IEB Inner Ear Biology Tübingen 2025

60th INNER EAR BIOLOGY WORKSHOP

14 – 16 September 2025, Tübingen

Symposium "Molecular Otology"
13 September 2025, Tübingen

ieb2025.eu

PROGRAMME and ABSTRACTS

Hosts: Hubert Löwenheim and Anthony W. Gummer

Universitätsklinikum Tübingen

EBERHARD KARLS UNIVERSITÄT TÜBINGEN

IEB Inner Ear Biology Tübingen 2025

Venues

Congress Venue

Innovationszentrum Westspitze GmbH | Eisenbahnstraße 1 | 72072 Tübingen

Lunch

Neckawa | Wöhrdstraße 25 | 72072 Tübingen

Welcome Reception | Saturday, 13 September | 17:00 – 19:00

Innovationszentrum Westspitze GmbH | Eisenbahnstraße 1 | 72072 Tübingen
Rooftop of the Congress venue (Innovationszentrum)
Fingerfood and drinks are provided

Afterwork Meeting | Sunday, 14 September | 19:00 – 23:00

AV Guestfalia Tübingen | Stauffenbergstraße 25 | 72074 Tübingen
Food and drinks are provided

By foot: approx. 30 min; we suggest avoiding high heels for the walk
By bus: Line 10 from Neckarbrücke (Neckar Bridge); Stop at Wielandshöhe. Bus every 30 min. Tickets are available from the ticket machines on the Neckar Bridge. Payment can be made by EC card, credit card, mobile phone (NFC) or coins
For a taxi: +49(0)7071-920555

Networking Event | Monday, 15 September | 19:00 – 23:00

Museum 1821 | Wilhelmstraße 3 | 72074 Tübingen
By foot: approx. 17 min
For a taxi: +49(0)7071-920555

Organisation

Local

Dr. Katharina Bader
PD Dr. Sven Becker
Dr. Aurore Brugeaud
Dr. Christopher R. Cederroth
Dr. Ernst Dalhoff
Prof. Dr. Anthony W. Gummer
Dr. Karina Gültig
Prof. Dr. Steffen Hage
PD Dr. Csaba Harasztosi
Prof. Dr. Marlies Knipper
Prof. Dr. Hubert Löwenheim
Prof. Dr. Ellen Reisinger
Prof. Dr. Lukas Rüttiger
Dr. Anke Tropitzsch
Dr. Stephan Wolpert
Gabrielle Zeller

International

Prof. Jonathan F. Ashmore (London)
Prof. Jonathan Gale (London)
Prof. Piotr H. Skarżyński (Warsaw)
Prof. Huib Versnel (Utrecht)

Conference Chairs

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Prof. Dr. Anthony W. Gummer
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External Conference Management

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Logo Design

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We would like to thank the following institutions and companies for supporting the Inner Ear Biology Workshop and Symposium

Research Organization:

Sponsors and Partners:

Saturday, 13 September 2025

Innovationszentrum Westspitze GmbH | Eisenbahnstraße 1 | 72072 Tübingen

08:30 – 09:30	**Registration**
09:30	**Opening** Hubert Löwenheim and Anthony Gummer
09:30 – 09:40	**Welcome** Boris Palmer (Lord Mayor of Tübingen)
09:40 – 09:50	**Welcome** Hendrik Rosewich (Speaker, Centre for Rare Diseases, Tübingen)
09:50 – 10:00	**Welcome and Introduction to the Symposium** Hubert Löwenheim
10:00	**Session 01: Advanced Genetic Diagnostics, Drug Delivery and Drug Therapy** Moderatos: Ronald Pennings, Anke Tropitzsch
10:00 – 10:30	Eliot Shearer (Harvard University) “Diagnostic Evaluation for Hearing Loss in the Era of Gene Therapy”
10:30 – 10:50	Anil K. Lalwani (Columbia University) “Microneedle-Mediated Safe and Precise Inner Ear Delivery”
10:50 – 11:10	Christoph Arnoldner (Vienna University) "Drug Delivery Studies in the Large Animal Model Pig"
11:10 – 11:30	Jonathan Kil (Sound Pharmaceuticals) “Drug Development and Clinical Trials”
11:30 – 12:00	**Round Table** Moderators: Ronald Pennings, Anke Tropitzsch Panelists: Eliot Shearer, Anil Lalwani, Christop Arnoldner, Jonathan Kil
12:00 – 13:00	**Lunch**
13:00	**Session 02: Current Gene Therapy Trials** Moderators: Hinrich Staeker, Hubert Löwenheim
13:00 – 13:20	Géraldine Honnet (Sensorion) "SENS-501 Gene Therapy in Young Children with Severe to Profound Hearing Loss Due to Otoferlin Mutations"
13:20 – 13:40	Carleton E. Corrales (Regeneron) “Safety and Efficacy of DB-OTO Gene Therapy in Children with Profound Deafness due to Otoferlin Variants: Data from the CHORD Phase 1/2 Open-label Trial”
13:40 – 14:00	Aaron Tward (Akouos/Lilly) "Clinical Development of AK-OTOF Gene Therapy for OTOF-mediated Hearing Loss: Preliminary Results"
14:00 – 14:20	Yuxin Chen (Eye & ENT Hospital, Fudan University) "Gene Therapy vs Cochlear Implantation in Restoring Hearing Function and Speech Perception for Congenital Deafness Individuals"

14:20 – 15:00	**Round Table** Moderators: Hinrich Staecker, Hubert Löwenhein Panelists: Géraldine Honnet, Carleton Corrales, Aaron Tward, Yuxin Chen
15:00 – 15:30	**Break**
15:30	**Session 03: Stem Cell Therapies & Organoids** Moderators: Athanasia Warnecke, Thore Schade-Mann
15:30 – 15:50	Heiko Locher (Leiden University Medical Center) "Mapping and Modeling the Human Inner Ear: RNA Atlases, Organoids, and Clinical Applications"
15:50 – 16:10	Pascal Senn (Geneva University) "From Regenerating Auditory Neurons To Drug Screening"
16:10 – 16:30	Marcelo Rivolta (Rinri Tx) “Recent Advances in the Development of a Pluripotent Stem–Cell Based Therapy for the Treatment of Hearing Loss”
16:30 – 17:00	**Round Table** Moderators: Athanasia Warnecke, Thore Schade-Mann Panelists: Heiko Locher, Pascal Senn, Marcelo Rivolta
17:00 – 19:00	**Welcome Reception** at the Rooftop of the Congress venue

08:15 – 08:20		**Opening** Anthony Gummer and Hubert Löwenheim
08:20 – 08:30		**In Memoriam A. James Hudspeth** Dáibhid Ó Maoiléidigh
08:30		**Session 1: Biophysics of the Cochlea – Stereocilia** Chairpersons: K. Charaziak, J. Ashmore
08:30 – 09:00	**O1**	**Target Lecture:** **Modelling the ear from the ground up: Physics, physiology, and pathology** **O. Tichráček*
	O2	Identification of TMEM145 as a principal component of outer hair cell stereocilia **D. Derstroff, M. Flook, A. Löhnes, S. Newton, V. Renigunta, S. Hanemaaijer, C. Aguilar, J. Holt, M. Bowl, D. Oliver, K. Reimann*
09:15 – 09:30	O3	PIP_2-Tmie interactions drive mammalian hair cell slow adaptation independently of myosin motors *G. Caprara, Y. R. Kim, S. Jun, S. Li, U. Kim, J. B. Shin, *A. Peng*
09:30 – 09:45	O4	Viscoelasticity explains fast adaptation in outer-hair-cell bundles *R. Chatterjee, *D. Ó Maoiléidigh*
09:45 – 10:00	O5	The stereocilia plasma membrane is actively regulated by TMCs to optimize mechanotransduction sensitivity **A. J. Ricci, S. George, T. Effertz*
10:00 – 10:30		**Break**
10:30		**Session 2: Biophysics of the Cochlea – OHC Soma to IHC Afferents** Chairpersons: D. Ó Maoiléidigh, A. Peng
10:30 – 10:45	O6	Sub-membranous chloride levels at the outer hair cell lateral membrane **J. Santos-Sacchi, W. Tan, D. Navaratnam*
10:45 – 11:00	O7	Hearing at high frequencies depends on piezo-electric outer hair cells **J. Ashmore*
11:00 – 11:15	O8	Cochlear dispersion shapes processing of dynamic sounds **K. Charaziak*
11:15 – 11:30	O9	Stimulus level, but not stimulus frequency, is spatially coded at the apex of the cochlea **A. Fridberger, W. Zaidi, P. Hakizimana, G. Burwood, A. Nuttall*
11:30 – 11:45	O10	*In vivo* spontaneous activity of type 1 spiral ganglion neurons in the pre-hearing mammalian cochlea **C. Palfrey, F. De Faveri, F. Ceriani, W. Marcotti*
11:45 – 12:00	O11	The properties of intrinsic lateral olivocochlear feedback to the cochlea throughout maturation, ageing, and hearing dysfunction **A. Carlton*
12:00 – 13:00		**Lunch**
13:00 – 14:30		**Poster Presentations I** P1 – P14: Biophysics of the Cochlea P15 – P25: Development P26 – P42: Hearing Loss 1/2

14:30		**Session 3: Molecular Insights to Make Auditory Connections** Chairpersons: B. Fritzsch, G. Pavlinkova
14:30 – 14:45	O12	Eya1-Six1-regulatory network in inner ear development and hair cell formation **P.-X. Xu*
14:45 – 15:00	O13	Restoration of Insm2 attenuates the abnormalities of Insm1-deficient cochlear outer hair cells *S. Li, *Z. Liu*
15:00 – 15:15	O14	SHANK2 establishes auditory hair bundle architecture essential for mammalian hearing **J. Bok*
15:15 – 15:30	O15	Development of ear and cochlea nuclei requires Irx3/5 and Lmx1a/b **B. Fritzsch*
15:30 – 15:45	O16	Regulatory networks of NEUROD1 and ISL1 shape auditory neuron development and tonotopic map formation **G. Pavlinkova, L. Gmiterkova, L. Lebron-Mora, R. Bohuslavova, K. Pysanenko, J. Syka, B. Fritzsch*
15:45 – 16:00	O17	Disentangling the various unknowns in the interface of sound and hair-cell electrical characteristics **E. Yamoah*
16:00 – 16:30		**Break**
16:30		**Session 4: Pathomechanisms of Hearing Impairment** Chairpersons: S. Pyott, E. Reisinger
16:30 – 17:00	**O18**	**Target Lecture:** **Which types of hearing loss can be reversed?** **K. Steel*
17:00 – 17:15	O19	Spectrotemporal deficits in patients with DFNB8/12 (*TECTA*), DFNB16 (*STRC*), or DFNA13 (*COL11A2*) **B. Bel Hach, M. van de Craats, R. Pennings, M. van Wanrooij, *C. Lanting*
17:15 – 17:30	O20	Identifying the mechanisms and potential new treatments for noise-induced hearing loss using metabolomics and lipidomics **G. Corfas, G. Wallace , L. Ji, C. Lyssiotis, C. Burant, M. Kachman*
17:30 – 17:45	O21	The TECTB-C225Y variant causes autosomal dominant deafness in a Nicaraguan family enhances sensitivity to noise-induced hearing loss in ageing mice *E. B. Hale, B. Vona, R. J. Goodyear, R. T. Osgood, S. S. Amr, K. Mojica, R. Vera-Monroy, K. Callahan, K. L. Gudlewski, R. Quadros, C. C. Morton, C. Gurumurthy, J. E. Saunders, G. P. Richardson, *A. A. Indzhykulian*
17:45 – 18:00	O22	Noise-induced hearing loss, cochlear synaptopathy, and a mouse mutant for increased inner hair cell presynaptic excitability **J. Engel, K. Blum, P. Schepsky, P. Derleder, P. Schätzle, F. Nasri, P. Fischer, S. Kurt*
19:00 – 23:00		**Afterwork Party** AV Guestfalia Tübingen, Stauffenbergstraße 25, 72074 Tübingen

08:30		**Session 5: Schwann Cells and Macrophages in Inner Ear Disease and Regeneration** Chairpersons: J. Kempfle, T. Kaur
08:30 – 09:00	**O23**	**Target Lecture:** **Peripheral myelin disorders and the mechanisms of hidden hearing loss** **G. Corfas, L. Cassinotti, L. Ji, D. Kohrmann, B. Borges, G. Wan*
09:00 – 09:15	O24	Auditory phenotype in neurofibromatosis type 2 is associated with Schwann cell dysfunction and peripheral myelinopathy **J. Kempfle, L. Cassinotti, S. Myoung, A. Zhang, R. Kuang, D. Montigny, B. Welling, G. Corfas, D. Jung*
09:15 – 09:30	O25	Important roles of auditory nerve glial cells and extracellular matrix molecules in noise-induced hearing loss **H. Lang, J. Barth, E. Fabrizio-Stover, K. Harris*
09:30 – 09:45	O26	Harnessing macrophages for the treatment of noise-induced hidden hearing loss **T. Kaur*
09:45 – 10:00	O27	Molecular signatures of cochlear injury: Unveiling oxidative and inflammatory drivers in acquired SNHL **A. Pisani, F. Paciello, V. Mohamed Hizam, B. Hassler, C. Grassi, A. R. Fetoni*
10:00 – 10:30		**Break**
10:30		**Session 6: MED-EL Symposium** **Investigating Inner Ear Health to Optimise CI Outcomes** Chairperson: A. Warnecke
10:30 – 10:32		**Introduction by the Chair**
10:32 – 11:02	O28	Pathological substrates in Meniere's disease and novel imaging-based stratification for predicting cochlear implant candidacy *A. H. Eckhard*
11:02 – 11:15	O29	The status of the auditory nerve in CI candidates *R. Glueckert*
11:15 – 11:28	O30	Perilymph sampling in human: The *state of the art* *T. Melchionna*
11:28 – 11:30		**Concluding Remarks by the Chair**
11:30 – 12:00	**O31**	**Spoendlin Junior Award Lecture** A single-nucleus atlas of human prenatal inner ear development reveals cell type vulnerabilities and regulatory mechanisms *W. H. van der Valk*
12:00 – 13-00		**Lunch**
13:00 – 14:30		**Poster Presentations II** P43 – P58: Hearing Loss 2/2 P59 – P67: Schwann Cells and Macrophages P68 – P82: Gene Therapy, Drug Delivery, Genetic Screening

14:30		**Session 7: Genetic Screening** Chairpersons: K. Steel, R. Pennings
14:30 – 14:45	O32	Divergent modes of hair cell bundle maturation in developing and mature mouse utricle **A. Cheng, A. Mahmoudi, T. Wang, R. Cai, K. Xu, K. Miller, J. He, N. Mohit, K. Giffen, T. Jan, N. Grillet*
14:45 – 15:00	O33	Cochlear implantation outcomes in genotyped individuals with sensorineural hearing loss: Reassessing the spiral ganglion hypothesis **C. Lanting, M. Fehrmann, L. Haer-Wigman, H. Kremer, H. Yntema, M. Thijssen, E. Mylanus, W. Huinck, R. Pennings*
15:00 – 15:15	O34	412 families' whole genome sequencing in early onset hearing loss: The French Reference Center's experience **S. Marlin*
15:15 – 15:30	O35	Single-nucleus multiomics and spatial transcriptomics define heterogoneity and spatial niches in the human fetal utricle **A. Dabdoub, W. Liang, R. Yamamoto, E. Luca*
15:30 – 15:45	O36	MDAtlas: A Meniere disease multiomic data resource of genes and cells *K. Bagheri, P. Cruz-Granados, *J. A. Lopez-Escamez*
15:45 – 16:00	O37	Cost-effective genetic screening for newborn hearing loss using a high-density SNV microarray **C.-Y. Tsai, Z. S. Shih, M. F. Chiu, Y. S. Lu, M. Y. Lo, Y. T. Chen, C. H. Lin, C. J. Hsu, P. L. Chen, H. J. Su, P. N. Tsao, C. C. Wu*
16:00 – 16:30		**Break**
16:30		**Session 8: From Basic Research to Novel Therapies** Chairpersons: W. Marcotti, J. Gale
16:30 – 16:45	O38	Investigating AAV-mediated gene therapies in a mouse model of progressive hearing loss **A. Zanella, M. Hool, W. Marcotti*
16:45 – 17:00	O39	Development of hiPSC-derived inner ear organoids harboring GJB2 mutation to model genetic inner ear hearing loss **W. van den Boogaard, D. Vinke, W. H. van der Valk, N. Geijsen, P. Shang, H. Locher*
17:00 – 17:15	O40	Optimizing tropism of human MSC derived extracellular vesicles for inner ear applications through peptide coating **A. Warnecke, P. Huang, X. Pan, Y. Li, H. Staecker*
17:15 – 17:30	O41	Tmprss3 expression in the mouse cochlea **R. Arora, L. Pifková, A. Deutschmann, E. Reisinger*
17:30 – 17:45	O42	Dose response and toxicity evaluation of TMPRSS3 gene therapy delivered either by AAV2 or novel next generation AAV capsid variant *J. Marx, P. Huang, A. Warnecke, H. Büning, *H. Staecker*
17:45 – 18:00	O43	A novel AAV capsid and promoter design for GJB2 gene therapy **K. Kamiya*
19:00 – 23:00		**Networking Event** Museum 1821, Wilhelmstraße 3, 72074 Tübingen

08:30		**Session 9: Protection, Repair, Regeneration** Chairpersons: I. Varela-Nieto, H. Löwenheim
08:30 – 08:45	O44	Single-cell RNA sequencing identifies organ-specific progenitor populations in the mouse otocyst *X. Bao, S. Chakraborty, J. Liu, *J. Waldhaus*
08:45 – 09:00	O45	The function of NFI transcription factors in cochlear differentiation and hair cell regeneration **A. Doetzlhofer, C. Morgan*
09:00 – 09:15	O46	Unraveling Notch-mediated regulation of hair cell regeneration in the auditory system **L. Khalaily, S. Kasirer, K. Domb, B. Shao, S. Taiber, M. Sperber, O. Loza, L. Tao, R. Elkon, D. Sprinzak, K. B. Avraham*
09:15 – 09:30	O47	Chemistry-dependent efficacy of antisense oligonucleotides for DFNA9 across human cell and mouse models *F. Aben, E. Fousert, D. Verdoodt, W. van den Boogaard, S. de Bruijn, J. Oostrik, L. Sels, K. Szewczyk, W. H. van der Valk, P. Ponsaerts, H. Locher, V. Van Rompaey, E. van Wijk, *E. de Vrieze*
09:30 – 09:45	O48	NOX4 as a key regulator of cochlear redox homeostasis during ageing **I. Varela-Nieto, M. Tapias-Martin, J. Contreras, J. M. Zubeldia, S. Murillo-Cuesta*
09:45 – 10:00	O49	Evidence of otoconia biogenesis outside of the macular organs **D. M. Correa, R. Osgood, A. A. Indzhykulian, S. Krämer, A. H. Eckhard*
10:00 – 10:30		**Break**
10:30		**Session 10: Speech Processing, Auditory Perception and Cognition** Chairpersons: A. Fetoni, M. Knipper
10:30 – 11:00	**O50**	**Target Lecture:** **Hearing and cognition across the life course: Evidence from behaviour, electrophysiology, and brain stimulation** **H. Nuttall*
11:00 – 11:15	O51	Use of OPM-MEG for auditory processing and cognition-related hearing disorders **R. A. Donoso-San Martín, S. M. Wolpert, S. Fink, P. H. Delano, C. Braun, L. Rüttiger, M. Knipper*
11:15 – 11:30	O52	Psychiatric comorbidity in patients with tinnitus or auditory hallucination and sleep evaluation, sound therapy and Shared Decision Making (SDM) **K. Kiyomizu, T. Nakamura, K. Takahashi, H. Funahashi*
11:30 – 11:45	O53	Alterations in the auditory cortex synaptic activity contribute to early noise-induced cognitive decline in a mouse model of Alzheimer's disease **F. Paciello, V. Mohamed Hizam, C. Vernamonte, A. Pisani, M. Rinaudo, A. R. Fetoni, C. Grassi*
11:45 – 12:00	O54	Hearing loss differentially impacts behavioral assays of memory *S. Hemachandran, *A. J. Ricci*
12:00 – 13-00		**Lunch**

13:00 – 14:30		**Poster Presentations III** P83 – P90: Protection, Repair, Regeneration P91 – P96: Cochlear Implants P97 – P105: Speech Processing, Auditory Perception, Cognition

14:30		**Session 11: Novel Approaches to Cochlear and Cortical Stimulation and Assessment in Primates** Chairpersons: M. Jeschke, S. Hage
14:30 – 15:00	**O55**	**Target Lecture:** **Neural coding mechanisms in auditory cortex** **X. Wang*
15:00 – 15:15	O56	Translational auditory gene therapy in non-human primates: From genetic modeling to optogenetic restoration **M. Jeschke, R. Behr, F. Benseler, A. Berenson, N. Brose, T. Bruegmann, C. Drummer, T. Kahland, K. Kusch, D. L. Lindenwald, T. Mager, A. Meyer, T. Moser, A. Rambousky, N. Rüger, O. Tkachenko Eikel, M. Uhl, B. Wolf, S. S. Yalamarthi*
15:15 – 15:30	O57	The electrically-evoked compound-action potential-derived Failure Index as neural health marker **W. Konerding, A. Günther, P. Baumhoff, C. Batsoulis, S. Vormelcher, S. Strahl, H. Benav, A. Büchner, A. Kral*
15:30 – 15:45	O58	Model-based characterisation of auditory nerve responses to electrical stimulation *R. Felsheim, *D. Sly, S. O'Leary, M. Dietz*

15:45 – 16:30		**Business Meeting**
		Close of Workshop

	Biophysics of the Cochlea
P1	Mitochondrial heat production in the apical region of outer hair cell
	**N. Harada, Y. Ito, A. Kawabata*
P2	Towards a unified structural understanding of ion transport by SLC26 anion transporters and force generation by mammalian prestin
	**M. F. Kuwabara, D. Oliver*
P3	The role of O-GlcNAcylation in the inner ear
	**A. U. Deutschmann, T. Bartling, E. Reisinger*
P4	Signal transmission at the ribbon synapses
	**F. Stella, G. Cheli, S. Masetto, M. Masin, M. Astolfi, G. Rispoli*
P5	PKCα-dependent interaction of otoferlin with calbindin or myosin VI modulates inner hair cell endocytosis
	**A. U. Deutschmann, E. Reisinger, A. P. Cepeda, H. Al-Moyed, C. Lenz, H. Urlaub*
P6	Motion of the organ of Corti during prolonged solution exposure
	**J. Ashmore, J. Hwang*
P7	Low-frequency acoustics biasing of distortion-product otoacoustic emissions in a cochlear model
	**V. Vencovský, A. Vetešník*
P8	Odd-even mode acoustic analysis of the bat cochlea: A theoretical model explaining hearing gaps in horseshoe bats
	**Y. Horii*
P9	Signal complexities in low-frequency short-pulse distortion product otoacoustic emissions
	**D. Zelle, K. Bader, E. Dalhoff*
P10	Determination of the pre-neural state of the inner ear with short-pulsed DPOAE level maps in normal and hearing-impaired ears
	**K. Bader, S. Kempa, E. Dalhoff*
P11	Ultrastructural analysis of the cellular and synaptic architecture of the mouse vestibular periphery, with consideration of quantal versus non-quantal transmission in the vestibular calyx
	**A. Lysakowski, M. Hameed, F. Imran, T. Madappallil, J. Oruganti*
P12	The contribution of KNa channels to peripheral vestibular structure and function
	**V. G. Paplou, N. M. A. Schubert, S. J. Pyott*

P13	Threshold tuning curves of utricular calyceal afferent neurons in guinea pig, with model extension to mouse, rat, sheep and human
	**R. Rabbitt, C. Pastras*
P14	Physiological limitations to parent-to-embryo acoustic communication in zebra finches
	**T. Anttonen, H. Loning, F. M. Felbo, S. C. Griffith, M. Naguib, J. Christensen-Dalsgaard, C. P. Elemans*

	Development
P15	K^+ currents of chicken embryo vestibular type-I and type-II hair cells do not involve the KCNQ4 subunit
	**G. Cheli, R. Giunta, F. Stella, F. Borgo, E. Guidi, G. Rispoli, G. Russo, D. Lazarevic, S. Masetto*
P16	Understanding the neural basis of hearing function and dysfunction in vivo
	**A. Aveta, W. Marcotti, F. Ceriani, A. Nikolaev*
P17	*In vivo* calcium imaging of spontaneous activity in the prehearing mouse cochlea
	**F. Ceriani, F. De Faveri, Y. Zhao, W. Marcotti*
P18	*In vivo* investigation of spontaneous activity in the prehearing outer hair cells
	**Y. Zhao, F. De Faveri, F. Ceriani, W. Marcotti*
P19	Exploring overlapping and distinct interaction partners of $G\alpha i_2$ and $G\alpha_{i3}$ in the inner ear
	**J. R. Tischlarik, G. Bauer, B. Nürnberg, E. Reisinger*
P20	Validation of potential G protein interaction partners in auditory hair cells of mice
	**L. Ording, G. Bauer, B. Nürnberg, E. Reisinger*
P21	Pertussis toxin, an inhibitor of Gαi/o proteins, impairs neurite outgrowth in cultured spiral ganglion neurons
	**A. Marimuthu, A. Deutschmann, V. Devanathan, B. Nürnberg, E. Reisinger*
P22	Molecular role of ISL1 in spiral ganglion neuron development
	**L. Gmiterkova, L. Lebron-Mora, R. Bohuslavova, M. Tavakoli, P. Abaffy, J. Syka, B. Fritzsch, G. Pavlinkova*
P23	Tmprss3 deficiency affects electrophysiology and morphology of SGN-like organoids
	*A. U. Deutschmann, *L. Pifkova, B. Findik, M. Klingenstein, A. Betz, M. Klimiankou, J. Skokowa, S. Liebau, E. Reisinger, S. Klingenstein*
P24	Developmental transcriptomic profiling of non-sensory vestibular epithelium reveals ionic contribution to endolymph formation
	**S. Kwak, S. Kim*
P25	A low-molecular-weight compound mimicking IGF-1 repairs excitatory amino acid-induced synaptic damage via *Klotho* suppression similar to IGF-1
	**T. Nakagawa, Y. Fukunaga, K. Nishimura, K. Omori*

	Hearing Loss 1/2
P26	Hearing loss characterization in DBA/1JRj and DBA/2JRj mouse models
	*D. Fefeu, M. Aincy, E. Rodrigues, C. Coyat, M. Souchal, *K. Toupet, S. Pucheu, G. Naert*
P27	Cochlear Kir4.1 regulation in aging and immunodeficient mice: Implications for sensorineural hearing loss mechanisms
	**L. Astolfi, S. Fracaro, A. Trotta, F. Hellies, S. De Simone, E. Simoni, G. Albertin*
P28	Delayed synaptic aging in the cochlea of long-lived naked mole rats (*Heterocephalus glaber)*
	**K. Castaño González, L. A. Buendía Sánchez, S. Pyott*
P29	Understanding high frequency vulnerability in age-related hearing loss
	**J. Y. Jeng*
P30	Longitudinal study of otoacoustic emissions in a rat model of age-related hearing loss
	**M. Petremann, V. Kaden-Volynets, K. Charaziak, H. Löwenheim, J. Dyhrfjeld-Johnsen*
P31	Hair cell apoptosis triggered by reduction in stereociliary PMCA2 Ca2+ pump density
	**R. Fettiplace, A. Rolseth, M. Beurg*
P32	Early detection of age-related hearing loss in mice using machine learning and auditory brainstem responses
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Session 01: Advanced Genetic Diagnostics, Drug Delivery and Drug Therapy

Diagnostic Evaluation for Hearing Loss in the Era of Gene Therapy

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BACKGROUND: A comprehensive diagnostic evaluation is required prior to administration of any gene therapy for patients with sensorineural hearing loss (SNHL). For pediatric patients with SNHL, imaging, congenital CMV testing, and genetic testing form the cornerstones of diagnosis. Although prevalence estimates for genetic SNHL approximate 50-60%, actual genetic testing yields average 43%. This discrepancy suggests a missing heritability in genetic SNHL using available sequencing methods. In addition, genetic testing may occur later than is optimal for treatment with gene therapy. Our lab is evaluating new methods to improve the diagnostic yield of genetic testing for hearing loss and to reduce time to diagnosis with genetic newborn hearing screening.
METHODS: We have recruited a cohort of pediatric patients with SNHL with varied audiologic phenotypes. We typically use a stepwise approach to diagnosis starting with either gene panels or exome sequencing. Short-read genome sequencing (srGS) and/or long-read genome sequencing (lrGS) are subsequently performed for undiagnosed patients. We have also begun a trial for newborns for whom we perform ultra-rapid comprehensive hearing loss genetic screening simultaneously with physiologic hearing screening.
RESULTS: 637 pediatric SNHL patients underwent genetic evaluation during the study period (2019-2024), including 234 gene panels, 465 exomes, 56 srGS, and 30 lrGS. The overall diagnostic rate was 30.9% with 42.3% for symmetric SNHL. Diagnoses were made in 65 genes, with *GJB2* (29.4%) and *STRC* (14.4%) being the most prevalent. 27.4% (n=54) of all diagnoses were syndromic. Gene panel diagnostic yield was 41.2% (n=63); performing ES on gene-panel negative probands added 9 additional diagnoses (13.0%). Diagnostic rate of srGS following ES was 1/56 (1.8%). LrGS following nondiagnostic srGS yielded 6/30 (20%). LrGS resolved variants in segmental duplications (*OTOA)* and genes with highly homologous pseudogenes (*STRC*). Results from a further ~50 patients sent for lrGS are pending. We have developed a platform for ultra-rapid genetic newborn hearing screening with automated analysis and results in less than 48 hours. We have performed this screening protocol on 105 subjects to date with enrollment ongoing.
CONCLUSIONS: When compared with gene panels, ES and srGS only marginally increase diagnostic yields. LrGS shows promise over srGS to better identify variants in difficult-to-sequence and noncoding genomic regions. Genetic newborn hearing screening holds promise as a method for earlier diagnosis for genetic hearing loss.

Microneedle-Mediated Safe and Precise Inner Ear Delivery

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Protected by one of the hardest bones in body, the cochlea is nearly an impenetrable structure frustrating both bacteria and clinician trying to gain access to it. As a result, a means for reliable delivery of agents into the inner ear for therapeutic purposes remains a formidable challenge. No method currently exists to provide effective and precisely dosed delivery of therapeutics to the inner ear without risking permanent damage to the patient's hearing. We believe that an elegant solution to overcome the difficulties of intracochlear delivery is to use microneedles to facilitate reliable and predictable intracochlear delivery across the RWM without anatomic or functional damage. Intracochlear drug administration has been shown to be superior to transtympanic injection and results in significantly higher and less variable drug levels. In addition, there is a much smaller concentration gradient from base to apex, as is typical of transtympanic injection, resulting in a more even distribution of material. With the availability of a reliable method of inner ear delivery, targeted delivery to hair cells, spiral ganglion neurons or other intra-cochlear structures could be accomplished for the treatment of variety of auditory and vestibular disorders such as sudden or progressive SNHL, Ménière's Disease, and tinnitus.

Drug Delivery Studies in the Large Animal Model Pig

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Question: With its complex 3-dimensional structure, its seclusion deep within the temporal bone, and its isolation from the systemic circulation through the blood-labyrinth barrier, the cochlea remains a challenging target for drug delivery. Moreover, there is an evident lack of preclinical models, which can be used to study drug delivery techniques as well as vector delivery to the cochlea.

Methods: We established the pig as a promising large animal model to study cochlear drug delivery in a humanoid inner ear. Using this model, we investigated different delivery methods including intratympanic and intracochlear delivery, using different delivery appliances such as catheter systems, hydrogel or PLGA formulations, and explored the delivery of separate compounds and particles, such as steroids, viral vectors, or new molecules such as AC102.

Results: Initially, we studied the porcine cochlea's size using histological and micro-CT analysis and found its basilar membrane's length of roughly 33mm closely resembling the human's, while its volume exceeding rodent cochleae's volumes by up to 10-fold. Subsequently, we investigated and compared different delivery drug techniques to the pig's cochlea. We found that formulation of drugs such as dexamethasone into carriers, which provide sustained drug release, e.g. hydrogels or PLGA-implants, can effectively prolong residual time of the compound into the cochlea. Alternatively, changing the delivery technique itself can influence intracochlear drug distribution, such as the use of an intracochlear delivery catheter boosting apical and total cochlear drug levels. Furthermore, by studying the innovative new molecule, AC102, which possesses highly different molecular properties compared to conventional otoprotective drugs, we observed interesting distribution patterns within the cochlea and its sensory and neural tissues, which may revolutionize the way we study inner ear drug delivery. Lastly, the pig model was found to be suitable to study viral vector-mediated cochlear transgene delivery. Here, different spatial and cellular transduction patterns were found depending on the specific serotype of viral vector used.

Conclusion: The pig cochlea's anatomical and physiological similarity to the human inner ear renders this model as a highly suitable large animal model to study cochlear drug and viral vector delivery. With the use of this model, we are able to close the apparent translational gap between small animals and humans facilitating research and translation of new delivery strategies and gene therapy approaches.

Drug Development and Clinical Trials

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The time and costs of developing a new drug are estimated to take 12-15 years and approximately $1.2-1.6B in the US. In addition, of 12-15 investigational new drugs that enter clinical trials, only one is ultimately approved by the FDA. In the field of inner ear drug development, only one drug has been approved over the last 30 years of clinical trials. In September 2022, PEDMARK® (sodium thiosulfate or STS), received FDA-approval to reduce the risk of hearing loss (ototoxicity) in pediatric patients 1 month and older being treated with cisplatin-based chemotherapy for localized, non-metastatic solid tumors (typically hepatocellular adenocarcinomas). STS first received FDA approval in 1992, 2011, and 2012 and is delivered intravenously to treat cyanide poisoning and other indications. STS's drug development path involving the prevention of cisplatin ototoxicity is an example of repurposing where an approved drug is tested and approved for a new indication. Over the last 20 years, 15+ companies have attempted to develop new investigational drugs or repurpose approved drugs for the treatment of a hearing loss or tinnitus indication. Unfortunately, all but one drug has failed to achieve Phase 3 study success. This presentation will highlight the development and clinical trial success of SPI-1005, the first investigational new drug to achieve its co-primary endpoints in a pivotal Phase 3 randomized double blinded placebo-controlled trial (RCT) to treat Meniere's disease (STOPMD-3). In addition, SPI-1005 has also achieved positive safety and efficacy results in four other RCTs involving MD, acute noise-induced hearing loss, and aminoglycoside-induced ototoxicity.
SPI-1005 is an oral capsule that contains ebselen, a novel selenorganic compound that mimics and induces glutathione peroxidase (GPx1) activity that crosses the cochlear and blood brain barrier. GPx1 is a critical enzyme that repairs injured and dying cells in the inner ear, retina, prefrontal cortex of brain, lung, and kidney, that is often reduced during and after exposure to environmental insults or aging. Consequently, neuroinflammation can progress throughout the peripheral and central nervous system leading to neurodegeneration. To date, no significant drug-drug interactions have been observed across multiple study populations that have enrolled 790 patients with 600+ more anticipated over the next year. SPI-1005 is being tested across 5 inner ear disease INDs and 2 neuropsychiatric indications.

