimulated distinct classes of presynaptic neurons and recorded the synaptic inputs. We also measured the effects of synaptic zinc on these same neuronal populations in awake mice during sound processing using in vivo 2-photon calcium imaging. We identify novel roles for synaptically released zinc in differentially enhancing AMPA receptor function at specific glutamatergic cortical synapses. We also identify a novel role for synaptic zinc in increasing the sound-frequency tuning bandwidth of specific layer 5 neurons in awake mice.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **Synaptic zinc enhances the response to oddball sounds by corticocollicular neurons in mouse auditory cortex**

Poster #: 56

Authors: Mason McCollum* & Charles T. Anderson

Affiliation: West Virginia University

The primary auditory cortex is crucial for the perception of sound. This structure integrates ascending auditory information with a variety of corticocortical and thalamocortical input and sends descending long-range axonal projections to subcortical auditory processing areas such as the striatum, thalamus, inferior colliculus, and brainstem. As a monosynaptic connection from the auditory cortex, these long-range axonal projections allow cortical input to directly influence the activity of neurons in subcortical auditory structures. In layer 5 of the auditory cortex, one major class of long-range projection neuron is pyramidal tract (PT)-type corticocollicular neurons. These neurons are tuned to a broad range of sound frequencies and have long and variable latencies in their sound-evoked responses, making them well-suited to integrate recent acoustic experience important for encoding acoustic context. The mechanisms supporting acoustic integration and context encoding are currently unknown. Although it is known that these long-range corticocollicular neurons are important for dynamic tuning, auditory learning, and auditory plasticity, much less is understood about the intracortical synaptic mechanisms that shape their activity and influence on auditory context encoding.

The auditory cortex is highly enriched with synaptic zinc. Zinc (as Zn2+) is loaded into presynaptic vesicles by the membrane zinc transporter protein ZnT3 and is coreleased with glutamate during synaptic transmission. Synaptic zinc is a potent modulator of synaptic signaling and refines the sound-encoding properties of layer 2/3 auditory cortical neurons in a cell-type specific manner. Since long-range projection neurons integrate information from a variety of local sources and convey these computations to multiple targets, they are the key link between cortical processing and subcortical signaling. However, the effect of synaptic zinc signaling on the context-encoding of corticocollicular neurons remains unknown. Deficits in context encoding are observed in psychiatric disorders such as schizophrenia which manifest as deficits in the ability to detect changes in the acoustic environment, deficits in processing the emotional content of speech, decreased tone matching performance, and deficits in corollary discharge. Since schizophrenia is associated with reduced ZnT3 expression in the cortex, this suggests a link between synaptic zinc and normal context encoding.

Here, we performed widefield and 2-photon calcium imaging from PT-type corticocollicular neurons in layer 5 of the auditory cortex in awake mice. We find that synaptic zinc signaling helps support the encoding of acoustic context by enhancing the responses to oddball tones in a train of standard tones. In mice with global and conditional deletion of ZnT3, these neurons have a greatly diminished ability to respond to these sounds. Our experiments reveal a novel role for synaptic zinc in shaping the context encoding of corticocollicular long-range projection neurons in the auditory cortex.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **The cellular environment after hair cell death is characterized by Fgf10 expression**

Poster #: 57

Authors: Elannah N. Venhaus*, Sydney N. Sheltz-Kempf, Paige V. Blinkiewicz, Elizabeth M. Ketchum, Jeremy S. Duncan