Session 02: Current Gene Therapy Trials

SENS-501 Gene Therapy in Young Children with Severe to Profound Hearing Loss Due to Otoferlin Mutations

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Background: Otoferlin mutation is responsible for 2-8 % of congenital severe to profound non syndromic hearing loss.
Objectives: This First in Man study intends to assess safety, tolerability, and efficacy of SENS-501 following unilateral intracochlear injection in children between the ages of 6-31 months with hearing loss due to a mutation in the Otoferlin gene.
Methods: The study consists of a dose-escalation part including two cohorts of three patients each, assessing sequentially a low and high dose of SENS-501 followed by a dose-expansion cohort at the selected dose recommended by the Data Monitoring Committee. While safety is the primary endpoint of the dose escalation study, Auditory Brainstem Response, twelve months following the injection, will be the primary endpoint for the dose expansion part
Results: To date 5 patients have received an intracochlear gene therapy injection (SENS-501). SENS-501 and the corresponding surgical procedure were uneventfully and well tolerated by all participants (aged 6 to 31 months and naive of cochlear implants at the time of the injection, as per study protocol) with no serious adverse events reported.
In Cohort 1 investigating the low dose, in one patient aged 11 months at the time of injection, early signs of hearing improvement were observed 3 months post injection using standard hearing tests. A positive response at ABR (Auditory Brainstem Response) was reported at two frequencies, with the best frequency reaching 70 dB compared to absence of response at baseline. An improvement of hearing levels of the Pure Tone Audiometry (PTA) across two frequencies with best frequency reaching 90 dB level compared to absence of response at baseline. Meaningful changes in responses to sounds and voices were reported by the parents with an IT-MAIS score increase of 16 points (145% relative improvement from baseline), and met expected auditory milestones based on an age-based parent questionnaire and according to the patient's age (LittlEARS).
Conclusions: The surgical procedure and perioperative period for each patient was uneventful. SENS-501 shows a good safety profile without any dose limiting event and preliminary positive data from the first cohort. The next step is to complete the recruitment of the second cohort at a higher dose.

Safety and Efficacy of DB-OTO Gene Therapy in Children with Profound Deafness due to Otoferlin Variants: Data from the CHORD Phase 1/2 Open-label Trial

Carleton E. Corrales
Regeneron

This talk will focus on DB-OTO. DB-OTO is a dual AAV1 vector designed for intracochlear delivery of *OTOF* to treat infants and children with profound deafness due to pathogenic *OTOF* variants. Twelve participants have been enrolled to date: 9 were dosed unilaterally and 3 were dosed bilaterally. DB-OTO was well tolerated, with no DB-OTO–related AEs or SAEs. The delivery has been well tolerated overall, with only transient vestibular AEs. 10 out of the 11 participants with ≥1 post-treatment assessment showed improved hearing. Among 5 participants with Week 24 assessments: 3 participants showed improvement in hearing thresholds to ≤40 dBHL and 2 participants showed improvement in hearing thresholds to ≤25 dBHL. In unilaterally treated participants, no ABR or PTA response was observed in the contralateral untreated ear (with the cochlear implant turned off when applicable). In the first participant, there were clinically meaningful improvements in the DB-OTO–treated ear across tested frequencies. Hearing thresholds were within normal limits in most speech-relevant frequencies (0.5–2.0 kHz) and were associated with positive ABR responses. Formal speech perception testing without a device was measured at 22 and 27 months of age; preliminary results are encouraging and are corroborated by the family's observations. The CHORD trial is enrolling in the US, UK, Spain and Germany (clinicaltrials.gov: NCT05788536).

Clinical Development of AK-OTOF Gene Therapy for OTOF-mediated Hearing Loss: Preliminary Results

Aaron Tward
on behalf of the AK-OTOF trial and study site teams and Akouos, Inc. (a wholly owned subsidiary of Eli Lilly and Company)

Background: The otoferlin gene (*OTOF*) encodes otoferlin, a protein critical for signaling at inner hair cell synapses; individuals with *OTOF* mutations initially present with congenital, Severe to Profound sensorineural hearing loss, with preserved otoacoustic emissions. Advances in gene therapy and intracochlear delivery support potential hearing restoration in individuals with *OTOF*-mediated hearing loss using a one-time, local administration of AK-OTOF (AAVAnc80-hOTOF). This multicenter Phase 1/2 clinical trial (NCT05821959) evaluates the investigational medicinal product, AK-OTOF, and the investigational medical device, the Akouos Delivery Device, used to administer AK-OTOF to the intracochlear space.
Materials and Methods: Eligible participants have Profound hearing loss, as assessed by auditory brainstem response (ABR), at baseline and receive, using a minimally invasive transcanal approach, a single intracochlear administration of AK-OTOF in one ear. Safety assessments and hearing restoration, including by ABR and behavioral audiometry testing, are assessed over the one-year trial and an additional four-year long term follow-up period.
Results: The first participant, an 11-year-old, experienced restored hearing within 30 days of AK-OTOF administration; behavioral thresholds were 65 to 20 dB HL. The second participant, an 8-year-old, also experienced restored hearing within 30 days of AK-OTOF administration. The surgical administration and AK-OTOF were well tolerated, and no trial-related serious adverse events have been identified as of the date of this report. Safety and efficacy data from these and additional participants will be presented.
Conclusions: Interim data suggest that AK-OTOF may be safely administered to patients with onset of hearing restoration as early as one month following administration.

Gene Therapy vs Cochlear Implantation in Restoring Hearing Function and Speech Perception for Congenital Deafness Individuals

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Question: Gene therapy (GT), a novel treatment for congenital deafness, improves hearing and speech in patients with *OTOF* gene mutations. Among patients with congenital deafness, is GT more efficacious than CI in auditory and speech perception? To investigate the issue, we compared the efficacy of GT and CI for congenital deafness comprehensively.

Methods: This is a single-center, observational study. Participants, with congenital hearing loss, aged 1-18 years, received GT or CI, were enrolled. They were matched on duration of deafness, hearing threshold, and speech ability at the pre-surgical baseline. The primary outcomes include auditory and speech perception evaluated by questionnaires including the Infant-Toddler Meaningful Auditory Integration Scale (IT-MAIS)/MAIS, Categories of Auditory Performance (CAP), Speech Intelligibility Rating (SIR), and Speech, Spatial, and Other Qualities of Hearing Scale for Parents (SSQ-P) and tests including audiometry, speech perception in quiet or noisy environment, and music. The secondary outcome includes auditory information processing ability analyzed by mismatch negativity (MMN) recorded by electroencephalogram.

Results: Between December 2022 and November 2024, 72 participants were enrolled, including 11 GT patients and 61 CI patients. Participants received follow-up at 3, 6, or 12 months. Based on the hearing modality, participants were analyzed: GT-only patients vs matched CI patients, and bimodal patients (unilateral GT + contralateral CI) including GT+CI vs bilateral CI and GT (CI-off) vs unilateral CI. The average auditory brainstem response threshold was restored from >95 dB to 55 dB in 9 GT patients at 12 months. For GT-only vs CI, GT patients performed better in IT-MAIS/MAIS, SIR, SSQP-speech, SSQP-other qualities, and SSQP noise related scores at 6 months, and better in IT-MAIS/MAIS and CAP scores at 12 months. GT patients showed shorter latencies of MMN at 6 months. For bimodal patients at 12 months, GT (CI-off) patients performed better than unilateral CI patients in speech perception in noise; GT+CI patients performed better than bilateral CI patients in singing in-tune rates, and showed shorter latencies of MMN at 12 months.

Conclusions: GT patients show stable hearing recovery, more rapid improvement in auditory and speech perception, and better speech in noise as well as music perception, compared with CI patients. It is the first systematic study comparing the efficacy of GT and CI for congenital deafness, offering preliminary functional evidence for future clinical decision-making.

Session 03: Stem Cell Therapies & Organoids

Mapping and Modeling the Human Inner Ear: RNA Atlases, Organoids, and Clinical Applications

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Our laboratory investigates the molecular mechanisms underlying human inner ear development and models this process using inner ear organoids derived from human induced pluripotent stem cells (iPSCs). This approach enables the study of early cell fate specification, the identification of key developmental pathways, refinement of organoid protocols, and the investigation of inner ear disorders and therapeutic strategies.

To provide a reference framework for normal development, we constructed HIEDRA, a single-cell RNA atlas of the human inner ear spanning the first and second trimesters. HIEDRA captures the emergence of sensory, supporting, neuronal, and secretory lineages, and will be made publicly available to support the broader inner ear research community. In addition to charting cellular diversity and developmental trajectories, the atlas highlights disease-relevant populations, offering insight into both congenital and acquired inner ear disorders.

Human inner ear organoids derived from iPSCs are emerging as a powerful in vitro platform to study sensorineural hearing loss. These 3D models recapitulate key aspects of human inner ear development and cellular complexity, potentially allowing investigation of many inner ear disorders. We applied this model to assess its potential for disease modeling and intervention. First, we demonstrated its use in studying ototoxicity caused by cisplatin and aminoglycosides. Second, we exposed organoids to congenital cytomegalovirus (CMV) infection to explore virus-specific inner ear tropism. Third, we modeled genetic forms of hearing loss using patient-derived iPSCs and tested therapeutic intervention through RNA-based antisense oligonucleotide (ASO) therapy.

Together, these efforts aim to establish robust, human-relevant inner ear models for understanding disease mechanisms and accelerating therapeutic development for auditory and vestibular disorders.

From Regenerating Auditory Neurons To Drug Screening

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Cochlear explants, the current gold standard for in vitro drug testing in the auditory field, are unsuitable for large-scale drug screens, as less than a dozen explants can be harvested from one animal. Billions of primary cell types would be needed to address all requirements needed to develop a new drug. To introduce a new molecule as a clinical therapy in humans, several thousands of candidate molecules have to be screened in the first place, which is not realistic using the gold standard murine cochlear explant models.
We have previously identified and characterized the phoenix auditory neuroprogenitors (ANPGs) as highly proliferative progenitor cells isolated from the cochlea of a specific strain, called A/J mouse. These ANPGs have a virtually unlimited capacity to self-renew >40 generations. In a subsequent study, we aimed at identifying signaling pathways responsible for the intrinsic high stemness. A transcriptomic comparison of traditionally low stemness ANPGs, isolated from C57Bl/6 mice and high stemness phoenix ANPGs from A/J mice was performed. Based on the differentially expressed pathways, we reprogrammed low-stemness ANPGs with a strategic pharmacological combination of a WNT agonist and TGFβ/Smad inhibitors, which resulted in a remarkable increase in the growth of presenescent neurospheres, effectively allowing the expansion of ANPGs on an extensive scale. The so-called stemness-induced ANPGs exhibited the favorable property of being freezable and thawable, facilitating their distribution to other research facilities. Importantly, even after more than 20 generations, stemness- induced ANPGs retained their capacity to differentiate into electrophysiologically active type I-like auditory neurons.
Both the stemness-induced and phoenix ANPGs represent a significant breakthrough in addressing a major bottleneck in auditory research. They offer an efficient, high-throughput, cost-effective, and 3R compatible approach for in vitro screening of potential otoprotective and otoregenerative drug candidates. The next steps include to upgrade this platform incorporating human auditory neuroprogenitors and to transfer the methodology to hair cell, which remains a formidable challenge today.

Recent Advances in the Development of a Pluripotent Stem-Cell Based Therapy for the Treatment of Hearing Loss

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Disabling hearing loss is a growing global concern with significant social, personal, and economic impacts. While sensory hair cells are essential for hearing, mounting evidence suggests that the loss of neural connections between hair cells and the brainstem often precedes and exceeds hair cell damage, as seen in conditions like presbycusis. Auditory neuropathy is another example highlighting the importance of cochlear neural health, characterized by neuronal loss despite preserved hair cell function. Currently, no disease-modifying therapies exist; hearing aids and cochlear implants are the only available interventions. Cochlear implants can partially compensate for hair cell loss, but no regenerative treatment for cochlear neuron degeneration exists to date.

To address this, we are using human pluripotent stem cells to target the auditory nerve. We have shown that we can restore auditory thresholds in a gerbil model of auditory neuropathy by transplanting hESC-derived otic neuroprogenitors (hONPs) into the cochlear nerve. To model cochlear implantation, we used a fully implantable rodent stimulator with an electrode activated by a magnetic field. Such studies demonstrated functional integration between the transplanted cells and the implant.

To evaluate safety, we conducted long-term studies exploring the distribution and behaviour of transplanted cells. Animals were followed for up to a year, with whole-body MRI scans performed at termination to identify any potential lesions. Additionally, biodistribution studies for human-specific DNA sequences were performed by QPCR. No tumours attributed to the test article were found and there was no detection of cells spreading systemically. Similar safety studies were undertaken using the Rag2/Il2rg (SRG) double knock-out rat. Adapting the research-grade process to industrial manufacturing standards has not altered the properties of the cell product.

Rinri is now preparing to initiate a first-in-human trial using Rincell-1, a preparation of ONPs derived from human embryonic stem cells. A novel surgical approach for injecting Rincell-1 into the cochlear nerve via the round window has also been developed. The initial clinical trial will focus on assessing the safety and early efficacy of Rincell-1 as an adjunct to a cochlear implant in patients with neural hearing loss.

Session 1: Biophysics of the Cochlea – Stereocilia

O1

Modelling the ear from the ground up: Physics, physiology, and pathology

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For auditory research to benefit fully from today's detailed biological data, our models must do more than fit input - output curves; they should link cochlear mechanics, hair-cell electrophysiology and neural coding in one testable framework. Sensorineural hearing loss complicates the picture, as it is a mosaic of pathologies - ranging from stereocilia damage and K^+-channel mutations to ribbon-synapse loss and neural-degeneration - all of which leave distinct fingerprints on the ear's mechanical and electrical output. Relying on one set of hearing-aid gains or cochlear-implant (CI) maps therefore risk "treating the average patient", missing the opportunity for precision audiology. Meeting these individual needs calls for detailed, bottom-up models of the auditory periphery.

To move in that direction, we have built a bottom-up computer model of the mammalian (human) peripheral auditory pathway that spans the outer and middle ears, a two-dimensional cochlear mechanics stage, a three-dimensional longitudinal electrical circuit of all three scalae, detailed hair-cell electrophysiology, ribbon-synapse dynamics, and an auditory-nerve front end. A key innovation is the explicit calculation of hair-cell receptor potentials from mechanotransduction currents, voltage-gated K^+ sub-types and the nonlinear outer-hair-cell (OHC) membrane capacitance. This enables a self-consistent feedback of the OHC receptor potential into the cochlear-amplifier force without ad-hoc assumptions. The model aligns with key experimental benchmarks: it reproduces the ~40 dB active gain, the characteristic level-dependent basilar-membrane input-output curves, and the measured tonotopic map. At the neural level, it also matches the observed distribution of spontaneous firing rates and the forward-masking patterns recorded in the auditory nerve.

The lecture will outline the model's architecture, demonstrate its agreement with key experimental data, and show how this integrated view reframes long-standing questions - for example, how a drop in endocochlear potential simultaneously weakens outer-hair-cell gain and raises inner-hair-cell thresholds, or how ribbon geometry and CaV1.3 channel distribution control neurotransmitter release at the inner-hair-cell synapse. It will also examine which approximations in the physical description are acceptable to capture these effects faithfully. The goal is to spark discussion about what a truly explanatory model of hearing should be and how it can guide future experiments and clinical tools.

O2

Identification of TMEM145 as a principal component of outer hair cell stereocilia

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Question: The superior acuity of mammalian hearing relies on cochlear amplification provided by sensory outer hair cells (OHCs). The amplificatory cellular process is governed by mechanoelectrical transduction of sound by the cell's stereocilia bundle. In OHCs, mechanical stimulation is accomplished through the physical attachment of the stereocilia to the overlying acellular tectorial membrane (TM) through an incompletely characterized attachment complex (TM-AC)[1].

Methods: Here we address the role of previously unstudied transmembrane protein, TMEM145, by in-vivo recordings of hearing function in genetically modified mouse models, high-resolution immunohistochemistry and heterologous expression systems.

Results: Mice with ablated TMEM145 were severely deaf and lacked cochlear amplification, indicating loss of OHC function. TMEM145 was selectively expressed in OHCs. Superresolution (STED) microscopy revealed the ringlike localization at the tips of the largest row of stereocilia, precisely outlining the sites of TM-ACs, and colocalizing with previously known intra- and extracellular TM-ACs components, tubby (TUB) and stereocilin (STRC)[2,3], respectively. Genetic ablation of TMEM145 resulted in the loss of TUB and STRC from OHC stereocilia and in disconnection of the hair bundle from the TM. Vice versa, in STRC knockout mice TMEM145 and TUB were absent from the hair bundle. Physical interaction between TMEM145 and TUB was shown by nanoscale pull-down. Moreover, TUB facilitated plasma membrane targeting of TMEM145, indicating functional interaction of both proteins.

Conclusions: Our results identify TMEM145 as the central organizer of OHC stereocilia attachment complexes. We propose that TMEM145 binds intracellular components (TUB) through its C-terminal intracellular interface and anchors the secreted component, STRC, through its extracellular Golgi-like domain.

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O3

PIP_2-Tmie interactions drive mammalian hair cell slow adaptation independently of myosin motors

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Auditory stimuli are transmitted to the inner ear, eliciting vibrations within the cochlear partition, comprised of hair cells and supporting cells situated atop the basilar membrane and covered by a tectorial membrane. Hair cells serve as auditory mechanoreceptors utilizing apically located hair bundles to transform sound-induced mechanical vibrations into electrical activity, a phenomenon referred to as mechano-transduction. This process is pivotal for cochlear amplification, which underlies exceptional sound level sensitivity, wide dynamic range, and notable frequency discrimination. This mechano-transduction is similar in the vestibular hair cells within the inner ear, which are responsible for balance. With a sustained step-like displacement, the hair-cell receptor current peaks followed by a decrease in the receptor current, termed adaptation. Among these adaptation mechanisms, slow adaptation, characterized by time constants typically ranging from 10-100 milliseconds, was previously hypothesized to operate via a motor model of adaptation. This form of adaptation relies on Ca^{2+} influx through MET channels modulating the attachment of myosin motors along the stereocilia core. Our recent investigations have challenged the motor model of slow adaptation, proposing instead a novel model reliant on the phosphoinositide PIP_2. We found that the reduction of hair bundle PIP_2 levels using PAO results in a reduction of slow adaptation. Previous work by others and us has shown that when the *Myo1c* motor is inhibited in vestibular hair cells, slow adaptation is reduced. Strikingly, we could rescue slow adaptation during *Myo1c* inhibition with exogenous PIP_2, indicating that PIP_2 is the critical component to slow adaptation, and that the myosin motors may only be required to help PIP_2 localize close to the MET channel. Slow adaptation in cochlear hair cells could also be rescued with PIP_2 when slow adaptation was reduced with myosin motor inhibition using intracellular sulfate. Our hypothesis is that PIP_2 directly modulates the MET channel complex as other ion channels, like the structurally similar TMEM16a, are directly modulated by PIP_2. Interestingly, TMIE, an MET channel complex protein, was shown to bind PIP_2. Using a point mutation in TMIE that reduces PIP_2 binding, we found these hair cells exhibit reduced slow adaptation. Our results provide the first data describing a new mechanistic model of slow adaptation in mammalian hair cells.

O4

Viscoelasticity explains fast adaptation in outer-hair-cell bundles

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Question: Outer-hair-cell bundles (OHBs) are required for normal hearing. They transduce their sound-induced deflections into the receptor currents that drive cochlear amplification. The cochlear amplifier is required for our ears' high sensitivity, broad dynamic range, and sharp frequency selectivity. Adaptation maintains the sensitivity of receptor currents to bundle deflections, but the mechanisms underlying adaptation in OHBs remain under debate and how adaptation works at physiologically-relevant frequencies is unclear. We propose and evaluate a mechanism for the fastest components of adaptation in OHBs.

Methods: OHB comprise stereocilia, filamentous rods protruding from the apical surface of the outer hair cell. Neighboring stereocilium pairs of different height are linked by gating springs, which are attached to mechanoelectrical transduction (MET) channels in the shorter stereocilia. To evaluate the feasibility of our proposal, we develop a new mathematical model of the OHB. The model OHB comprises pivoting stereocilia, gating springs, the kinetics of MET-channel gating, and two viscoelastic adaptation elements in series with the MET channels. The morphology of the model OHB is based on published experimental observations.

Results: To calibrate the model, we fit the mathematical model to twelve independent experimental observations. We validate the model by successfully predicting an observation not used for fitting—how much receptor-current sensitivity is maintained by fast adaptation. The experimentally-constrained model predicts the effects of fast adaptation for physiologically-relevant frequencies. The mathematical model shows that there is considerable deflection-current hysteresis, that the receptor current can lead the bundle deflection to a large extent, and that fast adaptation greatly high-pass filters the receptor current. Owing to viscoelastic fast adaptation, the dynamic range of the outer-hair-cell bundle depends on the stimulus frequency. These predictions and others are experimentally testable.

Conclusions: We find that viscoelastic elements in series with the MET channels can account quantitatively for fast adaptation in OHBs. Because viscoelastic fast adaptation in the mathematical model substantially affects receptor current sensitivity, hysteresis, phase leads, high-pass filtering, and dynamic range, we expect viscoelastic fast adaptation to greatly impact the cochlear amplifier and hearing.

O5

The stereocilia plasma membrane is actively regulated by TMCs to optimize mechanotransduction sensitivity

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It is becoming clear that the fluid mosaic model for the plasma membrane is at best an oversimplification of how the lipid bilayer is regulated and also that the lipid bilayer plays a critical role in a multitude of functions that incorporate, electrical, mechanical and biochemical processes. Within the inner ear, it was first suggested that the membrane along the lateral wall was more diffusive than the rest of the outer hair cell, in part due to a reduced level of cholesterol. A growing body of data suggests that the mechanosensory hair bundle represents a unique organelle where the plasma membrane is regulated independently of the rest of the cell. Flippase/floppase activity maintains membrane order while scramblase activity restores disorder creating a dynamic equilibrium. Here we present data demonstrating that transmembrane channel like proteins (TMCs) are scramblases regulated by the open probability of the mechanotransduction channel (MET). The potential dual role of TMCs as part of the pore forming domain of the MET channels and as lipid scramblases implies an overlapping functional relationship. The functional role of this regulations remains to be elucidated; however considerable evidence suggests that mechanotransduction sensitivity is regulated by the plasma membrane. We used reduction in membrane cholesterol as a tool to reduce membrane viscosity (increase diffusivity) and found an increased resting open probability of the MET channel with a reduction in sensitivity to stimulation. We also found a reversible reduction in maximum MET current. These changes occurred with little effect on MET adaptation. We are presently testing two hypotheses. The first suggests that the dynamic equilibrium creates a steady-state condition that sets the resting open probability and sensitivity of the MET machinery, regulating the effective viscoelastic response of the hair bundle. The second hypothesis is inclusive of the first but goes further and suggests that the local change in membrane mechanics controlled by TMC scramblase activity can regulate MET channel sensitivity on a cycle-by-cycle basis. This mechanism could account for fast adaptation and potentially be a mechanism associated with hair bundle amplification.

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Session 2: Biophysics of the Cochlea – OHC Soma to IHC Afferents

O6

Sub-membranous chloride levels at the outer hair cell lateral membrane

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Prestin is responsible for cochlear amplification which supports high frequency hearing. A fundamental property of prestin is its sensitivity to intracellular chloride. Boltzmann fits (2-state-Csa; Santos-Sacchi and Navarrette, Pflugers Arch, 2002) determine Qmax, Vh, z and dCsa, the latter thought to correspond to changes in membrane surface area/thickness that occur in sync with prestin conformational change across voltage. Here we explore the influence of chloride binding on prestin characteristics with the macro-patch technique, and compare to whole cell measures. In guinea pig membrane patches, first on-cell then excised, we measured prestin's Boltzmann parameters during changes under voltage clamp using AC measures of NLC (nonlinear capacitance) at 1 kHz. By comparing on-cell patch characteristics to subsequent excised inside-out patch characteristics perfused with 1, 10 and 150 mM chloride, we are able to estimate chloride levels in the intact OHC. Our data indicates that on-cell Vh is closest to those of excised patches when 1 mM chloride is perfused. Interestingly, dCsa appears unaffected by changes in chloride concentration, though previously salicylate had been shown to increase dCsa. Boltzmann characteristics are also evaluated at frequencies of 2, 4 and 8 kHz. dCsa, while variable, remains constant between 20 and 35 fF across frequency in our patches, with Qmax ranging between 15 and 22 fC at 1 kHz. Qmax and z roll off in frequency for all recording conditions, with Qmax decreasing by 8.3 dB from 1 to 8 kHz for on-cell conditions, like our previous macro-patch measures (Santos-Sacchi et al., J Neuro, 2023). Most evaluations of prestin properties have been garnered with whole cell patch clamp; for example, we estimated sub-membranous chloride to be less than 10 mM by leveraging the relationship between prestin's salicylate sensitivity and whole cell chloride levels (Santos-Sacchi et al., J Neuro, 2006). Our new data indicate levels near 1 mM chloride, and suggest that the sub-plasmalemmal space between the lateral membrane and subsurface cisternae may be tightly buffered by the high density of prestin, a poor anion transporter, within the lateral plasma membrane.

O7

Hearing at high frequencies depends on piezo-electric outer hair cells

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The hearing performance in mammals depends on the proper functioning of the outer hair cells (OHCs). The main cellular mechanism has been thought to depend on a fast potential-driven length change, "electromotility". A number of difficulties have always been recognised when including the electromotility into realisable cochlear models. One in particular is that OHC membrane filtering reduces the receptor potential. Electromotile mechanisms depend on the protein "prestin"/SLC26A5 and there may be bandwidth limits to the molecular conformational change, further suggested by experimental data from in vivo recording. It is shown here that high frequency basilar membrane tuning, particularly important for many mammals, may exploit 1) a piezo-electric property of OHCs ("reverse electromotility") that depends on longitudinal forces being applied to the cells; and 2) the variation of the angle between the tectorial membrane (TM) and the reticular lamina (RL) which increases significantly along the cochlear duct. In the mouse the angle increases to over 60 degrees at the 80 kHz point. This geometry suggests that the RL is severely restrained in vivo that but that large tensioning forces can be applied even to short basal cochlear OHCs by the BM motion acting through the Deiters cells. I shall describe how such sectional cochlear micromechanics can be integrated with the fluid macro-mechanics to produce effective BM amplification above 10 kHz. Although applicable to rodent hearing, such effects may even apply to the upper ranges of human hearing.

O8

Cochlear dispersion shapes processing of dynamic sounds

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The acoustical environment is often a superposition of many competing sounds that, through interactions within the auditory system, can mask or decrease audibility of target signals. While masking by sounds with stationary properties, such as pure tones, is relatively well-studied, the mechanisms of masking by dynamic sounds, which change over time, are not yet fully understood. For instance, upward frequency sweeps, where the instantaneous frequency increases over time, are typically more effective at masking tonal and speech signals than their time-reversed counterparts (downward sweeps). This sensitivity of the auditory system to sweep direction is believed to originate in the cochlea through the traveling wave dispersion that can be either enhanced or compensated for by sweeps. Here, we investigate "masking" as seen directly in cochlear vibrations in mice at ~9-kHz location, when a response to a characteristic frequency (CF) tone is suppressed by competing sounds with varied temporal characteristics, such as sweep direction and rate of frequency change. For sweep rates at least ~10 times slower than the dispersion rate, the up- and down-swept suppressors produced similar suppression. However, when the sweep rate was increased towards the rate of natural dispersion, the up-swept stimuli became more effective suppressors than down-swept ones, as seen in human masking data. While in general stronger suppression was seen for higher sweep intensities, the sensitivity to sweep direction was strongest for lower sweep intensities. These differences can be explained by changes in the frequency tuning of suppression, with the low-frequency side being primarily intensity-dependent, and the high-frequency side depending on the temporal properties of the sweep.

O9

Stimulus level, but not stimulus frequency, is spatially coded at the apex of the cochlea

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Vocal communication relies on the ability to perceive different sound frequencies. Two mechanisms purport to explain how this happens. Since afferent neuron firing can be synchronized with the stimulus waveform, the rate of action potentials in the auditory nerve informs about stimulus frequency. However, the cochlear place where the maximal response occurs also shifts with frequency. Whether rate or place cues are used at the low frequencies that are critical for communication is unclear. To investigate this, we developed a new method to measure hearing organ motion near the lower frequency limit of hearing. Anatomical locations several thousand microns apart had similar frequency response, and tuning curves with several peaks were common. Increasing stimulus level shifted the response to more apical locations, and traveling waves were absent at low frequencies. These results, which are fundamental for understanding how communication-relevant sounds are detected and processed, are incompatible with the place mechanism. Since a place-to-frequency map is used in auditory implants, consideration of these results may lead to better speech perception for implant users.

O10

***In Vivo* spontaneous activity of type 1 spiral ganglion neurons in the pre-hearing mammalian cochlea**

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In the developing peripheral auditory system, intrinsically generated calcium (Ca^{2+}) activity originating in the inner hair cells (IHCs) drives stereotyped bursting activity in afferent fibers of type 1 spiral ganglion neurons (SGNs). Propagation of this activity to the central auditory system is essential for neuronal survival and functional maturation of the auditory circuitry, including the refinement of tonotopic maps (Clause et al, 2014; Kersbergen and Bergles, 2024). The contribution of spontaneous activity to the development of peripheral auditory neurons, however, is yet to be fully elucidated, in part owing to the difficulty of studying cellular function within the cochlea *in vivo*. A novel surgical approach to optically access the intact cochlear sensory epithelium was developed, enabling single-cell resolution two-photon imaging of the genetically encoded Ca^{2+} indicator GCaMP6f expressed in SGNs (De Faveri et al., 2025). Synaptic terminals at the basolateral membrane of IHCs were semi-automatically segmented to quantify dynamics of Ca^{2+} transients. Importantly, it was found that the periodic synchronization of IHC Ca^{2+} activity was necessary to significantly increase the fraction of activated afferent fibers across both single and multiple adjacent IHCs of live mice. Such coordinated activity (previously unidentified in Ca^{2+} imaging studies of *ex vivo* Organ of Corti preparations) is likely essential to drive the patterned bursts of action potentials in developing auditory fibers (Sonntag et al, 2009; Tritsch et al, 2010). Spontaneous Ca^{2+} transients were evident at both the pillar and modiolar membranes of IHCs and demonstrated a trend towards increased size and frequency of activity on the pillar side. This pattern of activity may reflect development of a mature-like functional segregation of sensitive, high-spontaneous rate fibers contacting the pillar side of IHCs while less sensitive, low-spontaneous rate fibers preferentially contact the modiolar side (Markowitz and Kalluri, 2020). To further investigate the timeline of pillar-modiolar functional segregation, *in vivo* imaging of Ca^{2+} transients was paired with comprehensive immunohistological characterization of IHC-SGN synapse subtype and cellular localization across the tonotopic gradient and throughout the first two post-natal weeks. In conclusion, we demonstrate that approaches enabling *in vivo* investigation of cellular mechanisms in the physiologically intact cochlear epithelium are key to furthering our understanding of the development and function of the peripheral auditory system.

O11

The properties of intrinsic lateral olivocochlear feedback to the cochlea throughout maturation, ageing, and hearing dysfunction

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Inner hair cells within the cochlea are solely responsible for the transduction of all perceivable sound information, which is then transmitted to the brain by type I spiral ganglion neurons (SGNs). The activity of these SGNs is modulated by three components arising from the brainstem and, of these, the intrinsic lateral olivocochlear (iLOC) feedback from the lateral superior olive (LSO) has been shown to possess remarkable plasticity. For example, iLOC feedback is cholinergic and excitatory to SGNs by default, but can dynamically upregulate inhibitory neurotransmitters based on sound experience. These neurons also appear to form direct synaptic contacts onto inner hair cells during hearing dysfunction caused by ageing or genetically disrupted sound transduction. The function of this rewiring is unclear, but recapitulates the pre-hearing configuration where inner hair cells receive direct cholinergic input. However, the role and properties of iLOC feedback remains poorly understood, but is hypothesized to assist with sound localisation, hearing in noise, and resistance to sound induced damage. To investigate the underlying biophysical characteristics and better understand iLOC feedback across the lifespan, whole-cell patch clamp electrophysiology was employed at body temperature on mouse brainstem slices containing the LSO followed by fixation, immunolabelling, and biocytin based cell tracing. It was found that mature iLOC neurons possessed the same current threshold as pre-hearing neurons, and lacked the previously reported enhancement of spontaneous activity. However, through the downregulation of *I*A and upregulation of slower outward potassium current, mature iLOC neurons required less charge input to trigger an action potential and could fire at a higher rate with a smaller initial delay relative to pre-hearing neurons. Strikingly, despite how the aged cochlea recapitulates the immature cochlea in terms of iLOC innervation, aged (>1 year) neurons did not show any biophysical reversion, and instead showed further enhanced non-*I*A outward potassium currents resulting in even higher firing rates with faster spikes. Interestingly, while robust inner hair cell reinnervation is a common feature, sound transduction dysfunction caused by post-hearing Myo7a deletion did not shift the biophysics of iLOC neurons as much ageing, and instead only showed minor shifts in cell size. Taken together, this work provides new insights of how iLOC feedback adapts to optimally regulate hearing throughout life and during hearing dysfunction.

Session 3: Molecular Insights to Make Auditory Connections

O12

Eya1-Six1-regulatory network in inner ear development and hair cell formation

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During inner ear development, Sox2-positive proneurosensory progenitors must first be specified in the otic placode to generate the sensory hair cells and neurons essential for hearing. We demonstrate that the transcriptional co-activators Eya1 and Six1 recruit the SWI/SNF chromatin-remodeling complex to activate Sox2 and Neurog1, thereby initiating this lineage. Deleting *Smarca4*, the SWI/SNF ATPase, or simultaneously removing *Eya1* and *Six1* abolishes *Sox2* expression and neurosensory identity and induces apoptosis in the otic ectoderm. At later stages, Six1 binds stage- and subtype-specific cis-regulatory elements, partnering with downstream transcription factors to drive stepwise hair-cell differentiation and establish the distinct subtypes of the organ of Corti. Our recent results also indicate that Six1 is necessary for hair bundle morphogenesis and continues to play a role in hair cell maturation and maintenance.

O13

Restoration of Insm2 attenuates the abnormalities of *Insm1*-deficient cochlear outer hair cells

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The auditory epithelium comprises two types of sensory receptors: inner hair cells (IHCs) and outer hair cells (OHCs). Four transcription factors—Tbx2, Casz1, Insm1, and Ikzf2—are essential for specifying IHC and OHC identities. In *Insm1 -/-* mice, ~50% of OHCs ectopically express Tbx2 and transdifferentiate into IHC-like cells, with this fate switch more frequent in the base than in the apex. However, the underlying mechanism remains unclear. Here, we show that *Insm2* is expressed in OHCs with an apex-to-base gradient. While OHCs develop normally in *Insm2 -/-* mice, *Insm2 -/-; Insm1 -/-* double mutants exhibit a significantly higher frequency of OHC-to-IHC transdifferentiation than the *Insm1 -/-* mice alone. Notably, these ectopic IHC-like cells are enriched in the apex. Moreover, overexpression of *Insm2* partially rescues the OHC phenotype in *Insm1 -/-* mice. These findings identify *Insm2* as a supporting factor for OHC fate maintenance and suggest that it can partially compensate for the loss of *Insm1.*

O14

SHANK2 establishes auditory hair bundle architecture essential for mammalian hearing

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The precise architecture of hair bundles in mammalian auditory hair cells is critical for sound transduction. Each bundle comprises actin-filled stereocilia arranged in a staircase pattern, forming U-shapes in inner hair cells (IHCs) and V-shapes in outer hair cells (OHCs). This architecture develops through lateral movement of the microtubule-based kinocilium, regulated by lateral surface proteins like Gαi and GPSM2. While lateral mechanisms are well-characterized, medial side regulation remains unclear. Our study identifies SHANK2, a protein linked to synaptic function and autism spectrum disorders, as a key regulator establishing the bundle architecture from the medial side.

We investigated SHANK2's role using systemic (*Shank2–/–*), hair cell-specific (*Gfi1-Cre; Shank2lox/lox*), and spiral ganglion neuron-specific (*Bhlhe22-Cre; Shank2lox/lox*) knockout mutants. Protein localization was assessed by immunofluorescence, bundle morphology by scanning electron microscopy, and auditory function by auditory brainstem responses and distortion product otoacoustic emissions.