Affiliation: Department of Biological Sciences, Western Michigan University

The organ of Corti (OC), the highly organized auditory sensory epithelium of the mammalian inner ear, contains hair cells (HCs) and supporting Cells (SCs). Loss of HCs in humans can lead to extensive cellular rearrangement and eventual formation of a non-sensory flat epithelium. A comprehensive understanding of the cellular environment expressed after loss of hair cells will help guide future manipulation techniques designed to repair and regenerate a functional OC. We have found a methodology that consistently produces this phenotype. Here, we utilize the Pou4f3DTR mouse line and show that total HC loss occurs after a single diphtheria toxin (DTX) injection at one week of age. Inducing HC death at this time-point consistently results in the formation of a uniform flat epithelium across the mouse cochlea. Both inner and outer HCs and their corresponding SCs begin to undergo apoptosis 3 days after the initial injection and are completely lost within 5-7 days after the DTX injection. In situ hybridization and RNAscope were performed at various timepoints, revealing a high level of Fgf10 expression throughout the remaining cells, and the absence of Gata3, Sox2, and BMP4. This Fgf10 expression remains high until adulthood. These findings suggest that the gene expression of the remaining cells after hair cell death are molecularly unique and do not express typical supporting cell markers.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **The role of the calcium channel blocker verapamil on hearing**

Poster #: 58

Authors: Selin Yalcinoglu*, Amaar Wattoo, Rod Braun, Avril Genene Holt

Affiliation: Wayne State University

Background

Hearing loss affects approximately 48 million Americans. Increased spontaneous neuronal activity often occurs in auditory pathways following hearing loss. One of the leading hypothesis is that after hearing loss the reduction in the afferent input to the ear leads to central hyperactivity to compensate for the decrease of input (Schrode et al., 2018). Voltage-gated calcium channels function to moderate neuronal activity. Therefore, we examined the effect of the L-type calcium channel blocker, verapamil, in normal hearing and noise-exposed rats. The response of the auditory nerve and the inferior colliculus during auditory brainstem response (ABR; wave I and wave V respectively) was tested.

Methods

Twenty-five male Sprague-Dawley rats were divided into four groups (n = 5 - 7/group) and given either verapamil (30 mg/kg) or saline solution intraperitoneally. The treatment groups were: a) no noise exposure plus verapamil (n=5), b) no noise exposure plus saline (n=6), c) noise exposure plus verapamil (n=7), and d) noise exposure plus saline (n=7). The noise groups were unilaterally exposed to a 16 kHz, 106 dB SPL tone for one hour, while no noise control groups were maintained in ambient noise conditions for an equal amount of time. For ABR analysis both amplitudes (wave I and V) and thresholds were evaluated. The assessment was performed at two different frequencies (12kHz and 20 kHz) and time points (one and five days after treatment).

Results

Verapamil administration did not have any negative effect on the hearing threshold. In fact, when the noise groups had a temporary threshold shift (TTS) verapamil decreased the recovery time. The administration of verapamil had an effect on the amplitudes of both ABR waves assessed. In no noise conditions, administration of verapamil (n=5) caused a significant increase in wave V amplitude compared to the saline group (n=6) one day after treatment (8.4% at 12 kHz, $p<0.05$). The wave V/I ratio in the no-noise saline group at 12 kHz one day after treatment was 0.393 and in the no-noise verapamil group at 12 kHz one day after treatment was 0.667 ($p<0.12$). The wave V/I ratio in the no-noise saline group at 20 kHz one day after treatment was 0.523 and in the no-noise verapamil group at 20 kHz one day after treatment was 0.667 ($p<0.13$). The wave V/I ratio for the verapamil no noise group was significantly increased (95% at 12 kHz, $p<0.02$) five days after verapamil treatment.

Conclusion

Our results demonstrate that verapamil may increase gain in the inferior colliculus. In the future studies should focus on further understanding the relationship between changes in neuronal activity, voltage-gated calcium channels, and susceptibility to noise-induced hearing loss.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **The transcription factor Pou4f3 is essential for the survival of mouse cochlear hair cells at early postnatal ages through adulthood**

Poster #: 59

Authors: Jarnail Singh* , Michelle R. Randle, and Brandon C. Cox

Affiliation: 1.Department of Pharmacology, 2.Department of Otolaryngology, Southern Illinois University School of Medicine

The critical role of the transcription factor Pou4f3 in regulating survival of the mammalian cochlear hair cells (HCs) during embryonic development has previously been demonstrated using germline knockout and mutational studies. Here, we investigated the role of Pou4f3 in the survival of HCs at early postnatal ages through adulthood in the mouse cochlea. Additionally, we investigated the long-term effects of HC loss caused by Pou4f3 deletion, with a focus on the survival of surrounding supporting cells (SCs) and spiral ganglion neurons. We used the tamoxifen inducible CreER-loxP system (PrestinCreERT2:Pou4f3loxP/loxP) to delete Pou4f3 from outer hair cells (OHCs) at juvenile or adult ages; and Atoh1-CreERTM:Pou4f3loxP/loxP to delete Pou4f3 from both OHCs and inner hair cells (IHCs) at the neonatal age. Tamoxifen was injected on two consecutive days, in neonatal mice at postnatal day (P) 0 and P1, in juvenile mice at P12 and P13, and in adult mice at 4 weeks or 8 weeks of age. Auditory brainstem response (ABR) measurements were performed to assess hearing function. Significant loss of HCs and increased ABR thresholds were observed at all ages of Pou4f3 deletion. Many TUNEL-positive HCs were observed within 5-7 days after Pou4f3 deletion from HCs, which suggests that HCs die by apoptosis. Using real-time qPCR, we observed downregulation of genes associated with HC survival and the genes known to be downstream of Pou4f3, with upregulation of genes associated with apoptosis. At 16 weeks after Pou4f3 deletion, there was no significant loss of SCs irrespective of the age when Pou4f3 was deleted. However there appeared to be a significant loss of spiral ganglion neurons, quantification of these data is ongoing. In conclusion, Pou4f3 deletion from HCs, regardless of the postnatal age it was deleted, causes apoptotic death of HCs, decreased hearing function and loss of auditory neurons, but without significantly affecting the survival of surrounding SCs in mouse cochlea.

Funding: Supported by NIH/NIDCD R01 DC014441

FULL ABSTRACT
PODIUM PRESENTATION

Title: **Tonotopic differences in stereocilia remodeling confirm Its role in maintaining resting tension in the mechanotransducer of mammalian auditory hair cells**

Poster #: 60

Authors: Abigail K. Dragich*, Sara Gonzalez-Velez, Isabel Aristizábal-Ramírez, A. Catalina Vélez-Ortega, Gregory I. Frolenkov

Affiliation: University of Kentucky

In order to detect soft sounds, auditory hair cells tension their mechano-electrical transduction (MET) machinery at rest. A classical model developed for non-mammalian hair cells postulates that myosin motors at the upper end of the tip links climb along the actin cores of stereocilia, applying constant upward tension. However, some reports suggest that proteins associated with the upper end of the tip link may have limited mobility, at least in mammalian auditory hair cells. Therefore, we explored an alternative mechanism based on recent findings that transducing stereocilia in the hair bundle retract or elongate when resting current through the MET channels is decreased or re-established, correspondingly. We argued that MET-dependent retraction of stereocilia may either: i) not affect resting MET current if the upper end of the tip link is freely moved by myosin motors or, ii) increase resting MET current if it is somehow locked to the stereocilium actin core. Therefore, we have recorded MET currents in young postnatal cochlear outer hair cells (OHCs) using fluid-jet deflections of stereocilia while initiating MET-dependent stereocilia retraction by blocking MET channels either with tubocurarine or with negative hair bundle deflection. In both these experiments, presumable stereocilia retraction caused a prominent post-stimulus increase in resting MET currents which persisted for several seconds. Drugs disrupting actin polymerization inhibited recovery of these post-negative bundle deflection MET current “overshoots”. These data suggest that: i) actin polymerization is indeed involved in regulation of the resting tension within MET machinery; and ii) the upper end of the tip link is likely to be locked to the stereocilia actin core. Interestingly, our new experiments showed that these MET current “overshoots” have a prominent apex-to-base gradient. They are significantly larger in OHCs at the apex of the cochlea than at the base. Therefore, we used scanning electron microscopy to measure and compare the extent of MET-dependent stereocilia retraction in apical, middle, and basal OHCs after incubation of the organs of Corti for 2 hours with a saturating concentration of the MET channel blocker, tubocurarine. We observed more remodeling of transducing stereocilia in apical OHCs and only minimal remodeling in the basal OHCs. Thus, the observed tonotopic differences further support the role of MET-dependent stereocilia remodeling in self-adjusting the resting tension within the MET machinery of mammalian auditory hair cells.