SHANK2 localized specifically to the medial apical surface of developing hair cells, complementing lateral proteins like Gαi and GPSM2. In systemic and HC-specific, but not SGN-specific, *Shank2* knockouts, bundle architecture was disrupted, showing fragmented or wavy morphology while preserving kinocilium position and staircase arrangement. Despite widespread bundle defects, auditory impairment occurred specifically at high frequencies, primarily through compromised OHC amplification. Longitudinal studies indicated this architecture is essential for maintaining bundle integrity and hearing function over time. Yeast two-hybrid screening identified RAP1, a small GTPase, as a potential SHANK2-binding partner. HC-specific *Rap1* deletion caused SHANK2 mislocalization and similar defects including bundle abnormalities and high-frequency hearing loss.

These results demonstrate that SHANK2, localized on the medial apical surface, establishes characteristic hair bundle architecture essential for high-frequency hearing and long-term bundle integrity maintenance.

O15
Development of ear and cochlea nuclei requires Irx3/5 and Lmx1a/b

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The information outlines the developmental pathways and genetic influences involved in cochlear formation that depend on Irx3/5 and Lmx1a/b genes:

Development of the Cochlear Base and Saccule: The cochlear base originates from the saccule, which is normally separated by the ductus reuniens, allowing high potassium levels critical for generating the endocochlear potential (EP). In the absence of **Irx3/5** or **Lmx1a**, this separation is altered: These mutants exhibit a broad, uninterrupted continuation between the saccule and the basal cochlear region. **Lmx1a knockout (KO) mice** specifically show no documented EP. **Irx3/5 double knockout (DKO)** mice die early (~E16.5), preventing assessment of EP.

Structural and Cellular Changes in Mutants: Both **Irx3/5 DKO** and **Lmx1a KO** mutants demonstrate integration of the cochlear base with the saccule (a developmental anomaly). **Irx3/5 DKO mice** exhibit conversion of the cochlear base into vestibular hair cells with polarity reversal like the saccule. **Lmx1a KO mice** have a mixture of cochlear and vestibular hair cells. **Lmx1a/b DKO mice** show a complete absence of cochlear hair cells and spiral ganglion neurons (SGNs).

Spiral Ganglion Neurons (SGNs) and Innervation: SGNs project fibers to innervate cochlear nuclei in a topologically organized manner. In **Irx3/5 DKO mutants**, segmentation of these projections is incomplete. **Lmx1a KO mice** show shorter projections, whereas **Lmx1a KO/b heterozygotes** nearly lack rhombomeres 2 and 3 formation. **Lmx1a/b DKO** results in a complete absence of cochlear nuclei, dependent on **Atoh1** gene activity.

Developmental Continuation from Saccule and Lagena: The mutants demonstrate a developmental continuation from the saccule and lagena, which, in mammals, can develop into a cochlea. The extent of this continuation is influenced by the presence of **Irx3/5** and **Lmx1a/b**, affecting how the inner ear structures evolve.

In summary, mutations in **Irx3/5** and **Lmx1a/b** primarily disturb the separation and differentiation processes within the developing inner ear, hindering normal cochlear formation. The mutants reveal that the cochlear base can abbreviate or transform into vestibular-like structures when these genes are disrupted, highlighting their crucial roles in defining cochlear versus vestibular identity and the proper organization of neural innervation pathways.

O16

Regulatory networks of NEUROD1 and ISL1 shape auditory neuron development and tonotopic map formation

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The molecular regulation of neuronal development and the establishment of tonotopic connections to the hair cells or neurons of the hindbrain's first auditory nuclei, the cochlear nucleus, are not fully understood. A temporal regulatory cascade defines the initial inner ear precursors is represented by SMARCA4, SIX1, EYA1 followed by SOX2. Neuronal development is initiated by the expression of NEUROG1, which is followed by sequential activation of transcription factor regulatory networks, including NEUROD1, PAX2, GATA3, POU4F1, LMX1a/b, and ISL1. Using conditional deletion mutants of *Isl1* and *Neurod1* in developing auditory neurons, we compared inner ear phenotypes, the tonotopic organization of auditory pathways, and neuronal molecular transcriptomic characteristics. The transcription factor NEUROD1 is vital for the survival, differentiation, and segregation of inner ear neurons, while ISL1 plays a critical role in neuronal migration and axonogenesis. Deletion of either *Isl1* or *Neurod1* resulted in hearing deficits, disrupted central projections, and disorganized tonotopic maps. Single-cell RNA sequencing analyses of developing auditory neurons revealed distinct and complementary functions of these transcription factors. *Isl1* deletion resulted in an immature transcriptomic profile and impaired neuronal diversification programs. In contrast, delayed *Neurod1* deletion preserved neuronal survival and accelerated differentiation comparted to littermate control auditory neurons. Together, these findings demonstrate that ISL1 and NEUROD1 coordinate multiple aspects of auditory neuronal development and are both essential for establishing the functional tonotopic organization of the auditory pathway.

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O17

Disentangling the various unknowns in the interface of sound and hair-cell electrical characteristics

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The mechanoelectrical transducer (MET) channel acts as the gateway for the sound-balance-brain interface; however, the composition of the channel complex remains incompletely understood. Nonetheless, various strategies and abundant evidence have been used to identify cadherin 23, protocadherin 15 transmembrane inner ear protein, Ca2+/integrin binding family member 2 (Cib2), the Lipoma HMGIC fusion partner-like 5 protein, and the transmembrane channels 1 and 2 (Tmc1 and Tmc2), as well as recent identification of Piezo 1 and 2, as components of the MET complex. Here, we will provide a comprehensive progress report on efforts to dissect the functional roles of individual MET components.

Session 4: Pathomechanisms of Hearing Impairment

O18
Which types of hearing loss can be reversed?

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Progressive hearing loss is very common, particularly as people get older, and there are no medical treatments to slow down or stop the progression for the vast majority of cases. Hearing loss is highly heterogeneous, both in its causes and in the resulting pathology. Therefore, we are likely to need a range of different therapies for different causes and different sites-of-lesion within the inner ear. Some types of pathology may be treatable, even reversible, while other pathologies may not be treatable. Therefore, diagnosis of the cause, or at least the site-of-lesion, will be important to stratify patients for clinical trials, then to select the best treatment for each person. In this talk, I will summarise our understanding of the different ways we can lose our hearing, our progress using model systems to establish which types of pathology could be reversed, and how mouse mutants are giving us clues to how to distinguish different sites-of-lesion.

O19

Spectrotemporal deficits in patients with DFNB8/12 (*TECTA*), DFNA13 (*COL11A2*), or DFNB16 (*STRC*)

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Question

The tectorial membrane (TM), via mechanical interaction with outer hair cells (OHCs), is crucial for cochlear amplification, frequency selectivity, and nonlinear compression. Mutations in TM-related genes (TECTA, COL11A2) disrupt this function, causing elevated thresholds, degraded spectral resolution, and abnormal loudness growth. Temporal processing and speech recognition—mediated by inner hair cells and neural mechanisms—are thought to be preserved. In contrast, STRC (DFNB16) encodes stereocilin, which is essential for maintaining horizontal top connectors between OHC stereocilia. These connectors ensure bundle cohesion and proper mechanical transduction. STRC mutations impair OHC coupling and cochlear amplification but largely spare TM and inner hair cell pathways, suggesting preserved temporal processing. This study examines whether individuals with TM-related mutations (TECTA, COL11A2) and those with STRC mutations exhibit different auditory deficits—specifically in spectral versus temporal processing—and how these map onto functional outcomes like speech recognition in noise.

Methods

Ten participants with TECTA or COL11A2 mutations, nine with STRC mutations, and eight normal-hearing (NH) controls completed:

– A dynamic ripple reaction time (RT) task to assess spectrotemporal sensitivity

– DPOAEs to confirm OHC/TM dysfunction

– ACALOS for loudness growth

– The Dutch Matrix Test for speech-in-noise perception

Results

All groups showed absent DPOAEs, elevated thresholds, steeper loudness growth, and increased SRTs—most pronounced in COL11A2. In the RT task, STRC carriers showed normal promptness at low/medium ripple densities, but impaired performance at high densities—suggesting reduced spectral resolution. Temporal resolution, however, remained intact, with normal RTs across velocities. In contrast, TECTA and COL11A2 showed prolonged RTs across all velocities, indicating spectrotemporal deficits. COL11A2 carriers were most affected. RTs correlated with SRTs, supporting the functional relevance of spectrotemporal processing for speech understanding.

Conclusion

STRC-related hearing loss reflects a primarily spectral deficit, while TECTA and especially COL11A2 involve broader spectrotemporal impairments. Ripple-based RT testing offers a language-independent, functionally relevant tool to profile auditory deficits and distinguish genetic subtypes based on site-of-lesion characteristics.

O20

Identifying the mechanisms and potential new treatments for noise-induced hearing loss using metabolomics and lipidomics

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Hearing loss is a major global health concern, affecting more than 20% of the world's population, with noise exposure being a significant contributor to this burden. It is estimated that one-quarter of hearing loss cases worldwide are caused by noise overexposure, and more than 15% of U.S. teenagers show evidence of noise-induced hearing loss (NIHL) on audiological testing. While the impact of noise on inner ear structure and function has been well characterized, the molecular processes initiated by noise exposure that contribute to NIHL remain poorly understood.

To address this knowledge gap, we have developed metabolomics and lipidomics pipelines to investigate the effects of noise on inner ear metabolism. During my presentation, I will discuss:

1. Our progress in identifying the metabolic events triggered in the inner ear by different noise levels.
2. Insights into how noise alters inner ear energy metabolism.
3. Our advancements in identifying drugs that may reduce NIHL.

This research aims to enhance our understanding of the molecular mechanisms underlying NIHL and potentially lead to new preventive or therapeutic strategies.

O21

The TECTB-C225Y variant causes autosomal dominant deafness in a Nicaraguan family enhances sensitivity to noise-induced hearing loss in ageing mice

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The tectorial membrane (TM) is an extracellular matrix that lies above the mechanosensitive hair cells in the cochlea. Shear produced by the TM plays a major role in displacing stereocilia and facilitating hair-cell mechanotransduction. *TECTB* encodes a major non-collagenous protein of the TM. Here, we reveal a *TECTB-C225Y* variant causing an autosomal-dominant hearing loss in a Nicaraguan family. In a *Tectb-C225Y* mouse model, we carried out a longitudinal study of hearing function and histological assessment of the TM. We further utilized noise exposure to assess the TM under stress.

Methods: Exome sequencing, linkage analysis and audiometry were performed in family members. The hearing of $Tectb^{C225Y/C225Y}$, $Tectb^{C225Y/+}$, and wild-type mice on a corrected $Cdh23^{AHL+}$ background was assessed at 5 weeks, 3, 6, 9, 12, and 18 months. Samples were collected at 2, 6, and 14 months for histology. TM porosity was assessed on histological sections stained with Toluidine Blue. For noise exposure, $Tectb^{C225Y/+}$ and wild-type mice underwent auditory testing before noise exposure at 16 weeks or 18 months, and then at 2 days and 2 weeks after noise trauma (98 dB SPL for 8-week-old mice and 100 dB SPL for 18-month-old mice, for 2h).

Results: The *TECTB-C225Y* variant segregated in individuals with mild-to-severe sensorineural hearing loss. Auditory testing showed significantly increased thresholds in $Tectb^{C225Y/C225Y}$ mice at all time points, but were not significantly different at any tested time point in $Tectb^{C225Y/+}$ and WT mice. Histological analysis showed a significantly increased porosity in $Tectb^{C225Y/C225Y}$ TMs at all ages; and a lesser increase in $Tectb^{C225Y/+}$ at some cochlear locations when compared to WT. Following noise exposure of $Tectb^{C225Y/+}$ and wild-type mice at 16 weeks or 18 months, $Tectb^{C225Y/+}$ mice showed a greater temporary threshold shift, and a much larger permanent threshold shift in response to noise exposure than wild-type controls.

Conclusions: We propose *TECTB* as an autosomal-dominant non-syndromic hearing loss gene. Testing has confirmed that *TECTB-C225Y* disrupts TM structure and function. This effect appears recessive in the mouse model, contrary to human pathology. However, following noise exposure, the combination of age and noise insult reveals a difference between the robustness of the TM in $Tectb^{C225Y/+}$ and wild-type mice. This provides new information about the role of TECTB within the TM and further insight into its role in human hearing loss.

O22

Noise-induced hearing loss, cochlear synaptopathy, and a mouse mutant for increased inner hair cell presynaptic excitability

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Noise-induced hidden hearing loss is characterized by irreversible loss of synapses between inner hair cells (IHC) and spiral ganglion neurons (cochlear synaptopathy) despite normal hearing thresholds. We analyzed hearing performance and cochlear synapse structure in C57BL/6N wildtype (WT) mice that had been exposed to a noise trauma of either 100, 106 or 112 dB SPL for 2 h. To unravel mechanisms of synapse death, we studied "HA" mice with a modified $Ca_V1.3$ channel $Ca_V1.3DCRD^{HA/HA}$, which carries a 30 % larger peak Ca^{2+} current IHCs. We hypothesized that increased Ca^{2+} influx per se would cause damage at IHC synapses in HA mice, and that a noise trauma would aggravate this process.

Eight-week-old mice were exposed to broadband noise (8-16 kHz) for 2 h. Auditory brainstem responses (ABR) were assessed before, directly after and 28 days post trauma. Number, size, and pairing of IHC presynaptic ribbons and postsynaptic AMPA receptor scaffold (Homer1-positive) clusters were analyzed along the cochlea.

Four weeks after a 100 dB SPL trauma, a permanent threshold shift (PTS) was observed at 45 kHz in the WT group, which after the higher traumata extended towards middle to low frequencies. Frequency-specific loss of ABR wave I amplitudes scaled with trauma strength indicating loss of functional IHC synaptic connections. Synaptic pairs were reduced in the midbasal and basal cochlear region in all trauma conditions with ribbon loss up to 46 % compared to the control group. Surviving ribbons were enlarged and were paired with a postsynapse. In contrast, 4 to 6 unpaired postsynapses/IHC were found in the medial, midbasal and basal region irrespective of trauma strength.

Without trauma, IHCs of HA mice showed normal numbers of ribbons with a 30% reduction in size in the apical, medial and midbasal region whereas ~8 ribbons/IHC were lost in the basal region. The 106 SB SPL trauma affected ribbons and postsynapses similarly as in WT. Noise trauma led to orphan postsynapses per IHC in the range of 1-2 (medial), 4-6 (midbasal), and 3-5 (basal) in either genotype indicating that postsynapses were more stable than ribbons.

To summarize, ribbon synapses and ABR wave I amplitudes were reduced by a trauma of ≥ 100 dB SPL, and IHC postsynapses were more resistant than ribbons in C57BL/6N and HA mice. In the basal cochlea of HA mice, part of the ribbons degenerated even without trauma. The noise trauma did not further reduce ribbons in basal IHCs of HA mice suggesting that susceptible ribbons were destructed by Ca^{2+} excitotoxicity before.

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Session 5: Schwann Cells and Macrophages in Inner Ear Disease and Regeneration

O23

Peripheral myelin disorders and the mechanisms of hidden hearing loss

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Hidden hearing loss (HHL) is a recently identified auditory neuropathy believed to contribute to deficits in speech discrimination and intelligibility in people with normal audiological tests. In animal models, HHL presents as normal auditory thresholds but reduced sound-evoked potentials (ABR peak 1 amplitudes). Animal studies showed that age-related and noise-induced loss of synapses between the inner hair cells (IHCs) and spiral ganglion neurons (SGNs) correlate with HHL. Thus, IHC synaptopathy is often perceived as the only cause of HHL.

Since myelin impairments can also cause peripheral neuropathies, we tested whether myelin disorders cause HHL using three mouse models. First, Schwann cell ablation was induced by genetic means, causing a near-total loss of auditory nerve myelin within one week, followed by complete remyelination by four months. This demyelination does not alter auditory thresholds, yet results in a permanent reduction of the amplitudes and increased latencies of the first peak of the auditory brainstem response (ABR). Importantly, transient demyelination does not cause IHC synaptopathy but produces a permanent disorganization of cochlear heminodes. Moreover, HHL caused by transient demyelination and noise are additive, indicating that HHL can be caused by at least two distinct mechanisms. Similar pathologies, i.e., HHL and disorganized heminodes, were found in a mouse model of Charcot-Marie-Tooth disease type 1A (CMT1A) and a mouse line that has peripheral nerve hypomyelination due to loss of Schwann cell ErbB receptor signaling.

Together, our results show that peripheral myelin alterations can cause HHL that is very similar to that seen with synaptopathy, except that it also affects ABR peak 1 latencies. It also suggests that the heminodes play a critical role in the generation of the auditory nerve compound action potential, possibly acting as the action potential initiation site, and that patients with peripheral myelin disorder might also suffer from HHL.

O24

Auditory phenotype in neurofibromatosis type 2 is associated with Schwann cell dysfunction and peripheral myelinopathy

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Background:

Merlin, also known as neurofibromatosis type 2 (*NF2*), is a tumor suppressor gene. Its loss leads to development of Schwann cell tumors, predominantly of the vestibular portion of the 8th cranial nerve.

Schwannomas of the vestibular nerve are typically associated with varying degrees of sensorineural hearing loss (SNHL), but the etiology of this impairment remains to be elucidated. Leading current theories implicate the secretion of pro-inflammatory and potentially neurotoxic factors. In this study, we examined the auditory and vestibular nerves in a NF2 mouse model (Periostin-Cre; Nf2flox/flox) to investigate the underlying cochlear NF2 phenotype with an emphasis on cochlear Schwann cells.

Methods:

Periostin-Cre; Nf2flox/flox mice were compared to littermate controls with FVB/NJ background to characterize SNHL at 2 months and at later ages. For analysis of myelination, nodes of Ranvier and heminodes, cochlear whole mounts were stained for nodal (Gliomedin), paranodal (Caspr) and myelin (MBP) markers and imaged by confocal microscopy. Frozen cochlear sections were analyzed for glial and neuronal markers; Schwann cells and neurons were quantified per 100 µm area in Rosenthal's canal, Scarpa's ganglion, and the auditory nerve trunk on serial sections. Cochlear spiral ganglion mRNA was isolated and quantitative PCR was performed for Schwann cell markers.

Results:

At 2 months of age, months prior to onset of hearing loss and development of proliferating Schwann cell tumorlets, Periostin-Cre; Nf2flox/flox mice have normal ABR and DPOAE thresholds, but reduced Wave I amplitudes and longer latencies in the auditory brain stem response than control mice. Analysis of heminodes and nodes of Ranvier was consistent with myelinopathy, in addition to beginning inner hair cell synaptopathy. At later ages, ABR demonstrated significant hearing loss in all NF2 mice compared to control mice.

Conclusions:

Here we demonstrate that the Periostin-Cre; Nf2flox/flox mouse model displays a cochlear phenotype that may be associated with dysregulation of myelination and a subsequent neuronal dysfunction that initially resembles synaptopathy and progresses to loss of spiral ganglion neurons. These findings may, in part, explain the SNHL in patients with vestibular schwannomas.

O25

Important roles of auditory nerve glial cells and extracellular matrix molecules in noise-induced hearing loss

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Auditory glial cells ensheath a majority of auditory nerve fibers with myelin, protect spiral ganglion neurons, and allow for fast conduction of electrical impulses along the auditory nerve (AN). Previous studies from our laboratory and others have demonstrated that cochlear injury, such as that caused by noise exposure, results in near-immediate myelin abnormalities. RNAseq analysis of a widely used mouse model of noise-induced hearing loss detected altered expression of numerous genes in the AN, including QKI, a gene associated with myelination. These findings suggest an important contribution of myelinating glial cell dysfunction in noise-induced hearing loss. To further characterize the role of glial cells in noise-induced hearing loss, we examined transcriptional profiles of glial cells isolated from AN of young adult Plp1/tdTomato mice after noise exposure. Comparison of glial cells isolated from AN of noise-exposed mice to those of controls identified >500 genes that were significantly changed. Top biological processes affected by noise exposure included inflammation and stress response. Further bioinformatic analysis identified versican, an extracellular matrix (ECM) molecule and direct QKI target, as a regulatory candidate of inflammation and myelin dysfunction in AN following noise exposure. Evaluation of versican expression during AN postnatal development found that isoforms V0 and V2 had unique expression patterns. We then examined young adult mice having deletion of versican isoforms V0/V2 (V0/2 KO mice), finding that they exhibited abnormal myelination, a delay in hearing onset, a mild loss of hearing sensitivity, and expression changes in genes related to ECM function and inflammation. Additionally, reduced auditory function recovery was observed in adult V0/2 KO mice following noise exposure. Together, these observations suggest a causal linkage between ECM molecule dysregulation, myelin abnormality, and auditory impairment. Our study highlights the important roles of auditory glial cells in noise-induced hearing loss and other inner ear disorders.

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O26

Harnessing macrophages for the treatment of noise-induced hidden hearing loss

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Noise-induced hidden hearing loss is due to damage and loss of the ribbon synapses between sensory inner hair cells and auditory nerve fibers in the cochlea, which is not readily diagnosed by threshold audiograms, the standard clinical examination for hearing loss. The consequence of such synaptic loss is deficits in hearing acuity, leading to difficulty in speech recognition and listening in noisy environments. It is now well established that there is some degree of natural synaptic repair following noise trauma. Deciphering the mechanisms regulating synaptic degeneration and repair might inform interventions to preserve and/or restore the loss of synapses and hearing. The talk will focus on non-sensory immune cells, macrophages, and their interactions with the noise-damaged ribbon synapses and how such interactions influence synaptic degeneration and repair. It will also delineate a novel immunotherapy strategy to harness macrophages to regenerate lost ribbon synapses and hearing in an animal model of noise-induced hidden hearing loss.

O27

Molecular signatures of cochlear injury: Unveiling oxidative and inflammatory drivers in acquired SNHL

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Question: Oxidative stress and inflammatory processes are increasingly recognized as interconnected pathological processes contributing to the onset and progression of sensorineural hearing loss (SNHL), particularly when triggered by exogenous insults, such as noise exposure and ototoxic drugs (Fetoni et al., 2021; *Free Radic Biol Med.*; Paciello et al., 2021; *Antioxidants*). Gaining insight into the molecular mechanisms of cochlear damage is essential to identify therapeutic targets and develop effective strategies to limit sensory cell loss, preserve auditory function, and mitigate the progression of cochlear dysfunction.

In this study, we investigated the contribution of redox imbalance and inflammation to cochlear damage using *in vivo* models of acquired hearing loss induced by acoustic trauma and ototoxic agents.

Methods: We employed an integrated experimental approach to assess auditory function through electrophysiological recordings (ABR) and conducted extensive histological, immunofluorescence, and molecular analyses to evaluate oxidative and inflammatory responses within cochlear tissues.

Results: Our results revealed a consistent pattern of cochlear pathology across different models, characterized by elevated levels of reactive oxygen species (ROS), lipid peroxidation, and altered expression of antioxidant enzymes. These oxidative changes were accompanied by the activation of resident immune cells, including cochlear macrophages, as well as increased expression of pro-inflammatory cytokines (e.g., TNF-α, IL-1β) and chemokine receptors. Notably, we observed upregulation of inflammasome components, such as NLRP3, suggesting that inflammation processes contribute to hair cell loss and synaptopathy.

The activation of cochlear macrophages emerged as a prominent feature of the local immune response, characterized by increased Iba1 expression and morphological changes indicative of a pro-inflammatory state. Notably, this immune activation correlated with synaptic degeneration at the inner hair cell-spiral ganglion interface, reinforcing the link between macrophage-driven inflammation and auditory neural dysfunction.

Conclusions: Together, our findings highlight oxidative stress and inflammation as central drivers of cochlear injury in acquired hearing loss triggered by exogenous insults. By dissecting the molecular alterations at the cochlear level, this study provides a foundation for the development of targeted therapies aimed at restoring redox balance and modulating local immune responses to preserve auditory function.

Session 6: MED-EL Symposium
Investigating Inner Ear Health to Optimise CI Outcomes

O28

Pathological substrates in Meniere's disease and novel imaging-based stratification for predicting cochlear implant candidacy

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Meniere's disease (MD) is a chronic, degenerative inner ear disorder in which nearly 25 percent of patients ultimately suffer severely reduced speech discrimination (≤ 40 percent), and about 30 percent experience bilateral disease involvement—together representing a substantial and high-need cohort for cochlear implantation (CI). A hallmark of advanced MD is the pronounced gap between audiogram-predicted and actual word-recognition scores, reflecting extensive "hidden" neural degeneration that surpasses that seen in age-related or other sensorineural losses. Temporal-bone analyses confirm this signature, demonstrating loss of hair-cell ribbon synapses, depletion of dendritic fibers in the osseous spiral lamina and segmental demyelination of cochlear nerve fibers. Crucially, despite chronic-severe peripheral neural injury, spiral ganglion cell bodies remain at densities comparable to age-matched controls, preserving the cellular substrate for the dramatic speech-perception and quality-of-life gains achievable with CI.

From a public-health standpoint, early identification of those 25% of MD patients at risk for severe neural degeneration would be beneficial to reduce downstream disability, and social burden. Building on our insights into MD pathogenesis, we developed a CT-based screening protocol for endolymphatic-sac hypoplasia ("MD-hp"), a developmental-arrest endotype that accounts for virtually all bilateral cases and presents at a younger age. In a multicenter cohort, MD-hp patients were eight times more likely to progress to CI than those with the degenerative endotype, yet their preoperative audiometric profiles—mean pure-tone averages near 90 dB HL and speech-discrimination scores around 22 percent—were indistinguishable from other MD patients when measured within six weeks of implantation. By integrating this imaging-based endotyping into standard diagnostic pathways, clinicians can stratify MD patients at initial presentation, deploy targeted surveillance and counseling, and prioritize high-risk individuals for early CI—maximizing auditory rehabilitation, halting further neural loss and laying the groundwork for a scalable framework to alleviate the population-level burden of profound hearing disability.

O29
The status of the auditory nerve in CI candidates

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Increasing numbers of CI recipients and big changes in implantation criteria over the past decades aggravated variabilities in hearing fidelity with CIs. Causes for poor CI performance go beyond criteria such as “duration of deafness” and “age at implantation” and necessitate a better understanding of the health status of the cochlea before and after implantation.

The presented talk aims to shed some light on the causes of impaired sensorineural and metabolic functions and to summarize methods to determine cochlear health in animals and CI patients. An outlook on current and future therapies to improve cochlear function before and after implantation will be discussed.

Since spiral ganglion neurons are the primary targets of CIs, their integrity and function is most important. Differences in degeneration patterns and survival between human and animal models highlight the importance of deafening procedures in animal experimentation and demand alternative concepts to cover the full spectrum of neuronal decline in human. Residual low frequency hearing in patients improves CI performance but sets high demands to maintain function of hair cells and neurons. In order to keep up the electrochemical gradient in the scala media the cochlear blood supply is of utmost importance and pathological changes in vessel architecture are only poorly understood.

Pharmacological intervention before and after implantation becomes more important. In order to ameliorate the surgical trauma, corticosteroids do not present the only curative way. More potent drugs such as neurotrophins may be much more effective.

O30

Perilymph sampling in human: The *state of the art*

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Biomarkers are fundamental tools for the rational development of medical diagnostics and therapeutics and their analysis directly impacts on clinical decision-making. Therefore, the development of a biomarker that can be measured with sufficient precision and reliability is the impetus for substantial research efforts in a wide range of human diseases.

The pathophysiology of sensorineural hearing loss remains poorly defined, and its understanding is hampered by the lack of specific diagnostic tests that can precisely identify disease localization and disease mechanisms. The inner ear, in fact, due to its delicate structure and position, protected by the hardest human bone, does not allow a solid tissue biopsy. The fluid that fills the inner ear, however, can provide us with an opportunity for a liquid biopsy which, combined with inner ear imaging and auditory diagnostic testing, promises to yield insight into inner ear pathologies.

A small volume of perilymph, the most abundant and accessible inner ear fluid that bathes several cell types critical to sound transmission has been sampled intraoperatively in conjunction with procedures that involve opening the inner ear, such as stapedectomy in the past and cochlear implantation more recently. In this way, the proteome, metabolome, and transcriptome profile of the human inner ear has been established. Potential inner ear biomarkers with diagnostic, prognostic or predictive value start to be identified, though further validation is necessary to substantiate their clinical relevance. Also, drug concentration has been quantified in human perilymph following different administration routes, increasing our understanding of inner ear drug pharmacokinetics.

The presented talk will review strengths and limitations of current protocols for human perilymph sampling with the objective of identifying the desirable features of the perilymph sampling procedure. Furthermore, the main outcomes of several perilymph studies will be examined.

Spoendlin Junior Award Lecture

O31

A single-nucleus atlas of human prenatal inner ear development reveals cell type vulnerabilities and regulatory mechanisms

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The human inner ear contains diverse, highly specialized cell types that collectively enable hearing and balance. However, the precise mechanisms underlying their formation and function remain poorly understood, particularly in humans. This gap in knowledge limits our ability to interpret how genetic variants cause inner ear disorders and hinders the development of targeted therapies. To address this, we generated a high-resolution single-nucleus RNA-sequencing atlas of the developing human inner ear, named HIEDRA (Human Inner Ear Development RNAseq Atlas), spanning fetal weeks 7 to 15 and capturing both auditory and vestibular domains.

HIEDRA identifies 42 transcriptionally distinct cell populations, including sensory and nonsensory epithelia, as well as neuronal and mesenchymal cells. This also includes the under-characterized secretory cells, such as marginal cells in the stria vascularis and vestibular dark cells, which are essential for endolymph homeostasis and inner ear function. By integrating disease gene annotations, we map known deafness and balance disorder genes to specific cell types, revealing selective vulnerabilities. Moreover, we define developmental trajectories and gene regulatory networks that drive sensory and secretory lineage specification.

Among our key findings is the discovery that suppression of Hedgehog signaling is essential for secretory cell fate acquisition. These secretory cells are typically absent in existing human inner ear organoid models. Guided by our atlas, we applied targeted Hedgehog inhibition in human inner ear organoids, successfully inducing secretory cell differentiation and thereby enhancing the cellular complexity of this model. This establishes a more physiologically relevant *in vitro* system for studying disease mechanisms and evaluating therapeutic interventions.

Together, HIEDRA provides an essential human-specific framework for understanding inner ear development and disease. It enables cell-type-specific interpretation of genetic variants, guides regenerative and gene therapy approaches, and represents a significant step toward more complete and functional human inner ear models.

Session 7: Genetic Screening

O32

Divergent modes of hair cell bundle maturation in developing and mature mouse utricle

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Stereocilia are actin-filled organelles critical for inner ear hair cell function. As mechanoreceptors required for hearing and balance, mature hair cells are crowned by stereocilia that house the mechanotransduction machinery. In the developing mouse utricle—one of the five vestibular organs essential for detecting head position—sensory hair cells first emerge prenatally and continue to be added during the first postnatal week, spanning a two-week period. Previously, we fate-mapped postnatally born hair cells and found that they are primarily type II hair cells with short stereociliary bundles. However, how hair cells mature and develop bundles in the prenatal and postnatal mouse utricle remains unknown.

Here, we applied a newly established 3D image analytical tool to systematically and quantitatively examine hair cells in the mouse utricle at six time points from E13.5 to P180. We found that bundle and kinocilium lengths in both striolar and extrastriolar regions gradually increase with age, with type I hair cells exhibiting relatively longer stereocilia and kinocilia than type II cells. At E13.5, bundle and kinocilium heights were mostly uniform; by E15.5, their range began to diverge and remained heterogeneous from P15 onward.

In silico analysis of single-cell transcriptome of utricle hair cells from E14.5 to P70 identified candidate bundle-related genes that are dynamically expressed with age. We predicted and validated FSCN2 as a marker of mature bundles and PCDH15 as a marker of immature bundles. Using immuno-SEM, we observed that fate-mapped, postnatally added hair cells exhibit thin stereocilia lacking staircase patterning or tenting, contrasting un-mapped hair cells. Finally, we showed that postnatally added hair cells exhibit type II subtype morphology, molecular features, and relatively shorter, FSCN2-low stereocilia and kinocilia that fail to elongate with age, indicating a divergent maturation course between prenatally and postnatally born hair cells.

Together, our study presents a comprehensive dataset characterizing divergent modes of bundle and kinocilium maturation in the embryonic and postnatal mouse utricle, informing future work on hair cell development and regeneration.

O33

Cochlear implantation outcomes in genotyped individuals with sensorineural hearing loss: Reassessing the spiral ganglion hypothesis

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Question

Cochlear implantation (CI) is a proven intervention for individuals with severe-to-profound sensorineural hearing loss (SNHL), many of whom have a genetic etiology. While the "spiral ganglion hypothesis" proposes better CI outcomes when pathology is pre-synaptic (e.g., hair cells) rather than post-synaptic (e.g., spiral ganglion neurons), robust evidence across genotypes is lacking. This study assessed CI outcomes in a large, genotyped cohort (N = 220) with hereditary SNHL to investigate whether CI performance varies with the presumed cochlear site-of-lesion.

Methods

Participants underwent CI at Radboud University Medical Center between 2002 and 2021. Based on reported protein function and cochlear expression patterns, genes were classified into pre-synaptic and post-synaptic groups. Audiological outcomes were measured using phoneme recognition scores. Regression models evaluated associations between CI performance, site-of-lesion classification, age at implantation, and duration of auditory deprivation.

Results

Overall CI outcomes were favorable (median phoneme score: 90%), and early implantation (≤6 years) was the strongest predictor of outcome. While genotypes affecting pre-synaptic structures were associated with good performance, no significant difference was observed across site-of-lesion groups. Only one subject had a genotype considered post-synaptic (i.e., *OPA1*), limiting the ability to test the spiral ganglion hypothesis formally. A reanalysis of published data using the same classification showed similar limitations.

Conclusions

Notably, the genotype–phenotype correlation remains incomplete for most forms of hereditary SNHL. While hearing thresholds can often be linked to specific genotypes, suprathreshold auditory function—critical for speech perception and real-world hearing—remains difficult to predict. Furthermore, although gene therapy and regenerative approaches are under development, these are unlikely to benefit all genetic subtypes in the near future. In this context, CI remains the most effective and accessible treatment option for many patients with severe-to-profound SNHL, regardless of genotype. Future studies should incorporate human inner ear organoids, electrocochleography, and multicenter genetic databases to improve our understanding of genotype-driven pathophysiology and refine patient selection for current and future therapies.

O34

412 families´ whole genome sequencing in early onset hearing loss: The French Reference Center´s experience

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Background

Up to recent years, diagnosis of the genetic aetiology of early onset hearing loss (EOHL) relied on targeted gene panel analyses, with a diagnosis rate of about 50%. French clinical geneticists have now access to trio Whole Genome Sequencing (WGS) through the Plan France Medecine Genomique 2025 sequencing platforms for most patients presenting with EOHL

Methods/Results/Interpretation

Methods

Testing criteria for WGS differ according to the subgroup of EOHL indications. The strategy is genome-first for syndromic hearing loss (HL), WGS after normal array-CGH for HL associated with malformation(s) and WGS after normal GJB2/GBJ6 and HL gene panel for patients presenting with isolated HL (50% of positive results).

Results

From 2020 to 2024, **448 patients from 412 families** assessed in the French Reference Center for genetic HL underwent whole genome sequencing on the SeqOIA platform. The diagnosis yield was 35%. Class 4/5 variations were identified in 109 different genes.

Interpretation

We present the **diagnostic rate according to subgroups of the EOHL indication**, along with the advantages and limitations of our strategy.

O35

Single-nucleus multiomics and spatial transcriptomics define heterogoneity and spatial niches in the human fetal utricle

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The utricle, a sensory organ in the inner ear essential for balance, remains poorly understood at the molecular and cellular levels in humans, limiting the development of regenerative therapies for balance disorders. To address this, we integrated single-nucleus multiomic sequencing (RNA- and ATAC-seq) and spatial transcriptomics to generate a comprehensive molecular and spatial profile of the human fetal utricle at early (week 15) and late (weeks 18-19) gestational stages. Single-nucleus multiome profiling enabled simultaneous analysis of gene expression and chromatin accessibility, revealing distinct subpopulations of hair cells, supporting cells, and transitional epithelial cells, each defined by unique transcriptional and epigenomic signatures. Notably, we identified a proliferative supporting cell population at week 15, which declines by later gestational stages, and mapped stage-specific regulatory networks by correlating open chromatin regions with active transcription factors.

Building on these findings, we designed a customized 300-gene panel for high-resolution imaging-based spatial transcriptomics, enabling visualization of cell-type specific gene expression within the intact utricle. Spatial mapping validated the molecular identities of major cell types and confirmed the presence and localization of proliferative supporting cells. We also uncovered spatially distinct niches characterized by unique gene expression patterns and ligand-receptor interactions, further delineating the microenvironments that might govern cell fate and tissue organization.

Together, this integrative approach provides the first high-resolution molecular and spatial atlas of the human fetal utricle, uncovering its cellular heterogeneity, gene regulatory landscape, and spatial organization. These insights lay the groundwork for future studies of human-specific mechanisms underlying sensory cell regeneration and inform strategies for developing regenerative therapies to treat balance disorders.

O36

MDAtlas: A Meniere disease multiomic data resource of genes and cells

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Meniere Disease (MD) is an inner ear syndrome defined by episodes of vertigo associated with sensorineural hearing loss, tinnitus or aural fullness with a significant genetic contribution, including >20 genes reported in familial cases. Progress on genetic diagnosis is limited by the absence of integrated, accessible multi-omics data, hindering molecular insights and treatment development.

Methods

We designed and developed the MD Atlas as a full-stack web application. The platform architecture is containerised with Docker, comprising a React.js frontend for interactive data visualisation, a Node.js backend to manage API requests, and a MongoDB database for flexible data storage. For data processing, all datasets from global partners were standardised through bioinformatics pipelines. Genomic data was aligned to the GRCh38 reference genome and annotated against public databases like gnomAD, ensuring data integrity and interoperability.

Results

The MD Atlas integrates three datasets: genome aggregated MD variants (GRCh38-aligned, annotated for position, consequence, gnomAD frequencies); epigenomic data with methylated CpG sites from MD patients; and transcriptomic data including bulk RNA-seq from mononuclear cells and single-cell RNA-seq from B cells, CD4/CD8 T cells, monocytes, and NK cells of MD participants. Datasets were curated, standardized, and paired with visualization tools for each omics layer. The MD Atlas offers interactive visualizations of aggregated genetic variants, methylation patterns, and gene expression, revealing novel MD-specific signatures validated against public datasets from around 1000 individuals. Cross-omics queries support hypothesis generation, with planned comparative tools to enhance gene discovery and personalized diagnosis.

Conclusions

The MD Atlas is a valuable resource that successfully integrates multi-omic data for MD. It provides an essential tool for the research community to investigate the complex interplay between genetic, epigenetic, and transcriptomic factors in MD. By enabling cross-omics analysis, the platform facilitates hypothesis-driven research, supports the discovery of novel diagnostic markers, and fosters the global collaboration needed to advance personalised treatments.

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O37

Cost-effective genetic screening for newborn hearing loss using a high-density SNV microarray

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Background: Universal newborn hearing screening (UNHS) programs, primarily relying on automated auditory brainstem response (AABR) or otoacoustic emissions (OAEs), are crucial for the early identification of hearing loss. However, these physiological tests have limitations in detecting mild and late-onset hearing loss and identifying the underlying cause, which is paramount for counseling and targeted interventions. With over 150 genes implicated in non-syndromic hearing loss, genetic screening is a valuable complementary approach to improve diagnostic accuracy and clinical management.

Methods: We developed a high-density single-nucleotide variant (HD-SNV) microarray designed for the cost-effective genetic screening of newborns for hereditary hearing loss (HHL). The microarray workflow was optimized for minimal sample input, rapid turnaround time, and high analytical sensitivity and specificity, making it suitable for large-scale population screening. This microarray design leverages array-based hybridization to detect specific SNVs, deletions, and insertions, offering a significantly lower cost per test than next-generation sequencing.

Results: Our HD-SNV microarray robustly detects 51 deafness-related variants, across eight genes (*GJB2*, *GJB3*, *SLC26A4*, *OTOF*, *MYO15A*, *KCNQ4*, *POU3F4*, and *MYO7A*) and the entire mitochondrial genome, selected based on their high frequency in diverse populations. The platform exhibited 95% sensitivity and 92% specificity for 150 HHL patients and 93% sensitivity and 92% specificity for 300 newborn samples. This approach complements existing UNHS programs, as some cases may be missed or identified ambiguously by AABR/OAE screenings alone. Its significantly lower cost per test positions it as a viable and scalable option for widespread implementation in newborn screening protocols, offering a substantial advantage over more expensive, sequencing-based methods.

Conclusion: The HD-SNV microarray represents a rapid, cost-effective tool for genetic screening of hearing loss in newborns. Its ability to identify over 50 key deafness mutations complements current UNHS methods by facilitating earlier and more precise etiological diagnoses, enabling timely genetic counseling, and guiding personalized therapeutic strategies. This technology holds promise for improving the effectiveness of newborn hearing screening programs and enhancing outcomes for affected infants.

Session 8: From Basic Research to Novel Therapies

O38

Investigating AAV-mediated gene therapies in a mouse model of progressive hearing loss

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Progressive hearing loss is the most common sensorineural deficit in older adults, with over 700 million individuals projected to require intervention by 2050 (WHO). While rehabilitative devices such as cochlear implants and hearing aids offer some benefit, no curative treatments exist for disabling forms of progressive hearing loss. This underscores a critical need for therapeutic strategies targeting the underlying molecular causes.

Recent genetic studies have identified several key genes involved in the maintenance of stereocilia and mechanotransduction. Among them, *Baiap2l2* has emerged as a critical contributor to early-onset, severe progressive hearing loss. *Baiap2l2*-deficient mice display a progressive decline in auditory function, deterioration of hair bundle morphology, and disrupted mechanotransduction (Carlton et al., 2021). While AAV-mediated gene therapy has shown promise in models of congenital hearing loss, its potential to treat progressive forms has not yet been thoroughly explored.

Here, we evaluate AAV-based gene replacement therapy in *Baiap2l2-tm1b*$^{-/-}$ mice using local delivery of *Baiap2l2* via round window membrane injection. We used AAV/PhP.eB and AAV2 to deliver the gene at both neonatal and post-hearing stages, respectively, enabling assessment of the therapeutic window and translatability to clinical contexts. Our preliminary results demonstrate that neonatal AAV/PhP.eB-mediated delivery of *Baiap2l2* prevents progression of hearing loss by restoring BAIAP2L2 expression and correct localization to the tips of the shorter stereocilia rows in both inner and outer hair cells. This is mirrored by a partial rescue of hair bundle morphology and functions of inner and outer hair cells, as evidenced by behavioural and cellular assays.

These findings support the feasibility of AAV-mediated *Baiap2l2* gene therapy as a candidate strategy to prevent or delay progressive hearing loss in *Baiap2l2*-deficient mice, while also offering insights into the timing and delivery approaches for an effective intervention. Further studies are ongoing to evaluate the outcomes of AAV2-*Baiap2l2* delivery after hearing onset.

O39

Development of hiPSC-derived inner ear organoids harboring GJB2 mutation to model genetic inner ear hearing loss

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Hearing loss is the foremost common sensory disorder globally, impacting approximately 5% of the population. The current treatment options are limited to hearing aids or cochlear implants, which mitigate symptoms but are not a permanent solution. In the context of genetic hearing loss, gene therapy could be the ultimate solution.

In Europe, mutations leading to autosomal recessive deafness are most commonly found in the GJB2 gene, which encodes Connexin 26 (Cx26). Cx26 is a component of gap junctions, which facilitates ion transport between cells. The exchange of ions is an essential process for hearing as it is required for propagation of sound sensation of hair cells via supporting cells to neurons and the brain. Disease-causing mutations in GJB2 lead to aberrant or non-functional protein, probably leading to improper exchange of ions between cells and thereby reduced hearing.

The only available human model for inner ear research is the inner ear organoid model, generated from human induced pluripotent stem cells (hiPSCs). This model recapitulates the diverse cell types found within the inner ear, providing insights into hearing-related mechanisms. We are employing this model system to study how GJB2 mutations result in hearing loss.

In this project we have used CRISPR/Cas9 gene editing to create hiPSCs harboring the c.35delG mutation. This mutation was introduced in a healthy control hiPSC line to obtain the GJB2-c.35delG hiPSC line. Both the healthy control and GJB2 hiPSCs were differentiated to generate inner ear organoids (IEOs). Immunohistochemical analysis of IEOs was performed and revealed the lack of Cx26 in the GJB2-c.35delG inner ear organoids, while Cx26 could be observed in the supporting cells of healthy control organoids.

The GJB2-c.35delG hiPSC line will be used to test gene therapy, i.e. repair of the mutation by CRISPR/Cas9 gene editing, at both the hiPSC and IEO level to determine potential rescue of Cx26 expression.

The GJB2-c.35delG inner ear organoids will contribute to better understanding of the most common GJB2 mutation and its effects on cells of the inner ear and can aid in therapy development for GJB2-associated hearing loss.

O40

Optimizing tropism of human MSC derived extracellular vesicles for inner ear applications through peptide coating

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CRISPR technology is emerging as a novel treatment strategy that utilizes gene editing to effectively correct genetic defects. However, clinical translation of CRISPR technology is currently due to the lack of safe, efficient and targeted delivery methods. Extracellular vesicles (EVs) are also emerging as promising treatment strategy and as carriers for gene editing technologies due to their intrinsic biocompatibility, low immunogenicity and their ability to cross biological barriers. Thus, they can overcome challenges associated with viral vectors, such as insertional mutagenesis and immunogenicity. In the inner ear, we have shown that they display heterogeneous tropism, often leading to suboptimal delivery efficiency to the target cells.

To optimize tropism of EVs, we develop an AI-based engineering approach for identifying and incorporating specific surface ligands or receptors. We introduced a novel method to produce engineered EVs with controllable surface presentation and functional payload for targeted tissue delivery. Using a phage panning derived peptide library and review of the literature, an artificial intelligence (AI)-based peptide selection process was developed. The high-throughput scalable EV transfection together with AI- guided tissue targeting was used to develop groups of human umbilical cord derived MSC-EVs that were then tested for optimized delivery in the inner ear. Our approach may enable a next-generation platform for programmable and precise gene editing with focus on accelerated clinical translation.

O41
Tmprss3 expression in the mouse cochlea

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Cochlear implants (CIs) have shown variable performance outcomes in DFNB8/10 patients carrying pathogenic mutations in TMPRSS3. A study by Shearer et al, 2018 which involved electrical stimulation of spiral ganglion neurons (SGNs) in CI patients revealed on average smaller electrical responses in DFNB8/10 patients compared to ones with other deafness-related etiologies, indicating a loss of SGN function in these individuals. Another study by Fasquelle et al, 2011 had previously shown a loss of more than half of SGN cell bodies in Rosenthal's canal, observed between days 90 and 180, in a mouse model with a premature stop codon in Tmprss3 (Y206X). These observations indicate a loss of TMPRSS3 function-mediated damage to the SGN health which could plausibly explain such variability in CI performance. However, the cell-type specific expression and function of Tmprss3 in the cellular mosaic of Rosenthal's canal remains unclear. Here we carefully revisited the expression pattern of Tmprss3 in the murine cochlea, at mRNA and protein levels.

Combining an RNAscope assay for mRNA localization with immunohistochemistry using an anti-Tmprss3 antibody which we validated on knock-out animals, we semi-quantitatively assessed the cell-type specific expression levels of Tmprss3 mRNA in the murine cochlea, with a focus on SGN subtypes. We also performed an RT-qPCR assay with TaqMan probes on RNA isolated from mouse brainstem, and a direct few-cell RT-qPCR on the cells of the organ of Corti and SGNs.

In the brainstem of mice, hardly any Tmprss3 transcripts were detected. In the murine cochlea, we report a strong expression of Tmprss3 mRNA and protein in the cells of the organ of Corti with RNAscope, immunohistochemistry, and few-cell RT-qPCR. Specifically, we found the expression of Tmprss3 in the inner and outer hair cells, particularly in the stereocilia, and also in the pillar cells. In addition, Tmprss3 expression was detected in the root cells of the stria vascularis. Interestingly, in Rosenthal's canal, we observed Tmprss3 mRNA enrichment in the type-II SGNs with RNAscope and immunohistochemistry in the mature cochlea. Few-cell RT-qPCR revealed no abundance of Tmprss3 mRNA transcripts in type-I SGNs.

In conclusion, in contrast to our expectation, type-I SGNs do not or hardly express Tmprss3. Thus, the data suggest an indirect role of TMPRSS3 for the health and function of SGNs, which will need to be studied further to forward the course of treatment options for DFNB8/10 patients.

O42

Dose response and toxicity evaluation of TMPRSS3 gene therapy delivered either by AAV2 or novel next generation AAV capsid variant

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Question: TMPRSS3, a type II transmembrane serine protease, is necessary for normal hearing and mutations in *TMPRSS3* account for up to 9% of autosomal recessive non-syndromic deafness. Importantly TMPRSS3 mutations affect function and survival of hair cells and spiral ganglions, which significantly impact the only treatment in TMPRSS3 patients by cochlear implant that requires ganglion neurons. The development of gene therapy presents an opportunity to help patients who otherwise will be without any alternative intervention.

Methods: We constructed AAV2-TMPRSS3 for inner ear delivery in the *Tmprss3 (c.916G->A)* mice as AAV2 is widely used in clinic applications. Production of functional vector was optimized by comparing single stranded to double stranded constructs and including a stuffer sequence with the construct. In addition, we compared our standard AAV2 vector to a novel capsid variant, termed AAV V/6, that provides superior delivery to inner and outer hair cells. Toxicity was assessed by administering three different vector doses of AAV2 and AAV V/6 into the posterior semicircular canal, followed by evaluation of ipsi- and contralateral hearing one week post vector delivery. The TMPRSS3 vectors were also injected into the *Tmprss3* mutant mice by canalostomy at onset of hearing loss at 6-9 months of age in male and female mice.

Results: We found significant hearing improvement in the injected inner ears comparing to the contralateral uninjected control ears, shown by reduction in ABR and DPOAE threshold shifts, an indication of rescue of auditory hair cell function. A dose dependent toxicity level was established for the AAV2 vector. The AAV V/6 vector did not appear to induce any toxicity effects. Semi-quantitative PCR was used to establish the biodistribution of the vector.

Conclusions: These findings provide compelling preclinical evidence that AAV-mediated TMPRSS3 gene therapy, especially with the enhanced AAV V/6 capsid, can effectively restore auditory function with minimal toxicity, offering a viable therapeutic strategy for patients with TMPRSS3-related hearing loss who currently lack alternative treatment options.

O43

A novel AAV capsid and promoter design for GJB2 gene therapy

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Mutation in GJB2 (Connexin26) is the most common cause of hereditary deafness worldwide, accounting for up to 50% of non-syndromic sensorineural hearing loss. We previously demonstrated that GJB2 deficiency in mice impairs auditory function (Kamiya, *J. Clin. Invest.*, 2014), and that immediate postnatal delivery of wild-type GJB2 into the inner ear via an adeno-associated virus (AAV) vector successfully restored hearing in these mice (Iizuka, *Hum. Mol. Genet.*, 2015). However, GJB2 delivery to the mature cochlea failed to restore auditory function. Notably, the early postnatal period in mice corresponds to the embryonic stage of inner-ear development in humans.

To enable a clinically feasible gene therapy approach for humans, we engineered novel AAV capsids by shuffling sequences between wild-type serotypes, enhancing infection efficiency in cochlear supporting cells. Additionally, we engineered specific promoters to drive GJB2 expression selectively in target cells and facilitate proper gap junction formation. These vectors were evaluated in adult GJB2-deficient mice and in a supporting cell model derived from patient iPS cells harboring common GJB2 mutations (Fukunaga, *Stem Cell Reports*, 2016; *Hum. Mol. Genet.*, 2021).

Here, we report the development of AAV-Sia6e, a lead vector optimized through capsid shuffling and promoter refinement for targeted gene delivery to inner-ear supporting cells. To minimize off-target expression in hair cells, we employed a GJB2 cell-specific promoter. AAV-Sia6e-mediated GJB2 delivery significantly improved hearing in mature GJB2-deficient mice. Restoration of gap junctions was confirmed in both in vivo (mouse) and in vitro (patient-derived iPS cell) models.

These findings suggest that targeted AAV-mediated delivery of functional GJB2 using capsid/promoter modifications holds promise as a therapeutic strategy for GJB2-related hearing loss. Preclinical studies in animal models, including non-human primates, are currently underway.

Session 9: Protection, Repair, Regeneration

O44

Single-cell RNA sequencing identifies organ-specific progenitor populations in the mouse otocyst

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The inner ear anlage, or otocyst, gives rise to most of the epithelial cell types lining the fluid-filled spaces of the inner ear, including sensory epithelia such as the organ of Corti, the cristae, and the otolith-bearing maculae. While previous studies have identified numerous genes expressed across broad regions of the mouse otocyst at embryonic day 10.5, much less is known about progenitor populations specific to individual organs, such as the organ of Corti, at this developmental stage. We hypothesize that this gap in knowledge may stem from either the small sample sizes and limited sequencing depth of earlier single-cell studies, or the absence of organ-specific progenitors at this time point.

To investigate this, we generated a high-resolution single-cell RNA sequencing dataset comprising 1,556 otocyst cells, with an average sequencing depth of 17,010 reads per cell. Using a previously published approach, we reconstructed the otocyst in 3D space based on transcriptional profiles. Hierarchical clustering identified four distinct cell clusters, which were mapped to specific anatomical locations within the 3D otocyst model. Spatial reconstruction accuracy was confirmed using canonical marker genes. To link these clusters to future inner ear structures, we integrated our dataset with five previously published scRNA-seq datasets that span multiple developmental stages and provide organ-specific resolution. Applying trajectory inference algorithms and leveraging metadata from the sequencing libraries, we traced the lineage of organ-specific progenitors back to discrete regions on the digital otocyst surface. Notably, among other organ specific progenitors, we identified a small population of presumptive organ of Corti progenitors near the ventral pole of the otocyst.

In summary, by combining new and existing datasets, we achieved unprecedented resolution in mapping the emergence of organ-specific progenitor populations during inner ear development. These findings lay the groundwork for future efforts to generate organ-specific inner ear tissues in vitro, such as through the use of inner ear organoids or related technologies.

O45

The function of NFI transcription factors in cochlear differentiation and hair cell regeneration

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Background: Loss of cochlear hair cells (HCs) is a leading cause of hearing loss in humans. Research in mice shows that supporting cells (SCs) have the capacity to regenerate HCs at early postnatal stages. However, their regenerative potential sharply declines as SCs undergo maturation. Based on recent findings, we propose that the de-differentiation of SCs into progenitor-like cells enhances their mitotic and HC-forming potential. In our current study, we are investigating whether the downregulation of SC-specific transcription factors of the NFI family boosts the regenerative potential of cochlear SCs. NFI transcription factors promote the differentiation of neuronal and glial cells and were recently identified as the main repressors of damage-induced cell proliferation in the mouse retina. The function of NFI transcription factors in cochlear development and HC regeneration has yet to be addressed.

Methods and results: To determine the role of NFIA, B, and X in cochlear development and HC regeneration, we generated Nfia,b,x triple floxed mice that carried the Sox2-CreER transgene. Tamoxifen injections at E12.0 allowed us to selectively delete Nfia,b,x in pro-sensory cells before their terminal mitosis and differentiation into HCs and SCs. Tamoxifen injections at P4 allowed us to selectively delete Nfia,b,x in cochlear SCs at the onset of maturation. We found that deletion of Nfia,b,x genes at E12.0 severely delayed the cell cycle exit and differentiation of pro-sensory cells and led to patterning defects, with more inner HCs and fewer outer HCs being formed in Nfia,b,x triple knockout mice (TKO). Furthermore, we found that deletion of Nfia, Nfib, and Nfix significantly increased the mitotic and HC-forming capacity of postnatal cochlear SCs. Using cochlear organoid and explant assays, we found that the acute loss of Nfia,b, and x increased organoid formation efficiency, organoid size, and overall cell proliferation. Moreover, we found that organoids that were deficient for NFA, NFIB, and NFIX produced a significantly higher percentage of HCs per organoid than the control.

Conclusions: Our research identifies NFIA, B, and X transcription factors as essential for terminal mitosis and HC and SC differentiation. Additionally, our findings indicate that NFIA, B, and X expression in cochlear SCs has to be downregulated so as not to inhibit their ability to re-enter the cell cycle and form HCs.

O46

Unraveling Notch-mediated regulation of hair cell regeneration in the auditory system

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Hearing loss results from the irreversible loss of sensory hair cells (HCs) in the cochlea. Although supporting cells (SCs) possess a latent ability to trans-differentiate into HCs, this potential is largely lost after birth in mammals. Investigating this process at both the molecular and the epigenetic levels, using high-resolution cellular analysis and dynamic imaging techniques provides insights into the cellular mechanisms and functional dynamics underlying this transition, particularly given the rapid loss of regenerative potential after birth.

In our study, we systematically analyzed cochlear explants following Notch inhibition using both live imaging and single cell analyses. At neonatal stages, Notch inhibition led to an increase in HC numbers emerging specifically from the outer HC region but not from the inner HC region. However, at later stages, Notch inhibition failed to induce a comparable increase, suggesting developmental limitations on regenerative potential. A significant rise in responsive SCs was observed between 24 and 48 hours post-inhibition, although the number of new HCs formed was limited. Live imaging revealed dynamic SC behaviors, including migration, morphological changes, and limited direct SC-to-HC conversion events. Single-cell transcriptomics and chromatin accessibility analyses identified SC subpopulations with distinct trans-differentiation capacities. Deiters' and Pillar cells (DCs/PCs) showed greater plasticity compared to inner phalangeal cells (IPhCs) and border cells (BCs), with enrichment in pathways related to cell adhesion, migration, and cell projection organization, indicating for increased plasticity and dynamic cellular remodeling. Notch inhibition upregulated and increased chromatin accessibility at key transcription factor loci such as *Atoh1* and *Pou4f3*, promoting HC fate while repressing SC identity. A new subpopulation we termed Notch-Activated Supporting Cells (NASCs) derived from DC/PCs exhibit the necessary epigenetic and transcriptional features to undergo successful trans-differentiation.

These findings challenge the assumption that all SCs respond equally to regenerative cues, instead highlighting a distinct subpopulation with intrinsic regenerative competence. Recognizing and targeting such subsets offers a refined approach to promoting regeneration in the mammalian cochlea and may inform regenerative strategies for other non-renewing tissues.

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O47

Chemistry-dependent efficacy of antisense oligonucleotides for DFNA9 across human cell and mouse models

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The c.151C>T (p.P51S) mutation in *COCH* is highly prevalent in the Dutch/Belgian population and causes DFNA9 (hearing loss and vestibular dysfunction) in > 1500 individuals. The initial symptoms manifest between the 3rd and 5th decade of life, which leaves ample time for therapeutic intervention. The clear non-haploinsufficiency disease mechanism indicates that blocking or reducing the p.P51S mutant cochlin protein levels may alleviate or prevent the DFNA9 phenotypes.

Considering the broad expression of *COCH* by the fibrocytes of the inner ear, we designed "gapmer" antisense oligonucleotides (ASO) to specifically induce RNase H1-mediated degradation of *COCH* transcripts containing the c.151C>T mutation. We established several model systems to investigate the molecular efficacy of ASOs targeting the c.151C>T mutation or low-frequency mutant allele-specific SNPs.

Using overexpression models, we identified several gapmers that efficiently induce the degradation of mutant *COCH*transcripts. We employed different chemical modifications of the RNA wings to improve the affinity and selectivity for the mutation transcript. Several ASOs with a strong preference for the mutant transcript were identified. To investigate allele-specificity under physiological expression levels, we exposed patient-derived otic progenitor cells (iPCS-OPCs) and inner ear organoids to different ASOs for 8 days. This revealed a marked difference in ASO uptake between chemistries and models. Similar findings were obtained in our humanized mouse model that carries both a (partial) human mutant and human wild-type *COCH* allele. Studies with gapmers with 2"-OMe wings revealed poor uptake and molecular efficacy, whereas gapmers with MOE wings performed much better. In our ongoing studies, we are describing the intracochlear uptake of gapmer ASOs throughout the murine cochlea, and are performing a final in vivo comparison of the best-performing ASOs identified in our in vitro studies to define a lead molecule for further preclinical development.

O48

NOX4 as a key regulator of cochlear redox homeostasis during ageing

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Oxidative stress is one of the most extensively studied mechanisms in cochlear degeneration. The hair cells of the inner ear, which are responsible for the mechanoelectrical transduction of sound, are particularly vulnerable to oxidative damage due to their high metabolic activity. The accumulation of reactive oxygen (ROS) and nitrogen species (RNS) can damage lipids, proteins, and DNA, ultimately leading to cellular apoptosis. Thus, protection against redox imbalance can delay the onset of age-related hearing loss (1).

Objective. NADPH oxidase 4 (NOX4) is known for its constitutive production of hydrogen peroxide and its role in redox signaling in various tissues. While NOX3 has been implicated in cochlear oxidative stress and hearing impairment, the role of NOX4 in the inner ear remains poorly defined. This study investigates whether NOX4 contributes to the preservation of auditory function during ageing.

Methods. We assessed auditory function, cochlear morphology, and cellular integrity in *Nox4* knockout (KO) mice and their wild-type (WT) littermates at different ages (2–14 months). Auditory Brainstem Response (ABR) testing was employed to determine hearing thresholds. Histological analyses focused on the spiral ligament, organ of Corti, and spiral ganglion neurons.

Results. *Nox4*-deficient mice exhibited early-onset hearing loss compared to WT controls. ABR recordings showed elevated thresholds in KO mice at younger ages. Histopathological examination revealed significant degeneration of the spiral ligament (especially in the basal cochlear turn), deterioration of the organ of Corti, and a marked reduction in spiral ganglion neuron counts in KO animals.

Conclusions. Our results indicate that NOX4 plays a protective role in cochlear homeostasis and auditory function, likely through its contribution to redox balance. These findings support NOX4 as a potential therapeutic target in hearing disorders associated with oxidative stress.

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O49

Evidence of otoconia biogenesis outside of the macular organs

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Introduction

Otoconia are calcium carbonate crystals in the saccule and utricle that provide the inertial mass needed to detect gravity and linear acceleration. Disorders affecting these crystals are among the most common vestibular pathologies, especially with aging. Despite their clinical importance, the mechanisms underlying otoconia formation, transport, maturation, and maintenance remain poorly understood. Here, we apply novel histologic and imaging approaches, to elucidate otoconia biogenesis pathways, in both human and rodent specimens.

Methods

We examined vestibular tissue from mice and humans using a combination of histological preservation and advanced imaging techniques. Non-decalcified temporal bones were embedded in methyl-methacrylate resin (Technovit 9100) and sectioned with diamond wire cutting for crystal analysis by polarized light microscopy. Extracellular matrix was visualized by PAS-Alcian Blue staining in decalcified mouse ears embedded in celloidin and imaged with confocal microscopy. Immunofluorescence was performed for otoconin-90 and fibronectin. Focused ion beam scanning electron microscopy (FIB-SEM) was carried out on a human saccule to enable nanoscale 3D reconstruction.

Results

We identified two conserved otoconia delivery pathways. In the saccule, the non-sensory roof epithelium forms a glycoprotein-rich extracellular matrix conduit that extends across the endolymph and anchors to the anterior macula. Otoconin-90–positive otoconia are embedded along this matrix, suggesting nascent crystals are transported from the roof to an anterior "homing zone". These otoconia mature as they move posteriorly, forming an anterior-to-posterior size gradient. In the utricle, vestibular dark cells secrete a diffuse extracellular matrix "fog" enriched in nucleation proteins like otoconin-90. This fog seeds the macula in a top-down fashion, creating a depth-dependent gradient with larger, more mature crystals near the epithelium.

Conclusions

We describe two distinct strategies for otoconia biogenesis: a directed, conduit-based system in the saccule and a diffuse, matrix-mediated seeding mechanism in the utricle. These finely regulated systems may be prone to failure, providing new insight into disorders such as Benign Paroxysmal Positional Vertigo (BPPV) that may involve disrupted otoconia homing mechanisms.

Session 10: Speech Processing, Auditory Perception and Cognition

O50

Hearing and cognition across the life course: Evidence from behaviour, electrophysiology, and brain stimulation

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Hearing and cognition are tightly interlinked, supporting our ability to make sense of speech in complex environments across the life course. In this talk, I will present research from a variety of new studies from my lab that explore how the brain integrates auditory and cognitive processes in younger and older adults, drawing on evidence from behaviour as well as neurophysiological data from event-related potentials and neural oscillations. In the context of auditory perception, I will highlight differences in speech perception, working memory, and multisensory integration with healthy ageing and age-related hearing loss. Finally, I will outline our new research findings on how brain stimulation technologies might support listening in ageing populations. Together, this work aims to advance our understanding of the cognitive neuroscience of hearing and inform interventions that promote better communication and cognitive health throughout life.

O51

Use of OPM-MEG for auditory processing and cognition-related hearing disorders

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Acquired auditory processing disorders including age dependent hearing loss, speech discrimination deficits, tinnitus or hyperacusis, require a personalized diagnosis to assign the individual cause within the auditory hierarchy to either the periphery, subcortical, or distinct cortical or cortico-fugal neuronal dysfunctions. The good functioning of the feedforward and feedback PV-IN network is an essential precondition for audition that above all senses relies on high-speed information flow (Zajac IT and Nettelbeck T, 2018). Therefore, we hypothesize disease-specific deficits in temporal intracortical network function in auditory circuits. We studied fast auditory processing in tinnitus subjects with or without the comorbidity of hyperacusis using auditory stimuli evoking time-sensitive cortical responses. These responses were recorded using a time-sensitive OPM-MEG. We expect this method to become an efficent diagnostic strategy to fathom peripheral or central contribution of the distinct auditory impairments in the future to improve individualized targeted interventional therapies. Here we will present preliminary results demonstrating the usability and function of the OPM-MEG for hearing research in a clinical setting.

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O52

Psychiatric comorbidity in patients with tinnitus or auditory hallucination and sleep evaluation, sound therapy and Shared Decision Making (SDM)

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Background: We reported psychiatric comorbidity (1397/1934, 72.2%) in patients with dizziness.

Aims & Objectives: In this study, we investigated about tinnitus or auditory hallucination.

Method: The subjects were 417 patients (167men, 250 women) with tinnitus and 42 patients (14 men, 28 women) with auditory hallucination. Psychiatric comorbidity was revealed in 77.5% (323/417) with tinnitus and in 97.4% (41/42) with auditory hallucination. AhHI (Auditory hallucination Handicup Inventory), which is a revised version of THI, was used as an evaluation method for auditory hallucinations. In this study, we investigated about tinnitus or auditory hallucination.

Results: An evaluation of sleep disorders was effective using Insomnia Severity Index (ISI). Sound therapy was performed on 52 cases of tinnitus and 6 cases of auditory hallucinations. With sound therapy, Case 1 improved from THI 100 to 2 points and AhHI 98 to 0 points, and Case 2 improved from THI 32 to 0 points and AhHI 66 to 0 points.

A 36-year-old woman (Case 3) with schizophrenia, whose main complaints were dizziness, tinnitus, and auditory hallucinations, had a THI of 86 points, AhHI of 76 points, DHI of 96 points, and ISI of 24 points. With psychiatric treatment her score improved to THI 42 points, AhHI 20 points, DHI 58 points, and ISI 11 points. The 77-year-old man (Case 4) with right paraocular auditory hallucinations did not have psychiatric comorbidity. An evaluation of sleep disorders was effective using Insomnia Severity Index (ISI). Sound therapy was performed on 52 cases of tinnitus and 6 cases of auditory hallucinations. As a treatment for auditory hallucination, sound therapy, oral antipsychotic drug and long-acting injection (LAI) were effective. Shared Decision Making (SDM) was performed to increase treatment continuation rates.

Discussion & Conclusion: We believe that neuro-otological evaluation and sleep evaluation are useful for treating tinnitus or auditory hallucinations, and that improving insomnia, reducing anxiety, sound therapy and SDM are also useful.

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Keywords: psychiatric comorbidity, collaboration, sound therapy

O53

Alterations in the auditory cortex synaptic activity contribute to early noise-induced cognitive decline in a mouse model of Alzheimers disease

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The clinical association between hearing impairment and cognitive decline is well-documented. However, the complex pathophysiological relationship between auditory deprivation and dementia remains to be fully elucidated. We hypothesize that auditory and cognitive degeneration share common pathogenetic mechanisms, and that cochlear input influences cognitive processes by affecting neuronal networks that involve both auditory and cognitive brain structures, such as the auditory cortex (ACx) and the hippocampus (HP).

In previous work, we demonstrated that noise-induced hearing loss (NIHL) in an animal model of Alzheimer's disease (3×Tg-AD mice) accelerates memory deficits and causes persistent synaptic and molecular alterations in the ACx and HP (PMID: 34699347).

Using immunofluorescence and western blot analyses, in this study we found an imbalance of synaptic markers of excitatory and inhibitory neurons in the ACx of 3×Tg-AD mice in response to sound stimulation, indicating synaptic hyperactivity. Thus, based on these data, we investigated whether targeting synaptic activity in the ACx can mitigate the adverse cognitive effects of NIHL in 3×Tg-AD mice. To this end, we employed a chemogenetic approach using Designer Receptors Exclusively Activated by Designer Drugs (DREADDs). Specifically, the inhibitory hM4D(Gi) DREADD was expressed in the ACx via intracranial injection of viral vectors in 6-week-old 3×Tg-AD and wild type (WT, B6129sv) mice. Subsequently, mice underwent noise exposure sessions (pure tone at 100 dB, 10 kHz, for 60 minutes per day over 10 consecutive days) and 30 minutes prior to each session, animals received the selective DREADD ligand compound 21 (1 mg/kg, i.p.) to inhibit ACx synaptic activity. One and four months after noise exposure, we assessed recognition memory and auditory memory using the novel object recognition test and the Y-maze test, respectively.

Our results show that modulating ACx synaptic activity during noise exposure can prevent both recognition and auditory memory impairments in 3×Tg-AD mice with NIHL. These findings indicate that ACx synaptic activity plays a critical role in mediating the detrimental impact of noise on cognitive function.

O54

Hearing loss differentially impacts behavioral assays of memory

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Hearing loss in humans is linked to cognitive deficits in learning and memory and also to exacerbating dementia and Alzheimer's disease. Causal links between hearing loss and these outcomes are hard to nail down because data as to onset and severity of hearing loss are often missing as are details around cognitive decline. We have previously shown that using eight arm radial maze (8ARM), could identify significant deficits in working and spatial memory associated with total hearing loss and that the hearing loss was greater in older animals. A limitation to this work was the 8ARM required fasting which could induce a stress response and also was a single trial test forcing us to compare populations of mice. Our present work incorporates tests that do not require fasting or water restrictions and are repeatable so that each animal serves as its own control. We include here a Y-maze test as well as a Novel Object Recognition (NOR) test that we used for short term (1 hour) and medium term (24 hour) testing. Additionally, we tested for stress and used a social interaction test like NOR, but for using a novel mouse for interaction. Experiments were performed on 2-month-old c57BL6 mice (WT and Pou4f3DT). We selectively ablate the IHCs in mice by injection of diphtheria toxin (DT) IP. As previously demonstrated, animals were deafened within two days of DT administration as assayed by auditory brainstem responses. Results show a strong reduction in NOR at the 24 hour time period with a smaller effect on the 1-hour time point. Y-maze results show a very slow, small effect after 3 months. Thigmotaxis, used to assay stress showed no difference between groups. There was also no effect on social interaction. Together these data show a selective impact of deafening on longer term memory.