FULL ABSTRACT
PODIUM PRESENTATION

MIDWEST AUDITORY RESEARCH CONFERENCE
presented by the Kresge Hearing Research Institute

Title:	**T-stellate neurons provide excitatory synaptic input to NPY and VIP neurons in the inferior colliculus**
Poster #:	61

Authors: Yoani Herrera*, Michael Roberts

Affiliation: Kresge Hearing Research Institute, University of Michigan

The inferior colliculus (IC) is the auditory processing hub of the midbrain and is important for the processing of speech and other vocalizations. T-stellate neurons in the ventral cochlear nucleus (VCN) send information to the IC about sound frequency and fluctuations in the sound envelope and have been implicated in the processing of vocalization cues. In addition, T-stellate neurons are one of only three neuron types that receive input from the auditory nerve and project directly to the IC. However, the specific neuronal populations that T-stellate cells target in the IC, and how T-stellate cells contribute to IC cell excitability, remain widely unknown. Recently, our lab identified two novel classes of IC neurons: glutamatergic VIP neurons and GABAergic NPY neurons. NPY and VIP neurons together represent ~55-75% of stellate cells within the IC. With whole-cell patch-clamp recordings and optogenetic circuit mapping, we investigated the prevalence and dynamics of T-stellate cell projections to both VIP and NPY neurons to determine how T-stellate neurons influence distinct neuronal populations in the IC. We show for the first time that both VIP and NPY neurons receive functional synaptic input from T-stellate cells. Optogenetic stimulation of T-stellate terminals within the IC elicits excitatory post-synaptic potentials (EPSPs) in many NPY and VIP neurons, and these EPSPs undergo short term synaptic depression. Interestingly, the EPSPs evoked by T-stellate input to NPY neurons are typically larger in amplitude than those evoked in VIP neurons. In addition, activation of T-stellate terminals elicited both direct excitation and feedforward inhibition in several VIP neurons, suggesting that T-stellate afferents may recruit local inhibitory circuits in the IC. Together, these results show that T-stellate neurons functionally project to distinct populations of excitatory and inhibitory neurons within the IC, and these projections can also engage local inhibitory circuits. Since T-stellate projections to the IC may be involved in IC selectivity for vocalization cues, future steps include in vivo modulation of T-stellate projections to determine how T-stellate neurons contribute to frequency selectivity and amplitude modulation selectivity in VIP and NPY neurons.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **VIP signaling modulates the excitability of medial geniculate neurons in mice**

Poster #: 62

Authors: Luis M. Rivera-Perez*, Jina Patel, Michael T. Roberts

Affiliation: Kresge Hearing Research Institute, University of Michigan

Neurons in the medial geniculate (MG), the thalamic relay center of the ascending auditory system, express receptors for vasoactive intestinal peptide (VIP), a neuropeptide that plays significant signaling roles in several brain regions. Our lab uncovered that VIP neurons in the inferior colliculus (IC) project to the MG and express VIP mRNA, suggesting that these neurons are a potential source of VIP signaling to the MG. The somatosensory thalamus is potently influenced by VIP signaling, but it remains unknown whether VIP has a similar effect on the excitability of MG neurons. We hypothesized that VIP modulates the excitability of MG neurons and that VIP neurons in the IC are an important source of VIP signaling in the MG. To test this hypothesis, we are using slice electrophysiology, pharmacology, immunofluorescence, and anterograde tracing in MG slices prepared from VIP-IRES-CRE x Ai14 mice of both sexes. Puff applications of 2 µM VIP near the cell soma elicit depolarization in most MG neurons, evoking action potentials in some cells. Currently, we are testing VIP receptor antagonists to determine the mechanism by which VIP depolarizes MG neurons. In addition, by using Cre-dependent viruses to fluorescently label VIP neurons in combination with anti-VIP immunofluorescence we obtained preliminary data showing that IC VIP neuron terminals in the MG express the VIP peptide. Thus, VIP signaling from the IC to the MG may play an important and previously unappreciated role in modulating auditory processing in the tectothalamic pathway.