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Session 11: Novel Approaches to Cochlear and Cortical Stimulation and Assessment in Primates

O55
Neural coding mechanisms in auditory cortex

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The auditory cortex is situated at the top of a hierarchical processing pathway and plays a crucial role in speech and music perception as well as vocal communication. It has long been considered a challenging brain region to study and remains one of the least understood sensory cortices. Unlike other sensory systems, the auditory system has a longer pathway between sensory receptors and the cerebral cortex. This unique organization reflects the auditory system's need to process time-varying and spectrally overlapping acoustic signals, which enter the ears from multiple spatial directions. Our laboratory has developed a unique, highly vocal non-human primate model—the common marmoset—along with quantitative tools to investigate the neural mechanisms underlying auditory perception and vocal communication. Our research has shown that neural computation in the auditory cortex is highly nonlinear. We have identified regions of marmoset auditory cortex that exhibit selective responses to pitch and harmonically related frequencies. These findings suggest that a fundamental organizational principle of the mammalian auditory cortex is based on harmonicity. They also have important implications for understanding how the auditory cortex processes electrical stimulation through cochlear implant (CI) devices.

O56

Translational auditory gene therapy in non-human primates: From genetic modeling to optogenetic restoration

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Non-human primates (NHPs) provide a critical bridge between basic neuroscience and clinical translation, particularly for complex sensory disorders such as congenital hearing loss. In this work, we present two complementary approaches in common marmosets (*Callithrix jacchus*) to advance understanding and treatment of genetic deafness.

First, we established a CRISPR-Cas9-based knockout model targeting the OTOF gene, mutations of which cause DFNB9—an autosomal recessive form of auditory neuropathy. In vitro embryonic gene editing followed by embryo transfer to surrogates yielded offspring with complete OTOF deficiency, confirmed across germ layers without mosaicism. Affected animals displayed complete deafness, with preserved outer hair cell function and absence of otoferlin expression in inner hair cells. This model enables preclinical testing of gene therapies and investigation of auditory feedback in vocal behavior.

Second, we explored optogenetic restoration using ChReef, a red-shifted channelrhodopsin engineered for high conductance and fast kinetics. Delivered via rAAV through round-window injection, ChReef enabled cochlear transduction in a subset of animals. Functional optogenetic stimulation successfully activated the auditory pathway in one subject. Biodistribution was largely confined to regional tissues, and while no systemic toxicity was detected, mild gliosis and an evolving immune response to the viral capsid were observed.

These parallel efforts highlight the potential of NHP models to inform both the pathophysiology and therapeutic development of human auditory disorders. Together, they lay the foundation for future gene-based interventions—from modeling disease to restoring function.

O57

The electrically-evoked compound-action potential-derived Failure Index as neural health marker

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Objectives

Previously, we derived an electrophysiological marker for cochlear neural health, the Failure Index (FI), in an animal model of focal spiral ganglion neuron degeneration. This FI is a measure derived from electrically evoked compound action potentials (eCAPs) (Konerding et al., 2025, J. Neurosci) as the maximal excitable tissue (approximated by maximal eCAP amplitude) normalized by current spread (approximated by maximal current). A high FI indicates the failure to effectively transmit current into neural signals and the size of the elevation indicated the presence, site and size of a lesion. Here we translate the FI to human CI users.

Methods For this retrospective study, we selected clinical eCAP recordings based on quality criteria (e.g., clearly identifiable thresholds) and calculated the FI for 243 ears (postlingual, full-insertions). The eCAPs were recorded at Hannover Medical School (i.e., intraoperatively) and German Hearing Center Hannover (i.e., postoperatively, all MED-EL CI users) between January 2014 and August 2024. The FI was calculated based on the amplitude growth function (agf) parameters provided by AutoArt as FI = charge to achieve maximal eCAP amplitude / maximal eCAP amplitude.

Results Site-to-site differences in FI were individually distinct but stable for several years from 3 months post-implantation onwards . The mean FI over all recording electrodes was elevated with increasing age, and especially high for ears with known hypoplasia. Furthermore, the maximal FI was significantly correlated with speech in noise measures.

Conclusion

The FI reflects properties that do not extensively change over time (contrary to impedance or tissue growth) and changes with demographic factors indicative of neural health. Thus, we conclude that the FI is a promising marker for human cochlear health and may be used for predicting speech outcomes.

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O58

Model-based characterisation of auditory nerve responses to electrical stimulation

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Background: Cochlear implants restore hearing by stimulating the auditory nerve with electrical pulses. The discharge patterns of nerves encode speech features like onsets, offsets, and temporal gaps. However, current speech encoding strategies (e.g., ACE, CIS) do not take into account fine temporal structure, overlooking variability in nerve fiber responses, nor do they take account of neural features such as refraction and adaptation which occur at high rates of stimulation used in modern cochlear implants.

Gathering experimental data from new animal studies of electrical stimulation of the auditory nerve is limited by the difficulty of undertaking these experimental recordings, with few datasets available, and these are often constrained to population averages. Better computational models of existing datasets of individual nerve fibres are imperative for advancing CI efficacy.

Methods: We applied an adaptive leaky-integrate-and-firing probability (aLIFP) model (Felsheim and Dietz, 2024) to characterize individual auditory nerve fibers to clinically relevant biphasic pulse trains (100 ms biphasic, 25µs phase duration at rates of 200, 1000 and 2000 pulses/sec). Using Bayesian Adaptive Direct Search, we directly fitted model parameters to spike-rate data from 118 auditory nerve fibers from the guinea pig (Heffer et al., 2010).

Results: The outputs of the aLIFP model were highly correlated with the output from the nerve fiber recordings (overall mean correlation of 0.84 ± 0.05), validating the model's accuracy. We optimized the parameters of our model for each of these nerve fibers to reproduce the response patterns to pulses over time as close as possible. We also analysed the dependencies between different response phenomena of each individual nerve fiber.

Conclusion: By capturing neural firing heterogeneity at the fibre level, the model can quantify differences and similarities in nerve fibre response patterns. Such models could be used in future to optimise pulsatile stimulation and speech encoding strategies with temporal fine structure for cochlear implant patients.

Felsheim RC, Dietz M. An adaptive leaky-integrate and firing probability model of an electrically stimulated auditory nerve fiber. *Trends in Hearing*. 2024;28. doi:10.1177/23312165241286742

Heffer LF, Sly DJ, Fallon JB, White MW, Shepherd RK, O'Leary SJ. Examining the auditory nerve fiber response to high rate cochlear implant stimulation: chronic sensorineural hearing loss and facilitation. *J Neurophysiol*. 2010;104(6):3124-3135. doi:10.1152/jn.00500.2010

Biophysics of the Cochlea

P1

Mitochondrial heat production in the apical region of outer hair cell

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Objectives: Intracellular temperature is a vital physical parameter related to several cellular functions. Recent studies showed that mitochondrial thermogenesis is a process where mitochondria generate heat production in brown and beige fat cells. This heat production is facilitated by the uncoupling protein 1 (UCP1), which allows protons to diffuse across the mitochondrial inner membrane, the proton gradient disappearing and releasing heat. In the present study, we investigated whether mitochondrial uncoupling can induce thermogenesis in cochlear outer hair cells (OHCs).

Methods: Isolated OHCs were obtained from the guinea pig cochlea enzymatically. We monitored the changes of intracellular temperature in OHCs using a cationic fluorescent polymeric thermometer FDV-0004. To simultaneously monitor intracellular Ca^{2+} concentrations and mitochondrial Ca^{2+} concentrations, OHCs were loaded with fluo-4 AM and rhod-2 AM. To simultaneously visualize mitochondria and lipid droplets, OHCs were loaded with MitoBright LT Green, a mitochondria-specific dye and Lipi-Blue, a lipid probe.

Results: Labeling of MitoBright LT Green showed that mitochondria were accumulated in the apical region of isolated OHCs. FCCP, a mitochondrial uncoupler induced the increase of the FDV-0004 fluorescence intensities in the apical region of OHCs, demonstrating that UCPs induce heat production. As an uncoupler of mitochondria, FCCP collapses the mitochondrial membrane potential. In turn, this results in a rapid release of calcium from this store by the mitochondrial membrane depolarization. We showed colocalization of rhod-2 and MitoBright LT Green in the apical region of OHCs. Double-loading of OHCs with rhod-2 AM and fluo-4 AM showed that FCCP decreased rhod-2 fluorescence, indicative of decrease of mitochondrial calcium concentrations while the fluo-4 fluorescence was increased in the cytoplasm simultaneously. Double-labeling studies with the MitoBright LT Green and Lipi-Blue showed colocalization of mitochondria and lipid droplets in the apical region of OHCs. By pretreatments with oleic acid (200 µM), a fatty acid for 30min, the intensities of Lipi-Blue fluorescence were increased indicating the increase of LD production. Oleic acid (200 µM) enhanced the FCCP-induced heat production in the apical region of OHCs.

Conclusion: The present study demonstrates that mitochondrial uncoupling induces heat production in the apical region of OHCs. The regulation of thermogenesis by mitochondria-produced and processed lipids may affect cellular signaling in OHCs.

P2

Towards a unified structural understanding of ion transport by SLC26 anion transporters and force generation by mammalian prestin

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The mammalian inner ear achieves exceptional sound sensitivity through mechanical amplification of sound vibrations by outer hair cells (OHCs), a process known as cochlear amplification. This amplification depends on prestin, a voltage-driven piezoelectric actuator densely packed in the lateral OHC membrane. Prestin converts changes in membrane potential into rapid cell length changes, a phenomenon termed electromotility.

Using voltage-clamp fluorometry techniques and molecular dynamics simulations, we recently demonstrated that prestin's voltage-dependent conformational rearrangements share key features with the "elevator transport mechanism" characteristic of the SLC26/SulP anion transporter family to which prestin belongs. This mechanism, a form of the alternating-access model common to transporters, involves a substrate-binding transport domain moving across the membrane relative to a static scaffold domain, allowing the substrate to bypass structural barriers and be transported across the membrane. Our findings strongly support a model in which mammalian prestin functions as an area motor in the lateral membrane of OHCs by undergoing similar conformational changes in a voltage-dependent manner, rather than by transporting substrates. However, how voltage-induced conformational changes relate to those triggered by anion binding remains unclear.

In this study, we compared mammalian prestin (rat prestin; rPres) and its non-mammalian ortholog, zebrafish prestin (zPres), using whole-cell patch-clamp to assess the effects of substrate anions and introducing mutations at the anion-binding site. Our previous studies have shown that (i) despite high structural similarity, rPres retains voltage-sensing ability without transport activity, while zPres exhibits both, and (ii) negatively charged mutations at the anion-binding site of rPres (e.g., S396E) can substitute for chloride, which is normally required for the function of wildtype rPres.

Upon extracellular sulfate application, zPres exhibited chloride/sulfate antiport activity and concurrently showed a shift in the voltage-sensing operating point. The S399E mutant of zPres, homologous to the rPres S396E, abolished both chloride/sulfate and chloride/oxalate antiport activity. zPres, which combines electrogenic anion transport activity with voltage-dependent conformational flexibility, represents a powerful experimental model for investigating the voltage-sensing of mammalian prestin and the transport mechanism of SLC26 transporters. Ongoing studies aim to characterize prestin through comparative analyses.

P3

The role of O-GlcNAcylation in the inner ear

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Post-translational modifications are essential for fine-tuning protein function by altering their activity, localization, and interactions with other molecules. O-GlcNAcylation is a posttranslational modification present across various tissues. This modification is dynamically regulated by two key enzymes: O-GlcNAc transferase (OGT), which adds the GlcNAc moiety, and O-GlcNAcase (OGA), which removes it, making it a good target for pharmacological manipulation. However, its role in inner ear physiology remains largely unexplored. In this study, we demonstrate the presence of O-GlcNAcylation in multiple inner ear cell types, including auditory sensory epithelial cells. Pharmacological modulation of OGT and OGA activity alters O-GlcNAcylation levels in these cells, indicating that this modification is active and regulated in the inner ear. Moreover, patch-clamp recording after the inhibition of OGT and OGA reveals alterations in voltage-gated K^+ and Ca^{2+} currents in inner hair cells and spiral ganglion neurons. These findings suggest a potential regulatory role for O-GlcNAcylation in inner ear function and open new avenues for investigating its contribution to auditory physiology and pathology.

P4

Signal transmission at the ribbon synapses

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Some hearing, vestibular, and vision disorders are attributable to voltage-gated Ca^{2+} channels in sensory cells, which, in turn, affect afferent signal transmission. In vestibular hair cells and retinal photoreceptors, these channels sustain neurotransmitter release at ribbon synapses located at their basal pole. The ribbons characteristic of these peculiar synapses appear to be designed to support continuous exocytosis by accelerating the vesicles delivery to release sites, promoting molecular priming and the synchronous exocytosis of multiple vesicles, while preventing depletion or unnecessary release at inactive synapses. Electroactive neurotransmitters released at the synapse can be detected by Faradaic process by using electrochemical sensors, enabling the extraction of quantitative and kinetic information with high spatiotemporal resolution. In this study, neurotransmitter release was detected using a bare carbon fiber electrode, 5 µm in diameter, positioned as close as possible to the release site. The oxidation/reduction of active molecules on the fiber surface was achieved either via amperometry, by holding the fiber at a constant voltage, or via fast-scan cyclic voltammetry. However, carbon fibers cannot detect non-electroactive neurotransmitters such as glutamate directly. These can nonetheless be detected electrochemically by coating the fiber surface with specific enzymes capable of converting the neurotransmitters into electroactive molecules. For instance, glutamate release can be detected using fibers coated with glutamate oxidase, an enzyme that rapidly converts glutamate into H_2O_2. Here, neurotransmitter release was measured in hair cells isolated from the frog semicircular canal and in retinal cones isolated from the zebrafish retina. To minimize artifacts, release was elicited by depolarizing the cells via whole-cell voltage clamp while simultaneously performing faradaic recordings and ensuring the presence of a robust Ca^{2+} current. The precise location of the release site was identified by fast Ca^{2+} imaging conducted concurrently with patch-clamp and faradaic recordings, in order to determine the optimal position for placing the fiber as close as possible to the release site. As expected, cells exhibiting large Ca^{2+} currents (>100 pA) and, consequently, significant Ca^{2+} accumulation at their basal pole, did not release any electroactive neurotransmitter. So far, the glutamate oxidase-coated fibers have yielded unreliable results, likely due to the insufficient sensitivity of these electrodes.

P5

PKCα-dependent interaction of otoferlin with calbindin or myosin VI modulates inner hair cell endocytosis

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Otoferlin is essential for the priming, fusion, and replenishment of glutamatergic synaptic vesicles at ribbon synapses in auditory inner hair cells (IHCs). Since phosphorylation finely regulates synaptic transmission at conventional neuronal synapses, we hypothesized that protein kinases may similarly orchestrate these processes in IHC ribbon synapses. Upon strong depolarization, both PKCα and otoferlin relocalize toward the basal pole of IHCs. Proximity ligation and co-immunoprecipitation assays revealed that such stimulations significantly increase PKCα–Otoferlin interactions and induce phosphorylation of otoferlin at up to five distinct residues, a modification that can be blocked by PKC inhibitors. Moreover, we demonstrate that otoferlin interacts with Myosin VI and Calbindin in a PKCα-dependent manner. Patch-clamp recordings show that PKC inhibition alters exocytosis and endocytosis, particularly following prolonged depolarizations and elevated intracellular Ca^{2+} concentrations. These findings suggest that strong depolarizations trigger PKCα-mediated phosphorylation of otoferlin, promoting the formation of a Calbindin–Myosin VI–otoferlin complex that plays a regulatory role in synaptic transmission at IHC ribbon synapses.

P6

Motion of the organ of Corti during prolonged solution exposure

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The geometric structure of the organ of Corti (OoC) plays a significant role in the mode of excitation produced by a sound input. To investigate the articulation of the apical region of the mouse cochlea we have studied the way in which the adult organ deforms over time when the *ex vivo* temporal bone is maintained for experiments. Young adult C57BL/6 (P23-P40) mice were used and the bulla rapidly removed after death. A small opening was made to allow surface of the OoC to be studied by confocal microscopy. 5 µM FM143 in the bath defined inner (IHC) and outer hair cell (OHC) membrane outlines. The system was bathed in PBS, HBS or HBS with 10mM Na salicylate added iso-osmotically. Experiments were conducted at room temperature. Analysis of z-stacks using confocal fluorescent microscopy revealed hair cell displacements from their initial position over the 1 hour measurement interval. Specifically, 3D tracking of the hair cell apical surfaces revealed that the OHCs contributed most to the relative tilting of the reticular lamina. OHCs displayed significant swelling as well as patchy lysis in HBS and to a lesser degree in PBS. Salicylate conspicuously reduced swelling, consistent with its role in inhibition of chloride permeability of SLC26A5/prestin. IHCs did not exhibit swelling or lysis in any solution. The differential responses of the apical surface profiles during solution exposure are consistent with a pivot point between the hair cells to produce the observed tilting of the OoC.

P7

Low-frequency acoustics biasing of distortion-product otoacoustic emissions in a cochlear model

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High-intensity and low-frequency tones are used to bias a cochlear partition. This technique can modulate distortion-product otoacoustic emission (DPOAE) amplitude. It was suggested in the experimental literature that the DPOAE modulation patter can be used to estimate the position of the transducer operating point in the cochlea. The estimation method can rely on the *a-priori* assumed type of transducer characteristics, usually modeled as either 1st- or 2nd-order Boltzmann function. Here we use a nonlinear hydrodynamical cochlear model to study low-frequency biased DPOAEs. We use two model variants: one with 1st-order Boltzmann function and the other one with the 2nd-order Boltzmann function. We show that the 2nd-order BF predicts experimentally observed shift in the maximum of the DPOAE amplitude towards nonzero bias levels if the level of the evoking stimuli is increased. In contrast, the model with the 1st-order Boltzmann function does not show this level dependent shift. Therefore, the simulations result would favor the 2nd-order Boltzmann function for simulation of cochlear transducer. We use the model to interpret literature data of biased DPOAEs measured in Meniere's disease patients. If we assume the 2nd-order Boltzmann function then the simulations would suggest that the transducer operating point is shifted towards the point of symmetry of the function. This result could be interpreted as the shift of the cochlear partition towards scala vestibuli, which opens the mechanoelectrical transduction channels in outer hair cells.

P8

Odd-even mode acoustic analysis of the bat cochlea: A theoretical model explaining hearing gaps in horseshoe bats

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A simplified cochlear model was constructed by uncoiling the spiral structure and symmetrically aligning the scala vestibuli and scala tympani relative to the basilar membrane. This configuration enabled decomposition of the cochlear acoustic response into two components: antisymmetric odd-mode sound waves (O-SWs) and symmetric even-mode sound waves (E-SWs).

Main insights concerning the Odd/Even modes' approach:
1. The O-SW couple with an elastic-wave mode on the basilar membrane to generate a traveling wave.
2. Traveling wave amplitude peaks where O-SW energy is fully transferred.
3. The O-SW and traveling wave travel together at the same speed and in phase, forming a "slow wave".
4. E-SWs do not generate traveling waves.
5. E-SWs propagate through perilymph as "fast waves".
6. E-SWs reflect at the apex, forming standing waves.
7. These standing waves are influenced by total cochlear length.
8. The O-SW and E-SW interact at the scala vestibuli base, with matched pressure amplitudes.
9. E-SWs constrain O-SW amplitudes and determine auditory sensitivity.

Based on this theory, we analyze the cochlea of CF-FM horseshoe bats (*Rhinolophus ferrumequinum*), linking echolocation function to internal structure. This bat emits ultrasonic pulses composed of a constant-frequency (CF) component (80–85 kHz, 20–60 ms) and a frequency-modulated (FM) sweep (60–90 kHz, 2–5 ms). Its basilar membrane allocates an extended region to CF, achieving high frequency resolution, sufficient to detect Doppler shifts from wingbeats or target velocity.

However, decline in hearing sensitivity near 40 kHz and 78 kHz has been reported. To explore this, a cochlear model was built (basilar membrane: 16 mm; cochlea: 17 mm) using anatomical data. Structurally, the membrane exhibits a long region with gradual width and thickness change (CF region), followed by a short abrupt widening (FM region)—as supported by our simulations. Generally, assuming a sound velocity of 1520 m/s in cochlear fluid, the wavelengths at 40 kHz and 78 kHz are 38 mm and 19.5 mm, respectively. Accounting for wave compression due to flexible apex tissues, the cochlear duct length 16 mm corresponds to ~λ/2 at 40 kHz and ~λ at 78 kHz. E-SWs thus form standing waves with zero pressure nodes at the scala vestibuli base. As a result, O-SWs also reach zero pressure there, preventing the formation of traveling waves—potentially explaining reduced hearing sensitivity at those frequencies.

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P9

Signal complexities in low-frequency short-pulse distortion product otoacoustic emissions

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Introduction

Distortion-product otoacoustic emissions (DPOAEs) are widely used to noninvasively assess the mechanical function of the cochlea. According to a widely accepted model, the cubic distortion product at $f_{DP}=2f_1-f_2$ comprises two components: a nonlinear-distortion component generated in the region where the two primary tones overlap, and a coherent-reflection component originating from the f_{DP}-tonotopic place. Using pulsed stimuli, the two components can be distinguished in the time domain due to their differing latencies. However, at lower frequencies (f_2 < 1.1 kHz), the DPOAE time signals become increasingly complex, often exhibiting multiple peaks and notches. This study compares low-frequency DPOAEs recorded using two paradigms that employ 1) a short f_2 pulse during the steady state of a longer f_1 pulse, and 2) a short f_1 pulse during a longer f_2 pulse. Experimental results are compared with simulated responses generated by a hydrodynamic model of the human cochlea.

Methods

Short-pulse DPOAEs were recorded in normal-hearing subjects at five frequencies (f_2=0.7–1.1 kHz; f_2/f_1=1.2). For each frequency, nine L_2 levels (30–70 dB SPL) were used with frequency-specific L_1 levels. The f_1 pulse was 80 ms in duration. The f_2 pulse began 10 ms after f_1 onset and had a frequency-dependent half width ($T_{HW}=13.071/f_2$). DPOAE time courses at f_{DP} were extracted using primary-tone phase variation and band-pass filtering. Measurements were repeated with a second paradigm in which the roles of f_1 and f_2 pulses were swapped (i.e., f_1 was short-pulsed). Simulations yielded DPOAEs in the ear canal by numerically solving the equations of motion in the time domain. Simulated DPOAEs included only the nonlinear-distortion component, enabling the identification of signal complexities unrelated to wave interference.

Results

At stimulus levels of L_2≥60 dB SPL, both experimental and simulated DPOAE time signals exhibited considerable complexity when using the f_2-short-pulse paradigm. In contrast, the f_1-short-pulse paradigm yielded more regular responses, characterized by a single prominent maximum shortly after DPOAE onset.

Conclusions

At low frequencies and high stimulus levels, DPOAE time signals exhibit complexities and deformations that are not primarily caused by wave interference, but rather by nonlinear interactions such as mutual suppression between the two primary-tone traveling waves within the cochlea. Employing an f_1-short-pulse paradigm substantially mitigates these nonlinear effects, resulting in clearer and more interpretable DPOAE time signals.

P10

Determination of the pre-neural state of the inner ear with short-pulsed DPOAE level maps in normal and hearing-impaired ears

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Introduction

Objective determination of the pre-neural state of the inner ear in clinical settings typically involves comparing transiently evoked otoacoustic emissions (TEOAEs) or distortion product otoacoustic emissions (DPOAEs) to established normative data. However, these method are known to suffer from confounds such as sensitivity to sub-clinical conductive hearing loss or interference between competing source contributions within the cochlea. Recently, DPOAE thresholds derived from short-pulsed DPOAE level maps have been shown to reduce problems of two-source interference, of individually sub-optimal level combinations, and variability introduced by the state of the middle ear. Here, we investigate this technique in normal-hearing subjects and hearing-impaired patients.

Methods & Results

Short-pulsed DPOAE level maps were recorded in normal-hearing individuals and patients with sensorineural hearing loss across frequencies ranging f_2= 1 – 12 kHz. An adaptive measurement algorithm was employed to automatically sample the L_1–L_2 stimulus space within the individually relevant region, generating DPOAE level maps. By fitting a level map model to each dataset, virtual DPOAE input-output functions were reconstructed along an individually optimized stimulus path.

The results show that the state of the inner ear can be assessed with high accuracy in reasonable time up to hearing losses of 60 dB HL. Due to the capability of short-pulsed DPOAE level maps to independently characterize both cochlear function and also conductive components, this technique offers improved diagnostic specificity. As such, it holds potential for reducing false-positive rates in newborn hearing screening programs and improving ototoxicity monitoring programs.

P11

Ultrastructural analysis of the cellular and synaptic architecture of the mouse vestibular periphery, with consideration of quantal versus non-quantal transmission in the vestibular calyx

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Question

The mouse utricular macula is increasingly being used as a model preparation to study the vestibular periphery because we can generate transgenic mice to investigate molecular details of development and function. Yet, detailed knowledge of its synaptic innervation is lacking or inconsistent. Accurate ribbon synapse numbers and location are needed to quantitatively model quantal transmission in the mouse, as has recently been done for non-quantal transmission in the type I vestibular hair cell (HC) (1). The issue is that quantal transmission in the mature Type I HC-calyx synapse has recently been called into question (5).

Methods

We investigated this at the ultrastructural level, as we have done previously in chinchilla and squirrel monkey (2,3). The same methods that we used in those previous studies (disector and transmission electron microscopy, TEM) were used to confirm recent (4,6) confocal and TEM studies of synaptic ribbons contained in the 2 types of vestibular HCs, type I (enveloped by a large calyceal terminal) and type II (contacted by more conventional synaptic boutons). Because vestibular function varies depending on specific epithelial regions (central/striolar, peripheral/extrastriolar), the present study examined different regions and found regional and cell-type variations.

Results

Synaptic ribbon numbers were higher in type II than in type I HCs (almost double) in both the utricular macula and crista ampullaris. Previous work in chinchilla crista ampullaris had a gradient of synaptic ribbons in type I HCs, being more numerous in central zones versus periphery. In mouse crista, the opposite was true, ribbon numbers were slightly higher in the periphery. For comparison to mouse utricle, we also collected new data from chinchilla utricular macula. Outer-face synapses were consistent across rodent species at ~3/Type I hair cell in central/striolar zones with very few in peripheral/extrastriolar zones.

Conclusions

The findings have implications for synaptic transmission in the vestibular system and are relevant to the VOR being the fastest reflex in the body. These data should inform future functional and modeling studies of the vestibular sensory epithelium.

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P12

The contribution of KNa channels to peripheral vestibular structure and function

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Na^+-activated potassium (KNa) channels, including KNa1.1 and KNa1.2, are expressed in vestibular neurons, but their role in maintaining vestibular structure and function has not been characterized. While prior studies have highlighted the role of KNa channels in cochlear function and associated age-related pathology, their role in the peripheral vestibular system remains largely unexplored. This study aimed to determine whether deletion of KNa channels leads to vestibular dysfunction in mice, and to assess whether transcriptomic changes in vestibular structures precede or correlate with functional or structural impairment. Understanding these mechanisms could provide insight into the early pathophysiology of vestibular loss and inform strategies for intervention.

Next generation RNA sequencing was previously used to obtain transcriptomes from the vestibular sensorineural tissue isolated from wild-type (WT) and KNa (KCNT1/2) double-knockout (DKO) mice. Differential gene expression and gene ontology (GO) enrichment analyses were performed using DESeq2 version 3.14 and gProfiler2 in R. Vestibular structure was evaluated using immunofluorescent labeling and confocal microscopy of the sensorineural structures. Vestibular function was evaluated via vestibular sensory-evoked potentials (VsEPs) in 2 and 9-month-old mice to assess wave I thresholds, amplitudes, and latencies.

Transcriptomic analysis revealed minimal genotype-related changes in vestibular tissues, with only 21 DEGs between WT and DKO mice and no significant enrichment of gene ontology categories. 12 DEGs were downregulated, and 9 of were upregulated in DKO compared to WT mice. Hierarchical clustering and principal component analysis showed weak genotype-based separation in vestibular samples. Analysis of vestibular structure is ongoing but appears unaffected by gene deletion. Functionally, VsEP recordings showed no significant differences between WT and DKO mice in wave I thresholds, amplitudes, or latencies at either age. Age-related decline in VsEP responses between 2 and 9-month-old mice were observed but were comparable across genotypes.

Despite genetic deletion of KNa channels, the peripheral vestibular system in DKO mice remains functionally intact and shows minimal transcriptomic alteration, indicating relative resilience to early genetic and age-related disruption. These results suggest that KNa channels play a limited role in vestibular structure and function and highlight the relative resilience of the vestibular system to KNa channel loss.

P13

Threshold tuning curves of utricular calyceal afferent neurons in guinea pig, with model extension to mouse, rat, sheep and human

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Calyx bearing vestibular afferent neurons innervating the striola of the mammalian utricle are exquisitely sensitive to air conducted sound (ACS) and bone conducted vibration (BCV), and phase lock to stimuli up to ~3 kHz with vector strength exceeding auditory spiral ganglion neurons in guinea pigs. The latency from the onset of the mechano-electrical transduction (MET) current and the first action potential is ~0.3 ms, which is almost 4-fold shorter than the latency from the onset of inner hair cell MET current to APs in spiral ganglion neurons. Here, we used short transient stimuli to determine the threshold of utricular afferent sensitivity. The magnitude and latency of vestibular compound action potentials (vCAPs) correlated with the mechanical shear rate acting to deflect hair bundles, with a threshold of ~0.02 rad/s required to evoke a neural response. The threshold shear rate was independent of acceleration stimulus rise time and frequency, demonstrating that shear rate is the canonical mechanical variable that quantifies the adequate stimulus required to trigger action potential firing. Results for guinea pig using pulsatile stimuli were used to find parameters in a time-domain model, which was subsequently applied to predict frequency domain tuning curves. Tuning curves were band-pass for sinusoidal bone conducted vibration (BCV). At BCV frequencies below the characteristic best frequency (CF), the shear rate was proportional to linear jerk of the temporal bone (time derivative of acceleration), demonstrating why jerk is the adequate stimulus for rodent VsEP testing. For higher frequencies, the shear rate was proportional to acceleration near CF and to velocity above CF. Adjusting the model to account for differences between species predicts thresholds to sinusoidal BCV near -60 dB (re: 1 m s-2) across species, with narrowly-tuned CFs that increase in frequency with decreasing size of the utricle giving BCV CFs for human: 210, sheep: 286, guinea pig: 526, rat: 600, and mouse: 688 Hz. Thresholds in response to stapes vibration (SV) were predicted to be near -50 dB (re: 1 m s-2), but were broadly-tuned relative to BCV with the low threshold band starting near the BCV CF and extending about 1kHz above the BCV CF. (This work was supported by NIH DC018919 (RDR), Macquarie University Research Fellowship (MQRF) (CP) and the Army Research Office W911NF-25-2-0003 (SMR, RDR). The views and conclusions contained in this document are those of the authors and should not be interpreted as representing the official policies.)

P14

Physiological limitations to parent-to-embryo acoustic communication in zebra finches

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Question

Acoustic communication relies critically on the receiver's ability to hear. In precocial bird species hearing can already be functional during embryonic stages in the egg, while in altricial bird species hearing typically starts after hatching. Recent research suggests that zebra finch embryos, despite being altricial, engage in parent-to-embryo acoustic communication by detecting 7-11 kHz "heat calls" (Mariette and Buchanan, 2016 *Science*; Mariette et al., 2018 *Sci Rep*; Katsis et al., 2018 *Sci Rep*; Mariette and Buchanan, 2019 *Behav Ecol*; Pessato et al., 2020 *Sci Rep*; Udino et al., 2021 *Proc Biol Sci*; Katsis et al., 2021 *Behav Ecol Sociobiol*; Udino and Mariette, 2022 *Front Ecol Evol*; Katsis et al., 2023 *Anim Behav*). However, the acoustics of these calls are poorly described and their production mechanism is unknown. Furthermore, the auditory sensitivity of juvenile zebra finches remains to be determined.

Methods and Results

We recorded heat calls in adult zebra finches *in vivo* and show that they are extremely soft, frequency modulated calls with source levels of ~13.9 dB SPL at 1 m and with dominant frequencies ~ 6.8 kHz. We establish *in vitro* that these calls are aerodynamic whistles produced inside the avian larynx, not syrinx, during inspiration. Respiratory air flow during whistle production is higher than during regular song and consistent with thermal panting for evaporative cooling. Additionally, we measured auditory brainstem responses over early postnatal development and show that zebra finch hatchlings are deaf even to loud, broadband clicks with hearing responses emerging between 4-8 days after hatching. Auditory sensitivity develops progressively – high frequency sensitivity maturing last - and reaches adult levels by day 20. Furthermore, laser vibrometry indicates that egg vibrations induced by sound remain far below detection thresholds of vibrotactile senses.

Conclusions

Our data imply that earlier "heat call" playback experiments have been conducted several magnitudes over the physiologically relevant playback level. Furthermore, soft "heat calls" cannot function as adaptive signals or cues in parent-embryo acoustic communication due to the limited auditory sensitivity of developing zebra finches.

Development

P15

K^+ currents of chicken embryo vestibular type-I and type-II hair cells do not involve the KCNQ4 subunit

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Balance and gaze rely on the faithful and rapid signalling of head movements to the brain. In amniotes, vestibular organs contain two types of sensory hair cells, type-I and type-II, which convert the head motion-induced movement of their hair bundles into a graded receptor potential. Hair cell depolarization opens voltage-gated $Ca_V1.3$ Ca^{2+} channels which are functionally coupled to glutamate quantal release. Several distinct voltage-gated K^+ channels shape the receptor potential and therefore the dynamics and intensity of glutamate release. In type-I hair cells, low-voltage-activated K^+ (K,L) channels are additionally involved in non-quantal transmission, which is believed to speed up signalling. In adult mice, K^+ current through K,L channels ($I_{K,L}$) has been recently shown to involve the $K_V1.8$ alpha subunit, while in immature type-I hair cells the KCNQ4 ($K_V7.4$) alpha subunit seems to contribute to this current. In the present study we have investigated the nature of voltage-gated K^+ channels in immature vestibular (*crista*) type-I and type-II hair cells of the chicken embryo. We found that linopirdine, a specific blocker of $K_V7.4$ channels, did not affect the macroscopic K^+ current recorded from type-I hair cells, while it slightly reduced the macroscopic K^+ current elicited by large depolarization in type-II hair cells. By analysing the whole *crista* organ transcriptome, we found no evidence of K_V7 subunits expression, while we found the transcript for the $K_V1.8$ subunit. Therefore, we can conclude that in chicken immature type-I hair cells, $I_{K,L}$ does not involve the $K_V7.4$ subunit, and we provide support to the hypothesis that, like in adult mice, it involves the $K_V1.8$ subunit. Concerning the effect of linopirdine on the K^+ current recorded from type-II hair cells, it is likely due to a nonspecific effect on non-KCNQ conductances.

P16

Understanding the neural basis of hearing function and dysfunction in vivo

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Introduction: Hair cells in the inner ear convert acoustic stimuli into neural signals. In mammals, spontaneous hair cell activity is thought to guide early auditory circuit development [1, 2], but direct *in vivo* investigation remains limited due to the inaccessibility of the inner ear. Zebrafish offer a powerful alternative model, combining optical transparency, genetic accessibility, and a functionally similar auditory system [3].

Here, we use two-photon calcium imaging to investigate spontaneous and sound-induced responses in the zebrafish saccular macula.

Methods: Using two transgenic lines to image spontaneous neural activity (*HuC:GCaMP6s*) and hair cell responses (*m6b:GCaMP6m*), we performed two-photon calcium imaging on larvae aged 4–12 days post-fertilization (dpf). Fish were embedded in agarose, placed in a custom built 4-speaker water-filled chamber and exposed to pure tone stimuli (400–4000 Hz). Spontaneous activity data was processed with CaImAn to extract regions of interest (ROIs) and analyse activity.

Results: Spontaneous brain activity was prominent at 4 dpf but declined sharply by 6 dpf and was minimal by 10 dpf. Peak frequency dropped by ~75% over this period. Auditory stimulation of the saccular macula revealed frequency- and region-specific calcium responses. Stronger stimuli induced broader activation, with lower and mid-range frequencies triggering responses throughout the macula, while higher frequencies selectively activated caudal hair cells. At 4 dpf, responses to high-frequency tones were limited but became more defined with age.

Conclusions: These results show that spontaneous activity in the developing auditory pathway of the zebrafish diminishes rapidly over the first 10 dpf, while sensory-evoked responses become increasingly refined. The saccular macula seems to display early frequency-dependent spatial coding, supporting a key role for peripheral input in shaping central auditory circuits.

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P17

***In vivo* calcium imaging of spontaneous activity in the prehearing mouse cochlea**

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Background

Sensory-independent spontaneous activity regulates the development of mammalian sensory systems. Before the onset of hearing, the immature cochlea undergoes critical periods of spontaneous calcium-dependent signalling that spreads across supporting cells and inner hair cells. Our current knowledge of this complex process largely derives from *ex vivo* experiments, which has posed a significant barrier to fully understanding cochlear development, since *ex vivo* studies cannot replicate the intricate anatomy, innervation, and physiological environment of the cochlea.

We developed surgical and imaging methods that, combined with transgenic animals expressing fluorescent indicators, allow us to study how mammalian sensory hair cells function *in vivo*. Using this approach, we investigated the dynamics of spontaneous activity in the prehearing cochlea at cellular resolution *in vivo*.

Methods

We used transgenic mice expressing the genetically encoded calcium indicator GCaMP6f in inner (IHCs) and outer (OHCs) hair cells, supporting cells, or spiral ganglion neurons. Mice were kept under anaesthesia and placed on a heated mat. Following a surgical procedure to expose the cochlea, mice were transferred on the stage of a two-photon microscope equipped with long working distance (~2 mm) water immersion objectives and the fluorescence was visualised under two-photon excitation conditions.

Results

This approach allowed us to record from the same cochlear region spanning 100-250 µm along the tonotopic axis. Both IHCs and supporting cells exhibited spontaneous calcium activity *in vivo* throughout immature stages of development. Nearby IHCs displayed both independent and coordinated activity, which is likely caused by the modulation on IHC excitability exerted by calcium waves in the supporting cells. Moreover, we observed spontaneous calcium transients in individual OHCs during the first postnatal week, before their maturation. Finally, we compared *in vivo* calcium dynamics under different anaesthetic agents (ketamine/xylazine vs. isoflurane).

Conclusions

Our approach provides significant insights into the nature of spontaneous cochlear activity in prehearing mice, emphasizing its importance in the functional refinement of the auditory system. The application of two-photon imaging to study cochlear activity *in vivo* offers a promising avenue for future research.

P18

In vivo investigation of spontaneous activity in the prehearing outer hair cells

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Introduction

During pre-hearing stages of development, the mammalian cochlear hair cells elicit spontaneous electrical activity to induce neurotransmitter release onto the auditory nerve fibre, which is crucial to the refinement of both the cochlear sensory epithelium and the auditory pathway. Developing outer hair cells (OHCs), which later play a key role in cochlear amplification, have been shown to elicit spontaneous calcium activity in *ex vivo* preparations(Ceriani et al., 2019), but whether this activity is present *in vivo* and triggers activity in the afferent neurons, remains unknown. *Ex vivo* experiments cannot replicate the complex anatomy, innervation and physiology of the mammalian auditory system, causing a substantial barrier to our understanding of the developmental process in the prehearing cochlea. In this project, we have developed a novel experimental approach that combines a surgical procedure and 2-photon calcium imaging to record spontaneous calcium activity in the cochlea in neonatal mice *in vivo*.

Method

Anaesthetised mice (P3–P9) expressing the calcium indicator GCaMP6f in either the hair cells or the supporting cells were used for the *in vivo* experiments as recently described(De Faveri et al., 2025). We performed a surgical approach that allows us to optically access the cochlear sensory epithelium in live mice. This surgical procedure was performed without breaking the lateral wall membranes sealing the cochlear partition, retaining the separation between the endolymph and perilymph solutions The mouse was then placed on a stage of a 2-photon microscope for imaging using 16x objective.

Results

We found that pre-hearing OHCs elicit both spontaneous single and coordinated calcium transients during the first postnatal week (P3-P6), before their onset of maturation at P7-P8. Single calcium transient is the most frequent at P3 and P4, while coordinated calcium activity is the most frequent at P4 and P5. We have also found that the coordinated activity in the OHCs is appear to driven by spontaneous calcium wave in the greater epithelial ridge.

Conclusion

This project provides the first *in vivo* information into the nature of spontaneous activity in prehearing cochlear OHCs. In the future, this approach will be instrumental to uncover how this pre-hearing spontaneous activity regulates the functional development of the auditory system.

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P19

Exploring overlapping and distinct interaction partners of Gαi$_2$ and Gα$_{i3}$ in the inner ear

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The closely related G-protein alpha subunits Gα$_{i2}$ and Gα$_{i3}$ play crucial roles in the development of organ of Corti, particularly the planar cell polarity of hair bundles, yet the extent of their functional redundancy or specificity remains poorly understood. While both isoforms are known to inhibit adenylyl cyclase, increasing evidence suggests they may engage in distinct non-canonical signaling pathways via unique interaction partners.

To explore isoform-specific interactomes, HEK293 cells were transiently transfected with EE-tagged versions of Gα$_{i2}$ and Gα$_{i3}$. The respective cell lysates were co-incubated with lysate from the UB-OC2 cell line – an immortalized progenitor hair cell line and pulled down with an anti-EE-Sepharose. The eluated complexes were analyzed by mass spectrometry. Multiple parameters were tested, such as lysis buffers for optimal protein solubilization, and different activation states of the Gα$_i$ proteins (GTP-bound active vs. GDP-bound inactive forms).

Mass spectrometry analysis revealed several novel candidate interactors for both Gα$_{i2}$ and Gα$_{i3}$, including both shared and isoform-specific proteins. Among them are F-actin capping protein, Myosin-9, Myosin-10, and Moesin, all currently undergoing further validation in the developing inner ear. Additionally, we detected multiple actin cytoskeleton-associated proteins such as Tropomyosin, an F-actin uncapping protein, and unconventional Myosin-1. The identification of these proteins expands our understanding of Gα$_i$-coupled signaling beyond classical GPCR pathways and may suggest isoform-specific roles in inner ear development.

Our results indicate that Gα$_i$ might directly regulate cytoskeleton formation, which is in agreement with its localization at the tips of the stereocilia.

P20

Validation of potential G protein interaction partners in auditory hair cells of mice

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Heterotrimeric G Proteins of the Gαi family play a critical role in the development of stereocilia in auditory hair cells, involving non-canonical G Protein signaling pathways. Previous studies have shown that the combined loss of Gαi2 and Gαi3 leads to deafness in mice. However, the isoform-specific functions and interactions of Gαi2 and Gαi3 and their interaction with cytoskeleton-associated proteins remain poorly understood.

By pulldown and mass spectrometry analysis we identified several proteins - Moesin, Myosin 9, Myosin 15, and the actin capping protein CAPZB - as potential binding partners of Gαi2 and Gαi3. In this project, their localization and spatial proximity to Gαi proteins are investigated in explanted organs of Corti from wild-type mice (P0–P21) during the early postnatal development of the inner ear.

Expression patterns of the candidate proteins were first characterized via immunohistochemistry on whole-mount preparations. To validate protein-protein interactions, proximity ligation assays (PLA) are performed, allowing for the detection of proteins within less than 40 nm of each other - suggestive of a functional interaction.

This study aims to elucidate mechanisms of non-canonical G Protein-coupled signaling and to validate the role of newly identified interaction partners in stereocilia formation and maintenance. The results may contribute to a deeper molecular understanding of organ of Corti development.

P21

Pertussis toxin, an inhibitor of Gαi/o proteins, impairs neurite outgrowth in cultured spiral ganglion neurons

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This presentation highlights the role of $G\alpha_{i/o}$ protein-coupled signaling in regulating neurite development of spiral ganglion neurons (SGNs), the primary auditory neurons that relay sound information from cochlear hair cells to the brain. Despite their importance in normal hearing, the molecular mechanisms governing SGN neurite outgrowth and connectivity remain incompletely understood.

We utilized pertussis toxin (PTX), a pan inhibitor of $G\alpha_{i/o}$ proteins, to investigate how these signaling pathways influence SGN neurite morphology in dissociated cultures from early postnatal mice (P4). PTX treatment significantly reduced neurite elongation in cultured SGNs. This treatment increased neurite branching frequency without affecting the initiation of new neurites. In bipolar SGNs, PTX caused a significantly higher proportion of neurons to exhibit length differences between their two neurites, suggesting differential regulation of axonal versus dendritic development. Consistent with these morphological changes, cytoskeletal analysis demonstrated disrupted microtubule organization in growth cones following PTX treatment.

Our presentation will discuss how these findings enhance our understanding of $G\alpha_{i/o}$ signaling in auditory neuron development and neurite morphology regulation. In future, we aim to explore the cellular mechanisms underlying PTX-induced alterations in SGN neurite outgrowth.

P22

Molecular role of ISL1 in spiral ganglion neuron development

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The development of inner ear neurons is orchestrated by a complex network of transcription factors (TFs), each playing a distinct role at specific developmental stages. While the key TFs involved are already known, their precise functions and interactions are not completely understood. Among these, ISL1 emerges as an important regulator in early neuronal programming, profoundly influencing the morphological development of the spiral ganglion and hearing function.

Conditional knockout mice *Neurod1*-Cre; *Isl1*$^{f/f}$ (*Isl1CKO*) exhibit an abnormal fusion of vestibular and spiral ganglia, the spiral ganglion neurons have impaired migration, with most of them stuck in the modiolus. To elucidate ISL1's molecular role, particularly in neuronal differentiation and subtype specification, we employed single-cell RNA sequencing (scRNA-seq) on murine spiral ganglion neurons at embryonic day 14 (E14), a stage when spiral ganglion neurons have ceased proliferation and initiated differentiation.

Transcriptomic analysis revealed a distinct expression profile in *Isl1CKO* neurons compared to controls. While control neurons progressed towards spiral ganglion differentiation, *Isl1CKO* neurons displayed an altered transcriptional landscape, with populations expressing markers of both early and late developmental stages. Notably, some *Isl1CKO* neurons remained in the proliferative cycle, and there was a discernible population of neuronal progenitors, features largely absent in controls. Even the post-proliferative *Isl1CKO* neurons have an altered transcriptomic profile, simultaneously expressing early and late developmental markers. These findings suggest that ISL1 is indispensable for guiding spiral ganglion neurons through the differentiation process, ensuring proper developmental progression and functional maturation.

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P23

Tmprss3 deficiency affects electrophysiology and morphology of SGN-like organoids

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TMPRSS3 is a type II transmembrane serin protease that is expressed in the inner ear and its mutations are the cause of autosomal recessive hearing impairment with postlingual (DFNB8) and prelingual (DFNB10) onset. Patients with DFNB8 or DFNB10 show a variable cochlear implant (CI) performance. Since proper function of CI requires intact spiral ganglion neurons (SGNs), it was hypothesized that TMPRSS3 might be important for survival and function of SGNs. However, expression of TMPRSS3 is limited to type II SGNs, which make up only 5 % of all SGNs and are thought to be dispensable for auditory signal transduction. To investigate the role of Tmprss3 in SGN survival, we used an inner ear organoid model from human induced pluripotent stem cells enriched for SGN-like cells and introduced a TMPRSS3 knockout (KO).

Combining immunohistochemistry with RNAscope, we observed that control clones were positive for TMPRSS3 on both mRNA and protein level. Moreover, the cells expressing TMPRSS3 were also positive for peripherin, type II SGN marker, which is consistent with our expression data from mouse cochlea. Clones with TMPRSS3 deficiency displayed no immunofluorescence signal for the anti-Tmprss3 antibody.

When comparing Tmprss3-KO and controls, TMPRSS3-KO organoids appeared smaller and less differentiated. Moreover, Tmprss3-KO SGN-like cells formed fewer neurites. Electrophysiological characterization using a whole-cell recording approach showed that Tmprss3-KO SGN-like cells have smaller voltage-gated current and no action potentials. This confirms a crucial role of peripheral TMPRSS3 expression for the proper development of SGNs.

In conclusion, our organoid model is well suited to analyze SGN function, and might be used to assess more genetic causes associated with low CI performance.

P24

Developmental transcriptomic profiling of non-sensory vestibular epithelium reveals ionic contribution to endolymph formation

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Purpose: In the inner ear, various ion channels play a pivotal role in signal transduction, and any dysfunction in these channels can result in hearing and vestibular disorders. Endolymph, crucial for inner ear function, starts forming during embryonic development. We understand potassium ion is necessary for endolymph formation at postnatal. However, we still don't know which ion is responsible for endolymph formation at developmental stage.

Methods: We collected non-sensory epithelium specimens from the vestibule at different developmental stages: E16.5, E18.5, and P5. These specimens were categorized based on the presence of dark cells. We conducted RNA sequencing to analyze changes in ion channel expression during development. Candidate genes were selected for further functional studies.

3D live imaging and ion channel inhibitors were utilized to measure endolymphatic volume changes. Immunohistochemistry was employed to examine the localization of candidate ion channels. Finally, we measured ionic current from dark cell on non-sensory epithelium of utricle by vibrating probe.

Results: We identified 1613 differentially expressed genes (DEGs), primarily associated with ion transport. Clustering analysis revealed distinct gene expression patterns corresponding to different cell types and developmental stages. Our study highlighted sodium, chloride, calcium, and potassium ions as major players in endolymph formation. Functional studies demonstrated the effectiveness of chloride-free solution and amiloride in blocking endolymphatic fluid secretion at E16.5Interestingly, calcium-free solution did not affect secretion rate, while potassium ions influenced secretion only at P5, as confirmed by XE991 treatment. We found out that benzamil (ENaC blocker) act as lowering ionic current from dark cell at E16.5.

Conclusion: During inner ear development, sodium and chloride ion is strongly likely to be associated with endolymphatic fluid secretion. This finding may contribute to elucidating the mechanism of inner ear formation and possible mechanism for congenital hearing loss and vestibular disorders.

P25

A low-molecular-weight compound mimicking IGF-1 repairs excitatory amino acid-induced synaptic damage via *Klotho* suppression similar to IGF-1

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Question: Our previous studies have demonstrated that IGF-1 contributes to the maintenance and repair of ribbon synapses in cochlear explant cultures (Yamahara 2019; Gao 2020). Here, we tested the potential of a low-molecular-weight compound mimicking IGF-1 (IGF-1 mimic) for the repair of ribbon synapses using an explant culture model of excitatory toxicity, and examined the molecular mechanisms of its action using RNA sequencing.

Methods: Cochlear explant cultures of neonatal mice were prepared according to our previous studies (Yamahara 2019; Gao 2020), in which the sensory epithelium and spiral ganglions were maintained. The explants were exposed to 0.5mM N-methyl-D-aspartate and 0.5mM kainic acid for 4 h (EAA exposure) to induce the loss of ribbon synapses. Afterwards, the explants were incubated in the control medium or medium supplemented with IGF-1 or IGF-1 mimic. Explants cultured without EAA exposure were used as controls. The preservation of ribbon synapses was assessed using immunohistochemistry for CtBP2 and GluA2. Bulk RNA sequencing of dissected spiral ganglions from explants was performed for each condition.

Results: Immunohistochemistry revealed a significant loss of ribbon synapses in samples damaged by EAA exposure compared with control samples. Supplementation with IGF-1 or IGF-1 mimic significantly increased the number of ribbon synapses in comparison with samples damaged by EAA exposure. RNA sequencing revealed that EAA exposure upregulated the expression of *klotho* and downregulated that of *igf1*, compared to controls. Supplementation with IGF-1 or IGF-1 mimics downregulated *klotho* expression and upregulated *ndufa4l2*, which encodes a mitochondrial ROS-suppressing protein. RNA sequencing also demonstrated the difference in the gene expression patterns between IGF-1 and IGF-1 mimic. IGF-1 induced upregulation of cell cycle-associated genes, while IGF-1 mimic showed downregulation of these genes compared with IGF-1.

Conclusions: A novel low-molecular-weight compound mimicking IGF-1 induced the preservation of ribbon synapses in cochlear explants via suppression of klotho and mitochondrial ROS similar to IGF-1. In addition, this compound did not induce upregulation of cell cycle-associated genes differed from IGF-1, suggesting low risk for tumorigenesis.

Hearing Loss 1/2

P26

Hearing loss characterization in DBA/1JRj and DBA/2JRj mouse models

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DBA/1JRj mice are widely used as a model of rheumatoid arthritis while DBA/2JRj mice are more studied in cardiovascular biology or neurobiology. However, due to their homozygous Cdh23^{ahl} mutation, a gene involved in mechano-transduction in the inner ear, 10 months old DBA/1JRj mice develop severe hearing loss while DBA/2JRj exhibit hearing defects starting at 2-3 months old. The aim of this study was to compare and better characterize hearing loss in early ages in these two DBA mice strains, from a functional and histological point of view.

Auditory functions were assessed by two non-invasive measures: Auditory Brainstem Responses (ABRs) and Distortion Product Oto Acoustic Emissions (DPOAEs) at 5, 8 and 10 weeks of age for DBA/2JRj and DBA/1JRj mice, compared to CBA/JRj mice, used as normal-hearing mice. These functional measures were correlated with histological analyses: Spiral Ganglion Neurons (SGNs), Inner Hair Cells (IHCs) and Outer Hair Cells (OHCs) counts were performed after animal sacrifice, at 10 weeks of age.

Both DBA/1JRj and DBA/2JRj strains showed a reduction in DPOAE amplitudes and a significant elevation of ABR thresholds from 5 weeks of age, compared to CBA control mice. In DBA/1JRj mice, hearing loss was observed at highest frequencies (> 25 kHz), whereas DBA/2JRj mice displayed hearing deficits across nearly all recorded frequencies. Although both strains showed early-onset hearing loss, the phenotype worsened over time. At 10 weeks, severe impairments were still observed in DBA/1JRj mice at frequencies above 25 kHz and in DBA/2JRj mice across all frequencies. Interestingly, a complete loss of DPOAEs at all frequencies occurred from 8 weeks of age in DBA/2JRj mice.

Histological analyses supported the functional findings. A significant decrease in OHCs density was observed at high-frequency regions in DBA/2JRj compared to both CBA controls and DBA/1JRj mice. No significant IHCs loss was observed in any strain. However, a trend towards reduced IHCs density (approximately 10%) at 32 and 40 kHz was noted in DBA/2JRj mice. Finally, the SGNs analysis did not show a significant difference between strains.

In conclusion, both DBA/1JRj and DBA/2JRj mice exhibited early-onset, progressive hearing loss beginning as early as 5 weeks of age. The hearing impairments were more pronounced in DBA/2JRj mice, as demonstrated by both functional and histological outcomes. Our results suggest that hearing loss observed in DBA/1JRj and DBA/2JRj mice occurred earlier than previously reported and should be carefully considered for future studies.

P27

Cochlear Kir4.1 regulation in aging and immunodeficient mice: Implications for sensorineural hearing loss mechanisms

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Sensorineural hearing loss (SNHL), the most prevalent form of hearing impairment, is a leading cause of disability worldwide. Among the molecular factors involved, the inwardly rectifying potassium channel Kir4.1, encoded by the KCNJ10 gene, plays a central role in cochlear ion homeostasis and auditory transduction. Kir4.1 is highly expressed in the stria vascularis, organ of Corti, and spiral ganglion, where it contributes to the generation of the endocochlear potential and regulates potassium recycling and fluid balance. Dysregulation of Kir4.1 expression and localization has been associated with cochlear dysfunction and SNHL. To investigate age- and strain-dependent variability in Kir4.1 expression, cochlear tissues were collected from CBA/J, NOD, and immunodeficient NOD-SCID mice at 4, 8, and 12 weeks of age. Immunohistochemistry was performed to assess Kir4.1 expression, quantified using ImageJ. Morphometric analysis of stria vascularis and spiral ligament areas was also conducted. Statistical analyses included one-way and two-way ANOVA with post hoc testing using Excel and Jamovi. Compared to CBA/J, NOD and NOD-SCID mice exhibited a reduced stria vascularis area and an enlarged spiral ligament. Kir4.1 expression in the stria vascularis and organ of Corti progressively declined with age in NOD mice but partially recovered in NOD-SCID mice by 12 weeks. In contrast, Kir4.1 expression in the spiral ganglion increased over time in all strains. The age-related decline in Kir4.1 was less pronounced in CBA/J mice, suggesting that genetic background and immune status influence cochlear vulnerability. The partial recovery of Kir4.1 in immunodeficient NOD-SCID mice suggests a protective effect of immune deficiency against strial degeneration. The upregulation of Kir4.1 in the spiral ganglion may reflect compensatory or maturational responses to metabolic or neuronal stress. These findings highlight Kir4.1's critical role in cochlear metabolic balance and suggest its involvement in immune-mediated mechanisms of hearing loss. Modulation of Kir4.1 expression or activity could represent a promising therapeutic strategy for age- and immune-related SNHL. Further studies are warranted to elucidate the molecular pathways involved and explore translational potential. This work was supported by PRIN 2022 (Italian Ministry of University and Research).

P28

Delayed synaptic aging in the cochlea of long-lived naked mole rats (*Heterocephalus glaber)*

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The naked mole rat (*Heterocephalus glaber*) is an exceptional model for aging research due to its extraordinary lifespan (up to 31 years) and resistance to cancer and age-related diseases. While their hearing thresholds are elevated compared to other rodents, even at young ages—likely due to their subterranean lifestyle—the anatomy of their middle and inner ear, including the presence of inner and outer hair cells with ribbon synapses, is comparable to that of other mammals, including humans. Combined with their complex use of vocalizations for social communication, naked mole rats provide a unique model to study age-related changes in the cochlea in the absence of common age-associated pathologies.

In this study, we examined ribbon synapse counts in both inner and outer hair cells of naked mole rats ranging from 4 to 15 years of age. Synapse numbers remained stable throughout early and mid-life, with a noticeable decline emerging only in animals older than 12 years. Interestingly, the number of inner and outer hair cells themselves remains stable even in the oldest animals examined. This suggests that synaptic aging in the cochlea occurs independently of hair cell loss and may be a more sensitive early marker of age-related decline.

These findings offer initial insight into how cochlear synaptic aging progresses in a long-lived, disease-resistant mammal. They also raise important questions about the molecular and cellular mechanisms supporting this extended maintenance. Understanding how hearing is preserved in such species may help inform strategies to delay or prevent age-related hearing loss and its social consequences in humans.

P29

Understanding high frequency vulnerability in age-related hearing loss

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Age-related hearing loss (ARHL) affects billions of people worldwide, however currently available treatment options, such as hearing aids and cochlear implants, do not slow further degeneration or recover hearing loss. This is because hearing aids or cochlear implants rely on the amplification of sound but the mechanism underlying ARHL is still unclear. While genetic factors are known to influence the progression of ARHL, little is known about how these genes affect the function of hair cells especially prior to the onset of any age-related dysfunction. *Cdherin-23* (*Cdh23*) is one of the genes involved in ARHL, and C57BL/6N animals with the hypomorphic $Cdh23^{753A}$ mutation have been widely used for studying ARHL. Similar to humans, these mice show ARHL starting from the higher frequencies first evident at 1-3 months of age, but it is unclear how $Cdh23^{753A}$ initiates this. *Cdh23* is one major component for the mechano-electrical transduction of sound in cochlear hair cells; one of two components that form the link for the sound sensitive tip-link apparatus between the stereocilia. Here, I used electron microscopy to inspect the morphology of stereocilia, together with patch-clamp electrophysiology to investigate mechano-electrical transducer current in C57BL/6N mice. To understand the impact of $Cdh23^{753A}$, I compared recordings from C57BL/6N with those from genome edited C57BL/6N mice with repaired *Cdh23* (6N-Repaired). I also recorded mechanotransducer currents from both lower (9-12 kHz)- and higher (36-42 KHz)-frequency hair cells at P14 in mice to understand how hair cell function is affected prior to the first onset of high-frequency hearing loss at 1 month. Overall, by comparing physiology data acquired from hair cells at different frequencies and from C57BL/6N and C57BL/6N-Repaired mice, we have begun to build an insight as to how ARHL starts from higher frequencies caused by the genetic factor *Cdh23*.

P30

Longitudinal study of otoacoustic emissions in a rat model of age-related hearing loss

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As an early objective biomarker of subclinical hearing loss and cognitive dysfunction, outer hair cell (OHC) dysfunction receives increasing attention in age-related hearing loss (ARHL), speech discrimination and early onset dementia. ARHL progression has previously been reported in standard Wistar rats, including characterization of OHC function but limited to only non-linear distortion-product otoacoustic emissions (DPOAEs). Linear reflection-type stimulus-frequency otoacoustic emissions (SFOAEs) have not yet been established for this species and may provide additional information about cochlear amplification, tuning and onset of hearing loss. Based on human studies, inclusion of both OAE types in cochlear assessment may provide complementary information. Here we report a longitudinal study of male Wistar rats from 6 to 15 months of age, with an objective assessment of cochlear function using both swept-tone stimulus DPOAE and SFOAE measurements.

Thirteen male Wistar rats underwent monthly unilateral audiometric characterization in a soundproof chamber from 6 to 15 months: ABR (8/16/24/32 kHz, 90-10 dB SPL in 5 dB steps, closed field configuration), swept-tone stimulus OAEs from 4 to 42 kHz Including high and low level DPOAEs as well as SFOAEs. For DPOAEs parameters were: f2/f1 ratio = 1.2, L1/L2 = 80/70 and 50/40 dB SPL, for SFOAEs probe/suppressor level were 50/65 dB SPL, fs/fp ratio = 1.1.

At 6 months, animals demonstrated normal hearing with ABR thresholds from 19.2±0.8 to 22.5.8±1.7 dB SPL between 8-32 kHz. High level DPOAEs showed mean amplitudes from 13.7±1.1 to 33.01.6 dB SPL between 4-42 kHz, low level DPOAEs amplitudes ranged between -8.4±3.6 and 18.00.9 dB SPL. Half-octave averaged SFOAE amplitudes ranged from 2.7±2.0to 10.3±1.0 dB SPL.

Whereas ABR threshold shifts increased statistically significantly for 8, 16 and 32 kHz frequencies from 12 months, DPOAE amplitudes significantly decreased across the 4-32 kHz frequency range already from 11 months and even 10 months for low level DPOAEs at 8 kHz. SFOAE amplitudes also showed statistically significant losses from 11 months for 5, 7 and 20 kHz center frequencies.

These data confirm that SFOAEs and DPOAEs can be measured consistently in Wistar rats, offering a higher sensitivity for detection of the hearing loss onset with aging, compared to ABR thresholds.

Furthermore, in this model, the joint-OAE profile of DPOAEs and SFOAEs seems in accordance with published human data where SFOAE amplitudes appear to be more preserved that DPOAEs with aging.

P31

Hair cell apoptosis triggered by reduction in stereociliary PMCA2 Ca2+ pump density

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Sound transduction in cochlear hair cells arises by activation of mechano-electrical transducer (MET) channels for which transmembrane channel-like protein 1 (TMC1) is the pore forming subunit. We made mice harboring mutations in TMC1 that culminate in hair cell apoptosis and deafness by postnatal day (P) 21. However, mechano-transduction was normal at P6, when TMC1 is fully expressed. The goal is to understand when and how apoptosis is triggered.

We studied four mutations, *Tmc1* p.D569N, p.M412K, p.T416K and p.D528N. MET channels in all mutants were gated normally, but with diminished Ca2+ permeability at P6, T416K being the least affected and D528N the most. Early signs of hair cell apoptosis were assayed in neonatal *Tmc1* mutants by labeling with Calcein-AM, MitoTracker and Annexin V, the latter marking scramblase externalization of phosphatidyl serine. Reduced labeling with Calcein-AM was correlated with reduced MitoTracker, which indicated degraded mitochondrial function; the targeting of mitochondria was confirmed using MitoLight to reveal diminished mitochondrial membrane potential. These markers demonstrated mitochondrial dysfunction in *Tmc1* mutants, even at P6 when MET currents were still present. Acoustic brainstem responses established that *Tmc1* p.D569N, p.D528N and p.M412K mice were deaf by P15 but hearing was retained in *Tmc1* p.T416K until P21.

We hypothesized that apoptotic behavior may stem from increased cytoplasmic Ca2+ (Furuta et al 2021), which we tested by blocking the stereociliary PMCA2 Ca2+ pump to abolish Ca2+ extrusion. Scramblase activity was increased by elevating external pH to 9.0 or treating with 50 micromolar carboxyeosin, two methods known to block the PMCA2 (Beurg et al 2010). Scramblase activity was also augmented with 0.1 mM butylhydroquinone to inhibit the SERCA pump. The results suggest apoptosis is promoted by elevation in hair cell [Ca2+]. A reduced PMCA2 density was found in the stereocilia of all *Tmc1* mutants and was strongly correlated with a decrease in MET channel Ca2+ permeability. The path linking *Tmc1* mutation to reduced PMCA2 density is unknown but may reflect a homeostatic mechanism during the neonatal period due to reduced Ca2+ permeability in the mutants. In support of this notion, culturing cochleae in reduced (0.45 mM) external Ca2+ for 5 hours significantly reduced PMCA2 density. The results establish that hair cells in *Tmc1* mutants have embarked on apoptosis at P6 and suggest connections between stereociliary PMCA2 density, hair cell apoptosis and deafness. Funded by grant R01DC01362 from NIDCD

P32

Early detection of age-related hearing loss in mice using machine learning and auditory brainstem responses

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Background:

Progressive age-related hearing loss (ARHL) is the most common sensory deficit in adults, yet early detection remains challenging. Machine learning (ML) has shown promise in biomedical applications but remains underutilized in hearing loss diagnosis. Identifying early biomarkers of ARHL could facilitate timely intervention, improve treatment outcomes, and aide the discovery of genes linked with this pathology. The goal of this study was to use ML to detect progressive hearing loss in mice using the auditory brainstem response (ABR) as a non-invasive measure of hearing function.

Methods:

We evaluated different machine learning classifiers on two ABR datasets. First, we recorded ABRs from C57BL/6N (6N) mice, which exhibit early-onset ARHL due to a hypomorphic Cadherin23 ($Cdh23^{ahl}$) allele, and from co-isogenic $C57BL/6NTac^{Cdh23+}$ (6N-Repaired) mice, which maintain good hearing until later in life. Multiple ML classifiers were trained to distinguish between the two strains at an early time point (1 month old), when differences between the two genotypes are not yet evident to human experimenters. We then extended the models to a larger, publicly available, mouse ABR dataset containing >8000 ABRs from both WT mice and mice carrying mutations with known association with progressive hearing loss.

Results:

ML models accurately classified mice at the early stages of progressive hearing loss in both datasets. Moreover, we used the models trained on the larger dataset to make prediction on mice mutant for genes with no known association to hearing loss, highlighting new candidates for progressive hearing loss-linked genes.

Conclusions:

Our findings demonstrate that ML approaches can detect early signatures of ARHL, offering a promising approach for early diagnosis and intervention. These results support the potential translation of ML-based diagnostic tools to human ARHL screening, treatment planning and genetic discovery.

P33

A morphological and quantitative study of Nav 1.6 expression in the Ranvier nodes of C57Bl/6N mouse cochlea

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Age-related hearing loss (ARHL) is a multifactorial condition involving sensory-neural degeneration, where sensory cells and/or neurons are lost. Previous studies have described different subpopulations of neurons with different levels of susceptibility to ARHL. These subpopulations differ in morphology and in their expression of components relevant to action potential (AP) transmission. Voltage-gated sodium channel 1.6 (Nav 1.6) is a voltage-gated ion channel that is important for AP generation and propagation in spiral ganglion neurons (SGN). APs are generated at the heminode and are transmitted via saltatory conduction through the Ranvier nodes (RNs) present in the peripheral and central axons of the SGNs. Changes in the length, diameter or Nav 1.6 expression of the RN affect AP transmission and play a crucial role in ARHL.

The aim of this study is to describe whether there is a specific decline of a certain neuronal subpopulation that are more vulnerable to ARHL and the associated morphological changes in RN (length and diameter) as well as quantitative Nav 1.6 expression with age.

For this objective, image analysis and quantification of Nav 1.6 expression were evaluated in C57Bl/6N mice from 1 to 19 months of age. Immunostainings were performed using an automated immunostainer and quantification done with Image J. RNs were quantified in different areas of the SGN, including the peripheral axon, Rosenthal canal, central axon, transitional zone and RN from the cochlear nerve from the central nervous portion.

Results exposed a different spatial expression of Nav 1.6 in RN at different tonotopical turns. The middle turn showed the highest intensity of the staining which is consistent with the most innervated cochlear turn by the SGNs. The staining of Nav 1.6 at the soma of SGNs was quantified and compared with the changes in expression of this channel at the RN with age. Diameters and length of RN were also evaluated and appeared also to be regulated with age. The longest RN were found in the Rosenthal's canal at the central axon and length and diameters with age.

In C57Bl/6N mice, SGN with bigger caliber axons and longer nodes may be more vulnerable to age and may be lost at older ages accounting for ARHL. Increased Nav 1.6 staining with age may represent a compensatory mechanism against ARHL. These data present C57BL6 as a good model for a specific pattern of sensorineural decline with age, to test new therapeutic approaches and create new SGN models for cochlear implants.

P34

Nav 1.6 expression and Ranvier node morphometry changes in the ageing CBA/J mouse cochlea

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Age-related hearing loss (ARHL) involves a chronic sensory-neural degeneration leading to a loss of speech understanding, especially in challenging situations. Different subpopulations of neurons seem to have different levels of susceptibility to ARHL. These subpopulations differ in morphometry, firing rates and in their expression of components relevant to action potential (AP) transmission. Voltage-gated sodium channel 1.6 (Nav 1.6) is a voltage-gated ion channel is most important for AP generation and propagation in spiral ganglion neurons (SGN). APs are generated at the heminode close to the habenula and are transmitted via saltatory conduction through the Ranvier nodes (RNs) present in the peripheral and central axons of the SGNs. Changes in median length, diameter or Nav 1.6 expression of the RN affect AP transmission and may play a crucial role in ARHL.

The aim of this study is to describe whether there is a pronounced decline of certain neuronal subpopulations that are more vulnerable to ARHL and the associated morphological changes in RN (length and diameter) as well as quantitative Nav 1.6 expression with age.

For this objective, image analysis and quantification of Nav 1.6 expression were evaluated in CBA/J mice from 1 to 19 months of age. Immunostainings were performed using an automated immunostainer and quantification done with Image J. RNs were quantified in different areas of the SGN, including the peripheral axon, Rosenthal canal, central axon, transitional zone and RN from the central nervous part of the cochlear nerve.

Results showed changes of expression of Nav 1.6 with age in RNs dependent on the tonotopic localization. The staining of Nav 1.6 in the cytoplasm of the soma of SGNs was quantified and compared with the increased expression of this channel at the RN with age. CBA/J mice have a significant decrease in Nav 1.6 staining in RNs at older ages. This regulation is compared to the production site of the channel in the SGN soma. Composition of length and diameter of RNs were also evaluated showing changes in median RN length with age and changes in the composition of fiber subtypes.

In CBA/J mice, SGN with smaller caliber may be more vulnerable to age related changes since they are lost more than bigger at older ages. Decreased Nav 1.6 staining with age may further impair AP propagation safety. These data are useful to model this type of hearing loss in human, to find new therapeutic approaches and create new models for cochlear implants with residual hearing

P35

Frequency-specific mapping of auditory nerve fibers: Investigating G-ratio in serial sections

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The cochlea encodes sound via a precise tonotopic organization, where specific frequencies map to distinct locations along its length, ranging from 1 kHz to 100 kHz in mice. To achieve frequency-specific analysis on thin plastic sections, we generated a three-dimensional frequency map using high resolution microCT datasets and Greenwood function. Given the minimal anatomical variation in inbred mouse strains, this serves as a universal reference for precise frequency localization.