FULL ABSTRACT
PODIUM PRESENTATION

Title:	**Vocalization categorization behavior explained by a feature-based auditory categorization model**
Poster #:	63

Authors: Manaswini Kar * (1,2,4), Marianny Pernia (1,2), Kayla Williams (1), Satyabrata Parida (1), Nathan A. Schneider (1,2,4) , Isha Kumbam (1) , Madelyn McAndrew (1,4), Srivatsun Sadagopan (1,2,3,4,5)

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Vocal communication sounds (human speech or animal calls) are produced with a high degree of between- and within-subject variability in diverse listening conditions, over which listeners must generalize to accomplish call categorization. The behavioral strategies and neural mechanisms that support this ability to generalize are largely unexplored. A previous theoretical model from our lab proposed that call categorization can be accomplished by detecting features of intermediate complexity that best contrast each call category from all other categories. We further demonstrated that some neural responses in the primary auditory cortex were consistent with such a model. In the current work, we asked whether this feature-based model could predict call categorization behavior. To this end, we first trained both the model and guinea pigs on call categorization tasks using natural guinea pig calls. We next tested the categorization performance of the model and guinea pigs using temporally and spectrally altered calls. Both the model and guinea pigs were surprisingly resilient to a wide range of temporal manipulations including changes to tempo, reversal, and changes to inter-syllable intervals, but sensitive to moderate shifts in frequency content of the calls from the natural range. Critically, the performance of the model quantitatively matched guinea pig behavior to a remarkable extent. We gained further insights into possible strategies animals could be using to categorize calls by adopting different model training strategies and examining features that contributed to solving specific tasks. Our results validate the feature-based model, lending support to the idea that the auditory pathway might learn task-relevant features of intermediate complexity to optimally accomplish categorization tasks.

FULL ABSTRACT
PODIUM PRESENTATION

Title: **A framework for simplified, high-quality electrophysiology**

Poster #: 64

Authors: Alexis Paez, Rio Vetter, Daryl Kipke

Affiliation: NeuroNexus

Life science research encompasses a broad range of applications that are as unique as the labs performing them. Recent technological advancements in microelectrode arrays, multimodal data acquisition and data analytics introduced tools to create unique experimental workflows for each application. Here we present the Summa Framework, empowering both experienced and new neuroscience labs to conduct electrophysiology recordings with the most advanced and largest selection of multichannel electrodes and state-of-the-art data acquisition equipment. Supported by a well-defined consultative process, optimal tools from the framework are chosen before each experiment. Use of these tools then boosts researcher confidence in reliable data from the experiment's start to finish.

The core of the Summa Framework, silicon electrode arrays, have a nearly infinite design space, with specific arrangements developed for different targets, e.g., auditory cortex (Solyga and Barkat, 2022), auditory thalamus (Lohse et al., 2021), inferior colliculus (Land and Kral, 2022), deep posterior auditory field (Yusuf et al., 2021), both cortical and thalamic targets simultaneously (Ishizu et al, 2021), etc. We also expand silicon probe technology to include the new SiNAPS probe with active-pixel technology (Angotzi et al., 2019).

Three-dimensional visualization of each electrode array configuration within the target region is newly automated by the Radiens Analytics software suite. The software also controls data acquisition through the SmartBox Pro, hardware expressly designed to scale for high-channel count experiments in a cost-effective, off-the-shelf equipment solution. The simplicity of each component of the Summa Framework allows researchers to spend less time on configuring their equipment and more time on collecting data.