Age-related auditory nerve degeneration impacts cochlear implant outcomes, with frequency-specific patterns varying across strains, leading to selective loss and preservation of nerve fiber populations that influence hearing function. By integrating frequency-specific auditory brainstem response (ABR) audiometry with precise frequency mapping, we correlate physiological data with nerve fiber morphometry. Manual segmentation of the basilar membrane at the inner hair cells (IHCs) level enabled spatial mapping of nerve fibers into an "atlas" dataset, allowing the registration of the thin sections within the microCT dataset for an exact frequency correlated morphological analysis. The software-based segmentation and automated evaluation of the g-ratio using deep learning algorithms will provide an objective assessment of nerve fiber integrity across different frequency regions. By correlating ABR parameters, including hearing thresholds and latencies between young vs aged mice, we aim to refine our understanding of frequency-specific neural degeneration. These insights into cochlear pathology will inform strategies for optimizing cochlear implant electrode placement and stimulation paradigms, ultimately improving clinical outcomes by tailoring interventions to preserve and enhance neural function at critical frequency regions, ensuring better auditory performance for cochlear implant users.

P36

From sound to stability: Lessons learned from the CRUSH study on hearing loss progression and vestibular phenotype in Usher Syndrome type II

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Objectives

Usher syndrome type II (USH2) is characterized by sensorineural hearing loss, retinitis pigmentosa and traditionally normal vestibular function. This study aims to analyze the progression of hearing loss using prospectively collected data and to describe the vestibular phenotype of USH2.

Design

The Characterizing Rate of Progression in USHer Syndrome (CRUSH) is a longitudinal, prospective, observational natural history study of patients with Usher syndrome type 2A (USH2A) and *USH2A*-associated non-syndromic retinitis pigmentosa (nsRP). During four years of follow-up, hearing loss progression was measured by pure tone audiometry (PTA) and phoneme scores. Vestibular function was assessed by velocity step tests (VSTs), video head impulse tests (vHITs), caloric reflex tests and vestibular evoked myogenic potentials (VEMPs). Patient-reported symptoms were assessed by questionnaires (Usher Lifestyle Survey, SF-12, PHQ-9, DHI and SSQ).

Results

Thirty-three patients with USH2A and two patients with nsRP were included. Included subjects with Usher syndrome had bilateral symmetric high-frequency SNHL. $PTA_{0.5\text{-}4\ kHz}$ thresholds showed a significant decrease of 2.4 dB ($p = 0.017$) over the four-year follow-up period. The progression of hearing loss was most pronounced in the mid to high frequencies. No significant differences were observed in phoneme scores as measured by the Speech Reception Threshold (SRT). The two patients with nsRP had normal hearing. Vestibular evaluation showed abnormal cVEMPs in 34% and abnormal oVEMPs in 75% of USH2A patients. Velocity step tests, caloric reflex tests and vHIT results were within normal limits in more than 90% of subjects. Patient questionnaires revealed no major balance problems as indicated by the DHI. Based on the estimated marginal mean $PTA_{0.5–4kHz}$ progression, a power analysis showed that 12 patients per arm would be required for a two-year, placebo-controlled, blinded study with 80% power for a potential future therapeutic trial.

Conclusions

This analysis provides robust evidence of measurable hearing loss progression in $PTA_{0.5\text{-}4\ kHz}$ thresholds in USH2A patients. Speech understanding, as measured by SRT, remains relatively stable. Vestibular evaluation revealed no dysfunction of the semicircular canals, although VEMP results suggest a potential subclinical impairment of the otolith organs in USH2A patients.

P37

The CBA/J-Slc26a4p.R409H/p.R409H mouse: A new model for investigating DFNB4 and Pendred syndrome

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Pendrin (SLC26A4) is an anion exchanger mainly expressed in the inner ear, kidney, and thyroid. Defects in the ion transport function are associated with syndromic (Pendred syndrome) and non-syndromic (DFNB4) forms of deafness. The generation and characterization of a consistent animal model is crucial for the investigation of the pathophysiology of pendrin-related hearing loss and the development of therapeutic interventions.

A novel mouse model expressing the pendrin pathogenic variant p.R409H was produced by CRISPR-Cas. Hearing and vestibular functions were characterized. Auditory brainstem response (ABR) testing with clicks and tone bursts (decreasing dB levels from 90dB to 20dB, in 10dB steps), as well as DPOAE measurement at 60dB and 70dB were performed in general anesthesia with an RZ6 system (TDT, USA). Longitudinal measurements were performed from p20 to 4 months of age. For the investigation of the vestibular function, open-field tests were performed, where the mice were recorded for 15 minutes with a camera and analyzed with Ethovision XT (Noldus, NL). Different parameters were analyzed, including distance moved, velocity, and number of rotations.

Wild-type and heterozygous mice showed hearing thresholds in click and tone ABR at 20 – 30dB, while in the homozygous mutants no response could be detected at any dB level in either click or tone ABR. Similarly, positive DPOAE signals could be detected in wild-type and heterozygous mice but not in mutant animals. No significant changes in the hearing thresholds were measured over time. In the open field test, a significant difference in distance moved, velocity, and number of rotations was observed between mutant mice and heterozygous or wild-type littermates. Specifically, homozygous mutant mice moved more and faster than the wild-type and heterozygous littermates. Interestingly, mutant mice consistently showed a characteristic circling movement, which was not observed in any of the normal-hearing littermates.

In conclusion, a mouse strain carrying the common pendrin pathogenic variant p.R409H was successfully generated and characterized. Homozygous mutant mice recapitulated the human phenotype, namely profound hearing loss and severe vestibular dysfunction. These findings set the basis for future investigations of pendrin-related deafness in an animal model.

Acknowledgments

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Working towards human iPSC-derived vestibular-cochlear assembloids

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The human inner ear comprises two interconnected systems: the cochlea and the vestibular system. While recent advances have enabled the generation of cochlear and vestibular organoids from human induced pluripotent stem cells (iPSCs), these systems are typically developed in isolation, missing the anatomical unity seen in vivo. This separation poses limitations, especially when studying drug distribution or modeling diseases that affect the entire inner ear.

To address this, we propose a novel in vitro strategy to assemble a unified inner ear model by fusing separately differentiated vestibular and cochlear organoids. Organoids are initially directed toward vestibular or cochlear fate until day 22 (d22), corresponding to the stage when otic vesicle formation and lineage specification occur. At this point, we merge the organoids using different approaches, including direct co-culture in the same dish and a 3D-printed structure to push fusion of the two organoids.

To distinguish and track the developmental contributions of each organoid, we used a combination of a wild-type iPSC line and a SOX2+ reporter iPSC line. After 4 days in the same well, the vestibular and cochlear organoid fused together. These "assembloids" are cultured until day 90 (d90), when the majority of vestibular and cochlear cell types have matured. The tissues are then fixed and analyzed via immunohistochemistry to assess cellular composition, organization, and potential interactions, and compared to individually cultured vestibular and cochlear organoids.

As a next step, we aim to apply this inner ear assembloid platform to model genetic hearing and balance disorders. Using iPSCs derived from a patient with a mutation causing USH2A disease, we generate disease-specific cochlear-vestibular assembloids. Furthermore, we test the therapeutic potential of previously established antisense oligonucleotides to counteract the disease phenotype within this integrated system.

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Syndromic hearing loss in Slovakia: Genetic landscape and clinical insights

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Background: Syndromic hearing loss (HL) represents a clinically and genetically heterogeneous group of disorders affecting the auditory organ and at least one other organ system, accounting for approximately 30% of hereditary HL. This study investigates the phenotype variability and molecular background of syndromic HL in Slovak patients.

Methods: Subjects with suspected syndromic HL were selected from our DNA repository of individuals with hearing loss collected between 2010 and 2025. Genetic analysis included next-generation sequencing (whole exome and genome), Sanger sequencing and MLPA. Identified variants were classified according to ACMG guidelines. Special attention was paid to functional studies and to genotype-phenotype correlations, particularly in selected genes known for their variable expressivity (*EYA1*, *SIX1*, *SLC26A4*, and *CHD7)*.

Results: The genetic etiology of syndromic HL was identified in 81 probands, encompassing 19 different syndromes and 28 different causal genes. The most prevalent syndromes were Usher (18.5%), Pendred/Enlarged vestibular aqueduct (16.0%), Branchiootic/Branchiootorenal (13.6%), Maternally inherited diabetes and deafness (12.3%), Waardenburg (8.6%), CHARGE (6.2%), Deafness-infertility syndrome (6.2%) and Beta-mannosidosis (4.9%). Several novel variants were identified, including those in genes such as *EYA1*, *PAX1*, *SIX1*, *SLC26A4*, *CHD7*, and *GDF3*, as well as a variant in the *WFS1* gene, which is associated with Wolfram syndrome. To assess the pathogenic potential of selected variants, functional studies were performed for the *EYA1, SLC26A4* and *WFS1* genes. Notably, significant phenotype variability was observed in individuals with pathogenic variants in the *EYA1* and *SIX1* genes, ranging from classic BOR syndrome to isolated hearing loss. Similarly, non-syndromic presentations were identified in subjects with variants in *SLC26A4* or *CHD7* genes, despite their established links to Pendred and CHARGE syndromes, respectively.

Conclusions: This study highlights the genetic and phenotype heterogeneity of syndromic hearing loss in the Slovak population. The observed phenotype variability suggests that certain pathogenic variants may result in non-syndromic presentations. These findings emphasize the importance of recognizing variable expressivity and support the need for comprehensive, multidisciplinary diagnostic approaches, especially in subjects with atypical or incomplete syndromic features.

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P40

Methodology for correlated measurement of hair cell calcium, stereocilia function, and organelle ultrastructure in a noise-exposed guinea pig model

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Acoustic overexposure induces hearing loss through damage to cochlear hair cells, but the cellular mechanisms remain incompletely understood. Our focus here is on the effects of acoustic overexposure on intracellular calcium (Ca^{2+}) concentration, stereocilia function, and organelles in hair cells. Hair cell Ca^{2+} levels and sound-evoked Ca^{2+} responses depend on the function of stereocilia ion channels, which are essential for mechanotransduction and auditory signalling.

We present a methodological pipeline for correlated measurement of intracellular Ca^{2+} concentration, stereocilia function, and organellar ultrastructure in cochlear hair cells of noise-exposed adult guinea pigs. We begin the protocol with acoustic overexposure and auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE) recordings to assess hearing function before and after exposure. Next, we prepare a temporal bone preparation preserving the middle and inner ear at near in vivo conditions [1]. We load cochlear hair cells with the Ca^{2+} indicator Cal Red R525/650 for live imaging of intracellular Ca^{2+} concentration, or with glibenclamide-based ER-Tracker Green for imaging endoplasmic reticulum (ER) morphology using Airyscan detectors. This preparation allows us to simultaneously deliver sound stimulation via the ear canal, record cochlear microphonics, and image Ca^{2+} dynamics or ER structure in live cells. Finally, we assess stereocilia function via FM1-43 dye uptake, providing a measure of stereocilia ion channel functionality.

Following live imaging, we chemically fix and process the same preparation for ultrastructural analysis using expansion microscopy (ExM) on the cochlea [2]. In ExM, we apply pan-protein and membrane-specific staining to enable high-resolution visualization and segmentation of hair cell organelles and stereocilia.

This pipeline enables us to correlate single-cell physiological data with detailed subcellular structural features in the cochlea. It provides a platform to investigate how changes in calcium, stereocilia function, and organelle morphology contribute to hair cell dysfunction following acoustic overexposure. These insights may help in the discovery of early cellular events and mechanisms leading to hearing loss.

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P41

Susceptibility to Noise Trauma in Deaf *Otof* Knockout mice

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Congenital deafness due to mutations in the human *OTOF* gene (DFNB9) is due to a defect of vesicle release at inner hair cell ribbon synapses. Otoacoustic emissions, a measure of cochlear amplification, can be normal in newborn children but typically get lost within months to years after birth. In *Otof* knockout mice, we observed a moderate acceleration of age-related outer hair cell (Stalmann et al., Front. Cell Neurosci 2021). We hypothesized that the absence of protective reflexes (stapedial reflexes, olivocochlear reflexes) makes deaf ears more susceptible to noise damage. Alternatively or in addition, there may be age-related degeneration of the stria vascularis.

We applied lower- (15 min 103 dB) and higher-intensity (2 h 120 dB) acute noise trauma as well as a novel chronic noise trauma paradigm (80-85 dB, 8h/night, 5 days/week, 3 months) to *Otof* knockout and wildtype mice. Auditory function was assessed by recordings of auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE). The degeneration of inner and outer hair cells and of synapses and changes to vascular structures of the stria vascularis and spiral ligament were assessed by immunohistochemistry and confocal microscopy.

Acute noise trauma led to a similar loss of DPOAE and hair cells in mice of both genotypes. However, chronic noise trauma resulted in more severe loss of inner and outer hair cells in *Otof* knockout mice than in wildtype controls. Inner hair cell ribbon synapse numbers were reduced in wildtype mice following all three types of noise trauma. Ribbon synapse numbers were reduced in all *Otof* knockout mice, but noise trauma induced only a minimal further decrease. The vascularization patterns in the stria vascularis and spiral lamina in aged unexposed *Otof* knockout mice were mostly intact with only a slight decrease in vessel diameter in the stria vascularis.

Our findings are consistent with an increased susceptibility of hair cells to chronic noise trauma in *Otof* knockout mice, presumably due to the absence of protective reflexes. Consistent with an excitotoxic mechanism of noise-induced synaptopathy, the remaining inner hair cell ribbon synapses in *Otof* knockout mice were resistant to noise trauma. Unnecessary noise exposure (e.g. fitting of high-power hearing aids) in deaf children with DFNB9 who might be candidates for gene therapy should be avoided to preserve outer hair cell function.

P42

Male but not female *Otof* knockout mice are highly sensitive to noise trauma

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Deficiency in otoferlin, encoded by the gene Otof, typically results in severe to profound hearing impairment due to disabled synaptic transmission from inner hair cells to the auditory nerve. Patients with biallelic OTOF mutations typically display intact otoacoustic emissions (OAEs), which are lost mostly within the first two years, latest in the second decade of life. A similar effect was found in Otof-knockout mice, which display a loss of OAE function from 6 month of age on. We hypothetized that the missing activation of efferent inhibition might render patients and mice more sensitive to noise trauma.

We exposed anesthetized male and female wildtype and Otof-knock-out mice to a 15 min noise covering 4-16 kHz with 103 dB sound pressure level (SPL). We tested auditory function 3 days and 11-15 days after the noise trauma with auditory brainstem response (ABR) recordings and measuring distorsion product otoacoustic emissions (DPOAEs).

Noise exposure caused a ~30 dB ABR threshold elevation for 11 to 45 kHz after three days. After 11-15 days of recovery, this resulted in a permanent elevation of the auditory threshold of ~20 dB SPL in female and ~30 dB SPL in male wildtype mice. DPOAE thresholds were strongly elevated for 11, 16 and 22 kHz three days after the noise trauma in all mice. 11 to 15 days after noise exposure, wildtype mice of both sexes and female Otof-knock-out mice grossly recovered their DPOAEs at 11 kHz, but male mice displayed a broad variability, with a substantial number of male mice not recovering their DPOAEs. On average, DPOAE thresholds for 11 kHz F1 stimulus frequency were 30 dB SPL higher than before the noise exposure. For 16 and 22 kHz F1 stimulus frequency, the difference between female and male mice and between genotypes was less pronounced, since most mice only partially recovered their DPOAEs.

In conclusion, we confirm that Otof deficiency is indeed increasing noise susceptibility, particularly for male mice in the 11 kHz frequency area.

Hearing Loss 2/2

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The effect of KCNQ4 activator on acute vestibular dysfunction in mouse

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After the unilateral labyrinthectomy, spontaneous nystagmus was developed to the contralateral side of the labyrnithectomy in all mice. The mean slow phase velocity of spontaneous nystagmus was more significantly reduced in the retigabine injected mice at 48 hours (0.60 vs. 0.24 deg/sec) and 7 days (0.25 vs. 0.09 deg/sec) after the labyrinthecto Vestibular compensation can be stimulated by vestibular rehabilitation exercises, but there is no definitive therapeutic agent that can stimulate vestibular compensation as well as recovery of vestibular function. In this study, we tried to investigate the role of KCNQ4 stimulator (retigabine) in acute unilateral vestibulopathy mouse model. We performed unilateral labyrinthectomy in 8-week-old C57BL/6 mouse. Retigaine (10 μg/g) or DMSO was injected i.p. in the mice immediately after the labyrinthectomy. The mean slow phase velocity of spontaneous nystagmus and vestibulo-ocular reflex test was measured before labyrinthectomy, 1, 12, 48 hour, and 7 days after labyrinthectomy. The same experiment was performed in Kcnq4p.W277S/p.W277S mice to confirm the effect of retigabine in the wild type mice. To investigate the effect of retigabine on vestibular compensation, immunohistochemistry for c-Fos and KCNQ4 from peripheral vestibular nerve to vestibular nucleus was performed. After the unilateral labyrinthectomy, spontaneous nystagmus was developed to the contralateral side of the labyrnithectomy in all mice. The mean slow phase velocity of spontaneous nystagmus was more significantly reduced in the retigabine injected mice than DMSO injected mice. In sinusoidal harmonic acceleration test, gain value was significantly increased in the retigabine injected mice than DMSO injected mice at 7 days in 1.28Hz (0.13 vs. 0.20). In contrast, retigabine showed no effect on labyrinthectomized Kcnq4p.W277S/p.W277S mice. In immunohistochemistry, c-Fos protein and KCNQ4 expression was increased in the vestibular nucleus of retigabine injected mice when compared to that of DMSO injected mice. But Kcnq4p.W277S/p.W277S mouse doesn't have any difference. Based on the observation, KCNQ4 stimulator was likely to enhance vestibular compensation after acute vestibular injury. In addition, we will examine differences in C-fos expression in peripheral vestibular nerves in the direction of damage.

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Investigation of the interaction of stress hormone receptors and BDNF for hearing function in the animal model mouse

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To date, there are no standardized, scientifically proven treatment approaches for hearing disorders. One reason for this is the variety of different causes for hearing disorders. It is known that factors such as anxiety or stress, but also disturbed signal processing between auditory and non-auditory brain areas, contribute to hearing disorders. The extent to which stress or stress hormones are involved in the interaction between the auditory system and associated brain regions is unclear.

In preliminary studies we could show that a balanced interaction of the stress hormone receptors MR and GR, but also of excitation and inhibition, controlled by neurotrophins such as activity-dependent BDNF, is crucial for intact auditory function. The central deletion of MR or GR has effects on the peripheral auditory system that lead to altered stimulus processing and transmission as well as on central plasticity mechanisms that are important for adaptation to altered input activity. Further studies have shown that the same mechanisms are also associated with changes in activity-dependent BDNF.

Although it is known that glucocorticoids and BDNF share similar intracellular signaling pathways and regulate each other's function at multiple levels, the exact interplay for individual physiological processes such as in our case hearing function is not clear.

In the project presented here, new mouse lines are being investigated that enable both the inducible tissue-specific deletion of one or both stress hormone receptors (MR or GR) and the visualization of activity-dependent BDNF via intrinsically expressed fluorescent proteins (CFP and YFP).

These lines should provide information about the interaction of MR or GR and BDNF and how this can influence auditory function.

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Keywords: Stress, stress receptors, BDNF, hearing

P45

Gap pre-pulse inhibition of the acoustic startle in the objective assessment of tinnitus in humans using electroencephalography

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A limitation in current clinical trials on tinnitus in the reliance on subjective self-reported assessments as an outcome measure. Recently, it has been proposed that magnetoencephalography (MEG) recordings in humans of cortical evoked responses to the gap pre-pulse inhibition of the acoustic startle (GPIAS), a paradigm used pre-clinically to objectively assess tinnitus, offer greater sensitivity than the traditional blinking response. Here, we present preliminary results from the TInnitus DEtection (TIDE) project on a recently completed recruitment of 40 chronic tinnitus cases and controls at the Otolaryngology Head and Neck Surgery Department of the University Hospital in Tübingen. The Department is one of the 7 sites of a multi-centre study, using electroencephalography (EEG) more amenable to clinical routine. Following inclusion/exclusion criteria and informed consent, participants underwent series of auditory measures and on-line questionnaires (i.e., ESIT-SQ, several Visual Analog Scales, THI, TFI, HADS, PSQ-30, HQ) followed by EEG recording of the above-mentioned paradigm. We exposed participants to short sound pulses preceded or not by silent gaps embedded in a carrier sound of various frequency ranges (broad-band noise or BBN, 3 kHz and 5 kHz narrow-band noises). The paradigm was well tolerated by all participants. While nearly 75 % of suppression of pulse-evoked responses by gaps measured using MEG in a BBN carrier, we only achieved around 35 % using EEG, suggesting that EEG is less sensitive than MEG. Moreover, NBN were even less efficient than BBN in triggering inhibition. We present preliminary results from this first set of cases and controls and propose than BBN GPIAS may suffice as an objective outcome measure for tinnitus in humans using EEG, something that will be verified

across the other clinical sites. If successful, this novel approach may pave the way for an objective testing of drugs or medical devices with the aim of treating tinnitus.

Trial Registration: NCT06520565

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Towards objective tinnitus classification in mice: A multimodal, biomarker-based approach

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Investigating tinnitus mechanisms in animal models requires an objective method to identify tinnitus while disentangling mechanisms underlying associated hearing loss. Based on preliminary findings, $GABA_A$ receptor binding potential may be elevated in tinnitus patients. We aim to assess viability of this biomarker for objectively classifying tinnitus in animals and compare it to existing methods for tinnitus classification.

Awake mice were bilaterally exposed to 10-16 kHz noise at 110, 115, or 120 dB for 1h, followed by a 12-week assessment. Tinnitus classification was evaluated using Gap Prepulse Inhibition of the Acoustic Startle Reflex (GPIAS), Auditory Brainstem Response (ABR) wave V (inferior colliculus) to I (cochlear nerve) amplitude ratios to assess central gain, and PET imaging with [^{11}C]flumazenil to assess $GABA_A$ receptor binding. Inner ears and brains were collected for subsequent molecular analyses.

At 12.5 kHz, the 110, 115, 120 dB noise-exposed groups had permanent threshold shifts of 20, 38, and 63 dB, respectively. GPIAS revealed behavioral evidence of tinnitus in the 115 dB group one week after noise exposure, persisting throughout the follow-up. In this group, ABR measurements also demonstrated increased central gain. In total, from the 115 dB-exposed mice, 4 out of 9 were classified as tinnitus-positive based on behavioral evidence of tinnitus via GPIAS and 3 out of 7 based on elevated ABR wave V/I ratios. Importantly, these two methods did not yield overlapping classifications.

This study establishes 115 dB bilateral noise exposure as an optimal model for moderate chronic hearing loss. While GPIAS and ABR both detect evidence of tinnitus, their classifications differ, underscoring the need for a multimodal approach for robust and translatable tinnitus classification in preclinical research. Ongoing analysis of inner ear and brain tissues will reveal potential histological differences between classified groups. Ongoing PET analysis and upcoming autoradiography experiments will determine whether $GABA_A$ receptor binding potential correlates with tinnitus classification based on GPIAS and/or ABR.

P47

Complete loss of functional PHEX is required for development of Meniere disease in X-linked hypophosphatemia

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Question - The etiology of Meniere's Disease (MD) remains unclear, with genetic factors proposed among potential mechanisms. We focused on a distinct MD subgroup characterized by endolymphatic sac hypoplasia (MD-hp), presenting with early onset, male predominance, bilateral involvement, and familial clustering. Unexpectedly, several MD-hp patients were also diagnosed with X-linked hypophosphatemia (XLH), a rare disorder disrupting phosphate homeostasis and causing skeletal and renal abnormalities. We hypothesize a genetic link between XLH and this distinct MD-hp subgroup.

Methods - We recruited XLH patients and evaluated them for MD through clinical history and audiovestibular testing. Hypoplasia of the endolymphatic sac was assessed using a vestibular aqueduct surrogate marker on high-resolution temporal bone CT. We performed next-generation sequencing from blood or saliva samples and screened for novel and rare variants with loss of function (LoF) or missense consequences in PHEX gene or its downstream biological pathway. Variant prioritization integrated information from control and clinical public databases, in silico pathogenicity prediction, and 3D protein structural modeling.

Results - In our cohort of 33 XLH individuals, 6 patients (18.2%) met criteria for bilateral MD-hp. This co-occurrence rate (1 in 5.5) is over six million times higher than expected by chance (1 in 33.3 million), strongly supporting a causal association. None of the 26 females showed audiovestibular symptoms, whereas 6 of the 10 males presented with bilateral MD and radiologically confirmed bilateral endolymphatic sac hypoplasia. Two additional younger males showed hypoplasia without current MD symptoms and may develop the phenotype later in life. These 8 males carried hemizygous LoF variants in PHEX, in contrast to the heterozygous female carriers. The last two males did not show MD or hypoplasia and differed from the others by carrying their PHEX LoF variant in a mosaic pattern or by harboring a missense PHEX variant with milder functional impact.

Conclusions - Our findings support a dosage-dependent model in which the MD-hp phenotype in male XLH patients arises from complete loss of functional PHEX protein. In contrast, residual wild-type activity appears to prevent audiovestibular symptoms in other genotypes. We here identified a null-PHEX genotype, with a specific hypoplastic endotype, that defines a distinct subgroup of MD patients, providing a framework for mechanistic studies, biomarker discovery, and targeted therapies in genetically susceptible individuals.

P48

Divergent burden of rare missense and loss-of-function variants in inner ear-expressed genes in familial and sporadic Meniere disease

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Meniere disease (MD) is a chronic inner ear disorder characterized by episodic vertigo associated with fluctuating sensorineural hearing loss and tinnitus or aural fullness, with significant heritability according to familial aggregation and sequencing studies. This study compares the burden of rare high- and moderate-impact coding variants in an MD cohort to determine whether the genetic burden in sporadic MD (SMD) overlaps familial MD (FMD), potentially revealing hidden inheritance in SMD.

Exome sequencing was performed on 380 unrelated MD patients (93 FMD, 287 SMD) to assess the burden of rare missense and loss-of-function (LoF) variants. Gene burden analysis (GBA) identified enriched genes in each group, prioritizing candidates based on variant carriers, inner ear expression, and hearing/balance-related phenotype annotation.

FMD patients showed higher accumulation of missense and loss-of-function variants than SMD, especially in genes linked to auditory and vestibular function. GBA identified 269 enriched genes in SMD, with 31 annotated for inner ear phenotypes, while FMD had 432 with 51 prioritized. It should be noted that a total of 89 patients (95.7% of the FMD cohort) carried variants in the 51 enriched genes, while 270 patients (94% of the SMD cohort) carried variants in the 31 enriched genes. Sporadic and familial MD overlapped in 28.1% of enriched genes, with *ADGRV1*, *MEGF8*, and *MYO7A* most commonly shared. Functional evidence from auditory brainstem responses in knockout mouse models supported several novel candidate genes, with *NIN* and *CCDC88C* enriched in SMD, and *ANKRD24* enriched in FMD; these genes were not previously described as hearing loss genes.

In conclusion, SMD and FMD have a divergent genetic architecture. The differential burden of rare variants in genes related to specific inner ear structures suggests diverse pathogenic mechanisms and supports a multiallelic or recessive inheritance model in MD.

P49

Subtype-specific vulnerability of type I spiral ganglion neurons across cochlear injury models

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Introduction

Type I spiral ganglion neurons (SGNs), which synapse with inner hair cells (IHCs), are essential for sound perception. Recent transcriptomic and physiological studies have classified them into three molecularly and functionally distinct subtypes, Ia, Ib, and Ic, each characterized by different spontaneous firing rates and sound threshold sensitivities. These subtypes are not fully established at birth and undergo postnatal maturation during the first few weeks, a process believed to depend on afferent inputs from IHCs. Type I SGNs degenerate in response to various forms of cochlear damage, including ototoxic agents and aging. However, the susceptibility of each subtype to such damage remains unclear. This study aimed to investigate the subtype-specific vulnerability following cochlear injury using two models: aminoglycoside ototoxicity and genetic ablation of hair cells.

Methods

Cochlear injury was induced in adult mice using two approaches. First, gentamicin (1 µL, 40 mg/mL) was injected into the posterior semicircular canal of C57BL/6J mice. Second, Pou4f3-DTR/+ mice received intramuscular diphtheria toxin (25 ng/g) to selectively ablate hair cells. The mice were sacrificed 4-8 weeks later. The cochleae were cryosectioned and processed for immunohistochemical analysis. SGN subtypes were identified using antibodies against POU4F1, a well-established marker specific to type Ic SGNs, and TUJ1, a general neuronal marker. Quantitative analysis was performed to determine the proportion of POU4F1-positive SGNs among the total type I SGNs across the cochlear regions.

Results

A significant reduction in the density of type I SGNs was observed in the gentamicin-injected mice. Notably, the reduction in the density of type Ic SGNs, identified by a decrease in POU4F1 expression, was disproportionately greater than the overall loss of type I SGNs, indicating subtype-specific vulnerability. In contrast, the Pou4f3-DTR model showed overall SGN loss, but the proportion of type Ic neurons remained stable, implying non-selective degeneration across subtypes.

Conclusions

Our findings demonstrate that the subtype-specific vulnerability of SGNs depends on the type of cochlear injury. Type Ic SGNs are more susceptible to aminoglycosides, while genetic hair cell ablation affects subtypes uniformly. Recognizing SGN subtype diversity is vital for understanding cochlear pathology and designing targeted neuroprotective therapies.

P50

Zebrafish hair cells can survive prolonged exposure to aminoglycoside antibiotics

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Hair cells are sensitive to toxic insults from chemical agents such as nitrile compounds, cisplatin and aminoglycoside antibiotics. Cisplatin and aminoglycoside antibiotics are widely used to treat cancers and life-threatening infections, causing hearing loss and/or vestibular dysfunction as secondary effect. Hair cell loss is irreversible in most mammals, so these effects are permanent. In rodents, we have previously shown that chronic systemic exposure to a nitrile or streptomycin initially cause damage to vestibular hair cells that is reversible. Thus, reversible synaptic uncoupling between the hair cell and its afferent neurons occurs before the hair cell loss begins. The molecular basis of these phenomena remains largely unexplored.

Zebrafish larvae are a good model to study hair cells due to the external localization of part of them in sensory organs called neuromasts, forming the lateral line. They are accessible for ototoxins and for their assessment. Cisplatin and aminoglycoside antibiotics damage zebrafish larvae hair cells after short, acute exposures. However, in contrast to most mammals, the fish neuromasts contain support cells able to regenerate the lost hair cells already 24 hours post-exposure. Most studies have focused on acute exposures, finding that hair cells loss results from hair cell death by mitochondrial dysfunction and oxidative stress.

We aimed to develop a model to study long exposures to ototoxins using zebrafish larvae before the regenerative response is effective. To this end, we exposed 5dpf zebrafish larvae to low concentrations of four different aminoglycosides (kanamycin, streptomycin, neomycin and gentamicin) and, after 6h and 24h, we stained then with the fluorescent dye FM1-43X and assessed eight neuromasts of the anterior lateral line. All of the aminoglycoside antibiotics used caused a clear dose-response loss of hair cells. Then, we have confirmed these results by counting the number of hair cells after treatment using a transgenic line that specifically labels hair cell nuclei. Additionally, we have used a fluorescently labelled aminoglycoside to determine its accumulation inside hair cells and we have found that some zebrafish hair cells can survive even after 24 hours of continuous exposure of a low concentration with a noticeable amount in their cytoplasm. We will use this approach to study gene expression changes in these surviving hair cells.

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P51

Dose-dependent ototoxicity and hydration effects in a rat cisplatin hearing loss model

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Cisplatin is a platinum-based chemotherapeutic agent and is widely used in cancer treatment. It is accompanied by severe side effects, notably ototoxicity. Cisplatin-induced hearing loss (CIHL) results in permanent cochlear damage at both functional and cellular levels. Despite the extensive use of Cisplatin in clinical treatments, there are currently few protective strategies and no effective regenerative therapies available. This study aimed to optimize a reliable preclinical rodent model of CIHL.

We compared the effects of a single exposure to three doses (8, 10 and 13 mg/kg) of Cisplatin. Three days post Cisplatin administration, auditory function was assessed using non-invasive measures: Distortion Product Oto Acoustic Emissions (DPOAEs), Auditory Brainstem Responses (ABRs) and Wave I analysis, the combination of which allows a differential diagnosis of the sites of dysfunction. Auditory function (DPOAEs and ABRs) and transmission (Wave I) were altered in a dose dependent manner at 10 and 13 mg/kg indicating progressive hearing loss. In contrast, a single dose of 8 mg/kg did not produce significant alterations in auditory function or neural transmission compared to controls.

Immunostaining to quantify Inner and Outer Hair Cells (IHCs and OHCs) showed that the auditive deficits observed at 10 and 13 mg/kg were accompanied by a reduction in OHCs density in a dose dependent manner. Comparable reductions in body weight were observed across all doses (8, 10 and 13 mg/kg) indicating that the differential effects on auditory function were not associated with systemic health improvement.

Extending the viability of the CIHL model, through improved daily care, might provide a more translational model of hearing loss. To test this, we compared the effects of daily rehydration using Plasmalyte-A vs. Lactated Ringer's solution. Our findings demonstrate that these modifications did not impact body weight loss or recovery of animal health, meaning that neither Lactate Ringer's nor Plasmalyte-A conferred any systemic health benefits. However, auditory function was preserved in animals receiving Lactated Ringer's: both DPOAEs and ABRs remained at baseline levels, indicating a protective effect on hearing function.

An effective CIHL model requires an optimal Cisplatin dose combined with appropriate supportive care to maintain anticancer efficacy, while minimizing side effects. Preclinical testing is a critical phase in drug development, underscoring the need to refine and expand tools to support the advancement of protective or regenerative therapies.

P52

Emerging novel biomolecular targets of cisplatin-induced ototoxicity: The imbalance of the endocannabinoid system

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Cisplatin is a potent platinum-based chemotherapeutic agent used for treating several neoplasms in adults and children. However, it is associated with side effects including ototoxicity which irreversibly damages the sensory hair cells (HCs) in the organ of Corti leading to hearing loss. Strikingly, no effective treatment exists yet, underscoring the need for new targets. In this context, emerging studies indicate a key role for Endocannabinoid system (ECS) in cisplatin-induced ototoxicity. Here, we have systematically profiled for the first time the presence of the ECS in a mouse HC-like cell line (UB/OC1) and their effects post-cisplatin. We first verified the presence of Myosin 7a -a classic HC marker- in the cells to demonstrate the suitability of the cell line to study HC biology. In this HC-like model we explored the presence of ECS and later its modulation upon cisplatin-induced damage. Firstly, we detected the following ECS components; (i) principal endocannabinoids (eCBs), arachidonylethanolamine (AEA) and 2-arachidonylglycerol (2-AG); (ii) eCB-like lipids, *N*-palmitoylethanolamine (PEA), *N*-oleoylethanolamine (OEA), *N*-linoleoylethanolamine (LEA), *N*-stearoylethanolamine (SEA), *N*-palmitoleoylethanolamine (POEA), *N*-epoxyeicosatetraenoylethanolamine (EPEA); (iii) receptors, Cannabinoid receptors 1/2 ($CB_{1/2}$), Transient receptor potential vanilloid (TRPV 1), Peroxisome proliferator activated receptor α/γ/δ (PPARα/γ/δ); (iv) metabolic enzymes, Diacylglycerol lipase (DAGL α/β), Fatty-acid amide hydrolase (FAAH), α/β Hydrolase Domain-Containing Protein 4/6/12 (ABHD 4/6/12), N-acylethanolamine acid amidase (NAAA) in UB/OC1 cells. Afterward, a cisplatin-induced ototoxic model was established by treating cells with a range of cisplatin concentrations and IC50 (30 µM) was used for further studies. The model also showed key ototoxic features such as cell death, decrease in the Myosin 7a and activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) signaling. In such a model, the protein levels of receptor CB_2 and metabolic enzymes DAGL β and ABHD 6 were significantly reduced. Despite these changes, their sub-cellular localization patterns remain unaffected after cisplatin. Of note, CB_2 was unexpectedly detected in nucleus in both conditions, a finding further validated by live-cell imaging using a selective fluorescent probe. Altogether, our findings highlight the presence of ECS in HC-like cells and reveal their involvement in cisplatin-induced ototoxicity, uncovering novel biomolecular targets.

P53

Inhibition of cisplatin accumulation in the inner ear by CORM-2 protects against ototoxic damage

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Background: Cisplatin is a widely used chemotherapeutic agent effective against various solid tumors, but it causes irreversible hearing loss. Currently, there are no FDA-approved therapies to prevent cisplatin-induced ototoxicity.

Objective: This study aimed to determine whether tricarbonyldichlororuthenium (II) dimer (CORM-2), a carbon monoxide (CO)-releasing molecule, could alleviate cisplatin-induced hearing loss and reduce cisplatin accumulation in the mouse inner ear.

Methods: Six-week-old male BALB/c mice were divided into four groups: control (saline), CORM-2 only (30 mg/kg, i.p., four doses), cisplatin only (20 mg/kg, i.p., single dose), and CORM-2 + cisplatin. Auditory brainstem response (ABR) thresholds, cochlear platinum levels, and histological changes were analyzed.

Results: CORM-2 co-treatment significantly mitigated cisplatin-induced hearing loss and decreased platinum accumulation in the inner ear. It enhanced plasma membrane repair in the stria vascularis and suppressed inflammation, apoptosis, and necroptosis in cochlear tissues. CORM-2 also preserved the integrity of the cochlear microvasculature, including endothelial cells, pericytes, and perivascular macrophage-type melanocytes. FITC-dextran tracer leakage from cochlear vessels, observed after cisplatin treatment, was fully prevented by CORM-2, indicating restored vascular barrier function.

Conclusion: CORM-2 effectively protects against cisplatin-induced ototoxicity by reducing cochlear platinum uptake and cellular stress responses. These findings support the potential clinical use of CORM-2 as a co-therapy to prevent hearing loss in patients undergoing cisplatin-based chemotherapy

P54

Intratympanic injection of dexamethasone Mitigates Radiation-Induced Middle Ear Mucosa

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Radiotherapy (RTx) is a highly effective treatment for head and neck cancer that can cause concurrent damage to surrounding healthy tissues. In cases of nasopharyngeal carcinoma (NPC), the auditory apparatus is inevitably exposed to radiation fields and sustains considerable damage, resulting in dysfunction. To date, little research has been conducted on the changes induced by RTx in the middle ear and the underlying mechanisms involved. Dexamethasone (DEX) is widely used in clinical practice because of its immunosuppressive and anti-inflammatory properties. The present study investigated the effects and underlying mechanisms of DEX delivered via intratympanic administration on RTx-induced damage to the middle ear and human middle ear epithelial (HMEE) cells. Sprague–Dawley (SD) rats were exposed to fractionated RTx (6.6 Gy/day for 5 days), and middle ear samples were collected at 1 and 4 months. Rats that received RTx presented a significant increase in the thickness of the submucosal layer in the middle ear and disorganization of the ciliated epithelium in the Eustachian tube (ET) mucosa. Importantly, intratympanic administration of DEX 30 min before RTx resulted in a lower degree of damage than that in the control group. Furthermore, DEX pretreatment downregulated the expression of cell death pathway markers in HMEE cells. Our collective results potentially support the use of DEX to reduce radiation-induced damage in the middle ear and may contribute to the development of future studies.

P55

Dosis-dependent cytotoxicity of sodium hexachloroplatinate (IV), a model complex for corrosion of the CI-electrode contacts, in inner specific cell line and primary cells

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Introduction: Corrosion processes alongside the platinum (Pt) electrode contacts represent the potential limiting factor for long-term stability and functionality of the cochlear implants. As described in early studies Pt (II) and Pt (IV) compounds as corrosion products had been found around the electrode contacts and in the inner ear tissues. Especially hexachloroplatinate (IV) was shown to impede bacterial growth following electrical stimulation of Pt electrodes. Aim of this study was the investigation of the potential cytotoxicity of sodium hexachloroplatinate (IV) as model complex on HEI-OC1 (House Ear Institute-Organ of Corti 1) cells, a cell line of the mouse organ of Corti, rat primary spiral ganglion neurons (SGN) and the Organ of Corti explants (OCex).

Methods: In general, cell death induction, morphological and ultrastructural changings following Na2PtCl6 administration in varying concentrations were characterized by using fluorescence microscopy, cell viability assays, immunocytochemistry and transmission electron microscopy. The SGN and OCex were prepared from the cochleae of postnatal rats (P5) and cultivated for 48 h following exposition to Na2PtCl6. Cell survival rates and neurite outgrowth were quantified by staining of the neurofilament antigen. Hair cell loss of the OCex was determined following actin staining with phalloidine.

Results: The oxidative activity in HEI-OC1 cells decreased following increasing Na2(PtCl6) concentrations between 8 and 16 ng/µl. Accordingly, live cell staining with Calcein acetoxymethyl/Ethidium homodimer III demonstrated an increased number of cells with disrupted membranes. Furthermore, ultrastructural changes were related to mitophagy and subsequent induction of necroptosis. Exposure of the SGN to 15-35 ng/µl Na2(PtCl6) reduced neuronal survival and neurite outgrowth. In parallel, antigen specific staining of the fibroblasts and glial cells confirmed the dose-dependent cytotoxicity of Na2(PtCl6). Also, concentration-dependent hair cell loss in the OCex was observed following exposure to 25-45 ng/µl Na2(PtCl6).

Conclusion: It could be shown for the first time that the model complex induced oxidative stress in a concentration-dependent manner in all inner ear related cells and tissues resulting in cell death. Compared to the HEI-OC1 cell line, the resistance to the Na2(PtCl6)-induced stress in the SGN and the OCex may be related to the trophic support and regeneration mechanisms triggered by non-neuronal cells.

P56

Exploring the link between ototoxicity-induced hearing loss and cellular senescence: Effects of IDPN and senolytic intervention

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The sensorineural structures of the auditory system are vulnerable to ototoxic agents, which can cause permanent and irreversible hearing loss, tinnitus, and balance disorders. The molecular mechanisms of these ototoxic agents are varied and complex; therefore, no effective options currently exist to treat or mitigate the resulting hearing loss. Mechanisms involved in ototoxic hearing loss include increased reactive oxygen species (ROS) production, DNA damage, mitochondrial dysfunction, and endoplasmic reticulum (ER) stress response. These mechanisms overlap with the triggering of cellular senescence, which has also been shown to impair tissue homeostasis and contribute to various age-related diseases. To investigate the link between ototoxicity-induced hearing loss and cellular senescence, we examined the ototoxic effects of 3,3'-iminodipropionitrile (IDPN) and their mitigation using a combination of senolytic drugs (dasatinib and quercetin) in a mouse model. Mice were exposed to 30 mM IDPN solution in their drinking water for four weeks, with intraperitoneal injections of dasatinib and quercetin administered twice weekly. To examine ototoxicity and determine treatment efficacy, we used auditory brainstem responses (ABR) and immunohistochemistry to assess cochlear functional and morphological integrity. ABR analyses revealed that IDPN exposure resulted in high- and low-frequency hearing loss, as assessed by ABR wave I thresholds and amplitudes. The senolytic intervention did not protect against this hearing loss. Furthermore, immunohistochemistry was used to examine morphological structures, including inner and outer hair cells (IHCs and OHCs, respectively) densities and IHC paired synapses. We found that IDPN-exposed mice exhibited significant loss of OHCs and paired synapses between IHCs and auditory neurons in the higher frequency regions (22 and 32 kHz) in both vehicle-treated and drug-treated mice. In contrast, the IHCs appeared unaffected by the ototoxic damage. To further elucidate the molecular mechanisms underlying IDPN ototoxicity and cellular senescence, we plan to use differential gene expression analyses. Overall, these findings suggest that IDPN exposure results in permanent hearing loss, and treatment with senolytics is unable to mitigate this damage. Further investigation into the underlying mechanisms of this lack of efficacy is warranted.

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Establishment of a neural inner ear trauma model: A selective spiral ganglion neuron injury in a large animal model

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Introduction: As the primary neurons in the auditory pathway, spiral ganglion neurons (SGNs) play a crucial role in transmitting auditory signals from the inner ear to the brain, making them essential for hearing. Damage to these neurons, caused by mechanical trauma or ototoxic substances, can result in irreversible hearing loss. A detailed understanding of neuronal responses to trauma is critical for developing novel therapeutic approaches. Ouabain, a specific inhibitor of Na+/K+-ATPase, is frequently used to selectively induce SGN damage in small animal models. This study aims to develop a large animal trauma model to investigate the dose-dependent effects of ouabain on SGNs in a large animal model with human-like anatomy, physiology, and metabolism.

Methods: To determine the optimal dosage, piglets (final n = 4 per dosage group) are treated with an intratympanic injection of 0.1 mM, 1 mM, 2.5 mM, or 5 mM ouabain. Hearing function is assessed preoperatively and over one month postoperatively using auditory brainstem response (ABR) measurements. Subsequently, inner ears are histologically processed to quantify and compare the dose-dependent loss of SGNs.

Results: To date, 8 animals (n = 2 per dosage group) have been treated with an intratympanic application of ouabain. Preliminary ABR results indicate a dose-dependent shift in hearing thresholds, with higher ouabain concentrations leading to more pronounced and persistent hearing loss. Histological analysis of the inner ears, involving midmodiolar sectioning and hematoxylin and eosin staining, is ongoing, with comparative quantification of SGNs to follow.

Conclusion: The preliminary results confirm the suitability of the intratympanic ouabain application as a method for inducing selective, dose-dependent neuronal damage of SGNs in pigs as a large animal model. This model holds significant potential as a valuable foundation for future studies on the pathophysiology of hearing loss and the development of potential therapeutic strategies.

P58

Modulation of cochlear and renal endoplasmic reticulum stress by losartan in diabetic rats: A quantitative bioengineering study of GRP78 expression

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In recent years, lifestyle and dietary changes have increased the prevalence of metabolic diseases such as type 2 diabetes mellitus (T2DM) and obesity, often accompanied by complications like hypertension and vascular damage. These chronic conditions are also linked to less recognized issues, including sensorineural hearing loss. Evidence suggests a connection between diabetes and hearing impairment, with endoplasmic reticulum stress (ERS) and the chaperone protein GRP78 playing crucial roles in cochlear and renal damage. Hyperactivation of the renin-angiotensin system (RAS) contributes to organ injury, while ACE inhibitors like Losartan reduce ERS and inflammation, protecting kidney and ear tissues.

This study employed Zucker Diabetic Fatty (ZDF) rats, a validated T2DM model, divided into placebo and Losartan-treated groups (5 mg/kg/day for 42 weeks), alongside normoglycemic lean controls. Post-treatment, kidney and cochlear tissues were collected for histological and immunohistochemical analysis of GRP78 expression. Light microscopy images were quantitatively analyzed using a custom MATLAB algorithm to measure DAB-positive areas, spiral ganglion nuclear counts, and morphometry of the stria vascularis. Statistical analyses included t-tests and Kruskal-Wallis tests.

Results revealed significantly increased GRP78 expression in Losartan-treated tissues versus placebo and controls, with marked immunoreactivity in the renal cortex and spiral ganglion. The Losartan group showed greater morphological variability in the stria vascularis, while controls exhibited more uniform structures. No significant cochlear differences were observed among groups. In the kidney, GRP78 intensity was significantly higher in the cortical region at lower magnification in treated rats, whereas spiral ganglion nuclei counts were similar across groups despite GRP78 positivity differences.

These findings confirm the central role of ERS and GRP78 in diabetic cochlear and renal damage. Increased GRP78 expression following Losartan treatment suggests protective mechanisms via RAS inhibition. Regional renal differences highlight the complexity of ER stress regulation and therapeutic responses. Targeting the renin-angiotensin system and ERS pathways represents a promising strategy to delay diabetic complications. Future research should optimize treatment protocols to enhance organ protection.

Schwann Cells and Macrophages

P59

The story of FIRE and LYVE1 – The impact of macrophage subpopulations on hearing

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The immune system is known to critically regulate the central and peripheral nervous system during development, homeostasis, and repair after injury. The inner ear cochlea uniquely represents an intersection of the central and peripheral nervous systems. Several recent studies on mouse cochlea have reported diverse roles of macrophages including mediating drug uptake, promoting synaptic repair after noise exposure, and participating in tissue remodeling after cochlear implantation. Using single cell RNA sequencing, we have revealed three molecularly diverse populations of macrophages within the mature mouse cochlea, populations which are also observed in the human cochlea. Transcriptomes of two of these populations were similar to CNS microglia and to peripheral Lyve1+ sciatic nerve epineurial macrophages. To begin to ascertain the functions of these newly uncovered populations we examined FIRE mice (*Csf1r*$^{\Delta FIRE}$/$^{\Delta FIRE}$), which is deficient in microglia but retains other macrophages, and *Lyve1*$^{Cre/+}$*:Csf1r*$^{fl/fl}$ mice, in which Lyve1+ sciatic nerve epineurial macrophages are depleted. Surprisingly, FIRE mice display normal cochlea anatomy and function indistinguishable from age-matched, wildtype controls. In contrast, cochleae from *Lyve1*$^{Cre/+}$*:Csf1r*$^{fl/fl}$ mice showed a normal complement of inner and outer hair cells but fewer inner hair cell synapses, particularly in the middle turn of the cochlea. This finding was accompanied by mild but significant increase in auditory brainstem response threshold and decrease in wave 1 amplitude across frequencies. Taken together, these results reveal distinct functional roles for cochlear macrophage subpopulations and underscore the importance of studying macrophage heterogeneity in the cochlea to better understand the role macrophages play in hearing health and disease.

P60

Trophic and temporal dynamics of macrophage biology in human inner ear organogenesis

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Normal inner ear development requires the sophisticated orchestration of specialised cell differentiation and integration, which ultimately gives rise to the exquisite organs of hearing and balance. We are only just beginning to understand the timeline and dynamic nature of this process in the developing human inner ear. Recent studies using single cell transcriptomics have improved our understanding of the various cellular phenotypes present in the mammalian inner ear. During human inner ear development, macrophages have been identified as early as gestational week 7 by expression of IBA1 and CD45 and clearly populate the adult cochlea. Yet, there is little known about the origin of cochlear macrophages, or their functional contributions to inner ear organogenesis.

Using a transcriptional approach, we have identified seven distinct macrophage subtypes present over a broad window of human inner ear development which spans foetal weeks 7.5, 9.2, 16, 16.4 and adult. We describe differential gene expression in each of these unique macrophage subtypes, including how each subtype is closely linked to a specific developmental age. These data support and extend upon existing histological studies in the human inner ear, reporting the presence of both resident and non-resident macrophages in both the developing cochlea, and in adult human cochleae following cochlear implantation. In addition, we report that human inner ear macrophages are seeded from multiple sources, supporting the conclusions from recent studies in mice indicating yolk sac and foetal liver origins. We discuss the possible functions of the unique macrophage phenotypes identified at different developmental ages, by analysing their ligand-receptor interactions with other key cell types present during inner ear development.

Together, these data highlight for the first time, the breadth of macrophage phenotypes present throughout human inner ear development and their possible multi-disciplinary contributions to normal inner ear organogenesis. A more comprehensive understanding of the functional roles of human inner ear macrophages will accelerate novel therapeutic strategies targeting both immune-, congenital and age-related hearing loss.

P61

Immune cells and hearing disorders: Development of a short *in vivo* model for evaluating the anti-inflammatory potential of therapeutic compounds

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Following exposure to noise, ototoxic agents, or aging, immune cells, particularly macrophages, are recruited to the cochlea to contribute to tissue repair. However, their excessive activation can lead to chronic inflammation, resulting in cochlear damage and hearing loss. A better characterization of macrophage recruitment kinetics, localization and signaling pathways is essential for optimizing the therapeutic potential of anti-inflammatory compounds. This study aimed to establish a short and well characterized *in vivo* model of cochlear inflammation to efficiently evaluate anti-inflammatory compounds.

Cochlear inflammation was induced in mice by transtympanic (TT) injection of lipopolysaccharide (LPS) in both ears (T_0). Different parameters were evaluated: the dose of LPS (5 and 10 µg/ ear) and the timepoint for histological analyses of the immune response (T_{+2DAYS}, T_{+4DAYS}, T_{+7DAYS}). Auditory Brainstem Response (ABR) thresholds were measured at each timepoint to assess associated hearing impairments. Histological analyses were conducted on cochleae prepared either as flat surface or cross-sections, followed by immunolabelling to identify macrophages (Iba1), T lymphocytes (CD3) and B lymphocytes (PAX5). Immune cells quantification and qualitative assessment of their localization in the cochlea were performed to correlate with ABR outcomes.

LPS injection induced a significant increase in the number of immune cells in the cochlea, as demonstrated in flat surface preparations and cross sections. Immunolabelling of macrophages, T and B lymphocytes revealed that the immune response was mainly formed of macrophages, detected in the spiral ligament and spiral ganglion neuron regions. Macrophage recruitment in the cochlea was transient and increased at T_{+2DAYS} and T_{+4DAYS}, but not at T_{+7DAYS}, compared to controls. Interestingly, in mice injected with LPS, the number of macrophages correlated with both the dose of LPS and the increased ABR thresholds at T_{+2DAYS} and T_{+4DAYS}. At T_{+7DAYS} both markers completely recovered.

In LPS cochlea, a huge recruitment of inflammatory cells was reported, thanks to histological tools, and correlated with transient hearing loss. These results validate the local use of LPS as a rapid and efficient method to induce cochlear inflammation, providing a relevant in vivo model to evaluate anti-inflammatory compounds *in vivo* and their effects on hearing. Immune response characterization is now being applied to our noise- and cisplatin-induced hearing loss models to better define the therapeutic window and treatment efficacy.

P62

Macrophages around the reuniting duct and Bast's valve: Possible roles?

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Within the inner ear labyrinth, the saccule is connected to the cochlear scala media through the reuniting duct and to the utricle through the endolymphatic duct. Both ducts are potential sites of obstructions and other endolymphatic flow alterations but have been seldom studied, due to their thin and fragile nature and their deep position within the temporal bone.

By employing tissue clearing and lightsheet imaging (as in [Perin et al. 2019, doi: 10.3389/fnana.2019.00015]), we reconstructed both ducts in the rat inner ear, with their associated vessels and resident macrophage populations. The reuniting duct is supported by a lamina of Iba1+ macrophage-rich connective tissue and is coupled to the vestibulo-cochlear artery (VCA), which is visible as a tortuous bone channel in microCT scans. The endolymphatic duct is instead coupled to the vestibular aqueduct vein and is surrounded by Iba1+ macrophages throughout the vestibular aqueduct, up to the utriculo-endolymphatic (Bast's) valve. For both ducts, separate macrophage clusters were observed around the duct and around the associated vessel. In the reuniting duct, the macrophage population of the connective tissue lamina appeared to be continuous with the spiral limbus, suggesting a common origin. In the endolymphatic duct, several Iba1+ macrophages were observed in the loose connective surrounding Bast's valve.

The presence of macrophage populations associated to inner ear thin ducts calls for a role. The obstruction of the reuniting duct (e.g. by saccular otoconia) has been suggested as a cause of hydrops [Hornibrook and Bird 2017, doi: 10.1177/0194599816675843], and macrophages could be involved in this process, either by regulating the duct lumen through inflammatory changes in permeability, or by removing otoconial debris. For the endolymphatic duct, an important role of macrophages has been suggested, namely the coupling of immune protection and fluid homeostasis [Jansson and Rask-Andersen 1992, doi:10.1159/000276297]. The presence of macrophages around Bast's valve suggests a mechanism with which inflammatory processes could change the endolymphatic flow between the vestibular organs and the endolymphatic sac.

P63

Remodelling of the excitable domains of spiral ganglion neurons in ageing mice

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Proper passage of action potentials and regulation of conduction velocity along the spiral ganglion neurons (SGNs) are crucial for the precise transmission of sound information from the cochlea to the brain. The peripherally projecting SGN fibers are enwrapped by myelin produced by Schwann cells. Myelin organizes the neurolemmal nodal domains. The naked domains, the nodes of Ranvier (NRs), contain clusters of voltage-gated sodium channels that are crucial for rapid conduction. NRs are flanked by paranodes that provide a physical barrier between the nodal ion channel clusters. It is not understood if the loss of electric or trophic input from hair cells affects the organization of nodal domains. This disorganization might be an essential part of auditory nerve degeneration, the inevitable response to noise and ageing.

We used wild type C57BL6 mice and *Manf* (*Mesencephalic astrocyte-derived growth factor*) mutant mice to study if the progressive hair cell/synapse pathology affects the length of nodal domains, using βIVspectrin and contacting-associated protein (CASPR) antibodies to mark NRs and paranodes, respectively. We found elongation of NRs in ageing mice. It was most prominent in specimens with strong hair cell and nerve pathology. More subtle elongation was seen in ageing specimens with preserved hair cells, indicating that nodal remodelling is not just a consequence of advanced nerve degeneration. The length of paranodes was unaltered. We also studied if moderate noise exposure (98 dB, 2 h) affects nodal domains, assessed in C57BL6 mice 7-12 days postexposure. We did not find elongation of NRs. These results point to a difference in the response of the SGN excitable domains to an acute vs. progressive trauma.

We found upregulation of the c-Jun transcription factor in Schwann cells of the ageing auditory nerve. In other peripheral nerves, acute insult causes c-Jun upregulation that promotes demyelination. Demyelination is known to be a central part of auditory nerve degeneration, confirmed also in our study. Subtle myelin retraction from the nodal junctions may be an early step in the nerve degeneration process, causing elongation of NRs. If so, there must be intricate reciprocal crosstalk between SGNs and Schwann cells. Understanding this crosstalk and how it regulates nodal dynamics as well as the possible role of nodal length changes as a homeostatic mechanism could provide tools how to slow down nerve degeneration. This knowledge would be particularly important for the cochlear implant field, to improve the efficacy of the implants.

P64

Efficacy of rosmarinic acid and anakinra in mitigating oxidative and inflammatory damage

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Styrene exposure is known to cause ototoxic damage through mechanisms involving oxidative stress and inflammation. However, the molecular mechanism linking increased free radical production and inflammatory markers is still elusive. The objective of this study is to characterize cochlear damage induced by styrene, investigating the potential involvement of the NLRP3 inflammasome in mediating the relationship between oxidative stress and inflammation. Additionally, the study aims to compare the efficacy of two therapeutic strategies in alleviating styrene-induced auditory dysfunction. We used a model of styrene-induced ototoxicity by administering styrene (400 mg/kg, 5 days a week for 3 consecutive weeks) in adult male Wistar Rats. An antioxidant phenolic compound (rosmarinic acid, 10 mg/kg), and an IL-1β receptor antagonist with anti-inflammatory properties (anakinra, 30 mg/kg) were used to evaluate the protective effects on auditory function. At the end of treatments functional, morphological and molecular analyses were used to study the mechanisms underlying ototoxicity and otoprotection. Functional and molecular analyses revealed that both treatments significantly reduced oxidative stress, NLRP3 inflammasome activation, and inflammatory markers. However, anakinra provided the best protective effect, as evidenced by earlier auditory threshold recovery and greater suppression of inflammation. These findings underscore the pivotal role of inflammation in styrene-induced ototoxicity, our results suggest that targeting inflammation, particularly via IL-1β receptor antagonism, provides a more comprehensive and timely intervention for styrene-induced hearing loss. The dual mechanism of action of anakinra (reducing NLRP3 levels and directly modulating IL-1β) makes it a promising candidate for mitigating cochlear damage caused by environmental and toxic insults.

P65

Vascular and inflammatory changes in the aging human cochlea

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The stria vascularis, a highly vascularized structure in the scala media of the cochlea, is crucial for maintaining an electrochemical gradient fundamental for hair cell function. Structural and functional changes in this region are hypothesized to contribute to secondary sensorineuronal degeneration, particularly with aging. Understanding these alterations and influencing factors is essential for optimizing future therapeutic approaches. This study investigates vascular and sensorineuronal changes in the cochlea, cochlear nerve by combining 3D segmentation analysis and correlative histology.

Morphometrical changes of the stria vascularis come along with functional deficits, hence 3D segmentation of the entire stria vascularis was performed using ThermoFisher Amira software. For the thorough description of the method, we refer to approach by Mahdi Fallahtaherpazir from a collaborating FWF project which involved Savitzky-Golay filtering and computation of tangent vectors to generate perpendicular planes. These planes allowed for cross-sectional area profiling, enabling the assessment of tonotopic changes along the cochlea.

Correlated histological analysis of structural changes with additional immunohistochemistry (IHC) on paraffin and cryosections involved, targeting markers such as CD34 (angiogenesis), CD31 (endothelial activation), α-SMA (vascular smooth muscle and pericytes transformation), CD68 (inflammation and atherosclerosis) and IBA1 (inflammation). Thin-sections of epoxy-embedded cochleo-vestibular nerves served to assess neuronal degeneration. This integrative approach shall enhance our understanding of inner ear [RG1] vascular deterioration and its role in hearing loss, providing critical insights for future therapeutic strategies

P66

Vascular senescence as a key factor in vascular damage and microcirculatory pathology: Associated vascular degeneration and age-related hearing loss

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Background: Age-related hearing loss (ARHL) is increasingly understood as a manifestation of systemic vascular aging. Beyond progressive sensory cell degeneration, accumulating evidence implicates microvascular dysfunction, low-grade inflammation, and cellular senescence as key contributors to cochlear decline. Type 2 diabetes mellitus (DM II), which is known to accelerate vascular pathology, provides a clinically relevant model for investigating these mechanisms in the aging inner ear.

Objective: This study aims to determine whether vascular alterations in the cochlea reflect broader systemic microangiopathic processes in aging individuals. We seek to characterize endothelial dysfunction, inflammatory activity, and senescence-associated changes within cochlear tissues and compare these findings to corresponding vascular features in the internal carotid artery and, when available, kidney and skin.

Methods: Postmortem tissue samples are being collected from a targeted cohort of 30 donors aged over 60 years (including both diabetic and non-diabetic individuals), all with documented cardiovascular risk factors.Tissue retrieval is performed within a 12-hour postmortem interval to ensure optimal preservation. Cochlear and carotid artery specimens are processed as cryosections and paraffin-embedded sections; kidney and skin tissues are included where available. Ongoing histological and immunohistochemical analyses include PAS, Sudan III, and H&E stains, as well as markers of endothelial integrity (CD31, AGE), vascular remodeling (α-SMA), inflammation (CD45, CD68, PDGFR), and cellular senescence (p21). Lipofuscin, as a histological hallmark of senescence, is evaluated via morphological criteria in H&E-stained sections and enhanced through Sudan Black B staining. Quantitative assessments will be performed using digital image analysis with nuclear segmentation, targeting anatomically defined cochlear substructures and arterial wall layers. Planned advanced imaging includes microCT following MicroAngiofil perfusion to reconstruct the cochlear microvasculature in 3D and to generate quantitative data on sensorineural decline.

Outlook: Our results support the hypothesis that vascular senescence plays a critical role in age-related hearing loss. The cochlea, due to its complex vascular architecture and metabolic sensitivity, may serve as an early indicator of systemic microangiopathy in aging and diabetic individuals. Future work will include neuron quantification, image-based vessel quantification, and validation of therapeutic targets in vascular modulation.

P67

Cell death type-dependent interactions between immune cells and sensory hair cell regeneration programs

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Regeneration following injury requires the concerted response of multiple cell types, including immune cells and stem cells. The cellular and molecular components of regeneration programs can be affected not only by the severity of the injury, tissue identity, but also the type of cell death. Here, we established a comparative approach to specifically interrogate the influence of hair cell necrosis versus apoptosis on the macrophage response and zebrafish lateral line hair cell regeneration. This comparative approach allows us to specifically interrogate the influence of the cell death modality on regeneration. Using high resolution live imaging we visualized and characterized the rapid recruitment of tissue-resident macrophages, uncovering intricate differences in their phagocytic behavior depending on the cell death modality. Additionally, neutrophils were rapidly recruited to HC necrosis but not apoptosis, suggesting that the inflammatory response is influenced by the type of cell death. These cellular differences were accompanied by distinct transcriptional changes in phagocytosing macrophages and lateral line support cells, as evidenced by single-cell RNA sequencing. While HC necrosis triggered a robust injury response in support cells, it was largely absent following apoptosis. Despite these differences in the stress response to injury, both paradigms eventually converged on similar genes involved in HC regeneration, question prior assumptions that inflammation is required to trigger regeneration. Lastly, blocking immune cell migration using a dominant-negative approach increased injury response gene expression in support cells in both paradigms, suggesting that immune cell recruitment attenuates the injury response to cell death. Time lapse imaging and EdU incorporation experiments revealed that blocking immune cell recruitment increases injury-induced proliferation of support cells and hair cell differentiation specifically in response to HC apoptosis, but not necrosis. Our findings have important implications for our understanding of the earliest cellular and transcriptional responses to hair cell loss and how these potentially have to be manipulated to trigger regeneration in mammals.

Gene Therapy, Drug Delivery, Genetic Screening

P68

Large animal model for human inner ear gene therapy: Transgene expression of viral vectors in pigs

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Introduction: Recent clinical data demonstrate the potential of inner ear gene therapy using viral vectors in patients with *OTOF* mutations causing congenital hearing loss. For the first time, children with autosomal recessive *OTOF* mutations were treated successfully by delivering adeno-associated viruses (AAVs) into the inner ear. However, detailed analyses of viral vector efficacy have so far been limited to a few large animal models. This study evaluated AAV-mediated transduction patterns in the cochleae of pigs – a model with inner ear anatomy comparable to humans.

Methods: In three pigs, AAV2 vectors carrying either a CAG or CMV promoter were injected through the round window membrane under general anesthesia. Two additional pigs received AAV-PhP.B vectors with a CMV promoter using the same procedure. Auditory brainstem responses (ABR) were measured pre- and postoperatively. One week post-injection, inner ears and key peripheral organs were harvested and analyzed via immunofluorescence and confocal microscopy.

Results: AAV2 transduced cochlear hair cells efficiently, with outer hair cells showing a decreasing base-to-apex gradient, while the opposite was observed in inner hair cells. No contralateral transduction was observed after unilateral injection. Transduction rate of total outer hair cells exceeded 50% with AAV2 vector (and 70% of inner hair cells), while the PhP.B vector transduced at a substantially higher rate. ABR thresholds remained unchanged in all injected ears. Preliminary data indicated no off-target transduction in peripheral organs, including kidney, heart, liver, lung, temporal lobe, and cerebellum.

Conclusion: With a cochlear size similar to that of humans, the pig serves as a promising large animal model for inner ear gene therapy. Our findings support AAV2 and PhP.B as effective vectors for targeting cochlear sensory hair cells. These results reinforce the utility of the porcine model for preclinical optimization of vector delivery, distribution, and cellular targeting – critical steps toward developing effective gene therapies for human hearing loss.

P69

Neuron-specific gene delivery into the mouse inner ear using an adeno-associated virus vector

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Introduction:

Recent advances in gene therapy for congenital sensorineural hearing loss have focused on targeting cells within the organ of Corti using adeno-associated virus (AAV) vectors to restore function lost due to genetic mutations. In parallel, AAV-mediated gene delivery to spiral ganglion neurons (SGNs) has gained increasing attention. These strategies offer potential for the protection or regeneration of SGNs. In this study, we evaluated three key aspects of neuron-specific AAV-mediated gene delivery to both peripheral and central auditory pathways. First, we assessed gene transduction efficiency in SGNs. Second, we examined auditory brainstem response (ABR) to evaluate the invasiveness of the AAV injection. Lastly, we investigated off-target transgene expression in the central nervous system (CNS) to assess the safety and specificity of the approach.

Methods:

AAV9-hSyn-GFP was injected into the cochlea via the round window membrane in neonatal C57BL/6N mice. Four weeks post-injection, ABRs were recorded, and inner ears and brains were harvested. Immunostaining was performed using anti-βⅢ-tubulin for type 1 SGNs, anti-Pou4f1 for type 1c SGNs, and anti-Gata3 for type 2 SGNs.

Results and Discussion:

GFP expression was observed in type 1 SGNs throughout all cochlear turns on the injected side. Notably, no significant difference in transduction was found between the injected and non-injected ears. Strong GFP expression was detected in type 1 SGNs, including both type 1a/1b and type 1c subtypes, whereas type 2 SGNs showed no detectable expression. ABR thresholds were comparable across AAV-injected, saline-injected, and non-injected groups at all tested frequencies, indicating that the vector and injection procedure did not induce hearing loss. However, widespread GFP expression was observed in several CNS regions, including the brainstem, cerebellum, cerebral cortex, hippocampus, and midbrain. This suggests that AAV vectors administered via the inner ear can disseminate to the CNS and contralateral cochlea, likely through the cochlear aqueduct and cerebrospinal fluid pathways.

Conclusion:

The neuron-specific AAV9-hSyn vector efficiently and selectively transduces type 1 SGNs in the cochlea and represents a promising platform for gene therapy aimed at hearing preservation and restoration. Future studies should focus on minimizing off-target gene expression in the CNS to improve the safety profile and clinical translatability of this approach.

P70

Safety and efficacy of GJB2-GT, an adeno-associated vector-based gene therapy treatment candidate for the autosomal recessive non-syndromic deafness 1A (DFNB1A)

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The estimated prevalence of severe or profound deafness in humans is 1/1000 neonates, and genetic factors account for half of the cases. Pathogenic variants of GJB2, the gene encoding Connexin 26 (Cx26), are involved in 50% of congenital deafness and are mostly associated with an autosomal recessive non-syndromic DFNB1A. In the cochlea GJB2 is largely expressed in the majority of supporting cells (SCs) but not in sensory hair cells (SHCs). Gene therapy is a promising therapeutic strategy for autosomal recessive forms of deafness, and Adeno-Associated Vectors (AAVs) are being developed to this aim.

We have developed GJB2-GT, an AAV vector for DNFB1A with proprietary cis-regulatory elements for a safe and targeted transgene expression. GJB2-GT efficiently transduces human cell lines to produce Cx26 protein that is adequately addressed to the plasma membrane and allows functional gap junctions.

A conditional model was generated to elude embryonic mouse lethality caused by complete loss of Gjb2 expression, mimicking the most common forms of DFNB1A and severe/profound hearing loss. Intracochlear injections of GJB2-GT lead to improvement of hearing thresholds as early as 3 weeks post-injection in a dose-dependent manner. Microscopic examination confirmed that GJB2-GT efficiently and adequately restores Cx26 expression. Current dose-response experiments show that intracochlear GJB2-GT injection is safe over a wide range of doses administered and provides a significant therapeutic benefit in our DFNB1A mouse model. Restoration of the endocochlear potential is currently being addressed.

Early tolerability and biodistribution of GJB2-GT, or GJB2-FLAG-GT, studies were conducted in mice and Non-Human Primates (NHP) after injections through the round window (RW). Transgene expression was observed in most cells that naturally express Gjb2 along the tonotopic axis of the cochlea, with good local and systemic tolerability. In particular, no SHCs express the transgene, confirming the specificity of the expression cassette in both species. GJB2-GT administration in NHP was performed using the surgical procedure and injection device intended for clinical use. Long-term safety studies conducted in wild-type mice demonstrate persistence of transgene expression with no impact on auditory function or cochlear cytoarchitecture. High-dose intravenous injection of GJB2-GT does not induce any adverse events or changes in behavior.

Overall, efficacy, biodistribution and early safety data package support GJB2-GT development to restore physiological hearing in DFNB1A patients.

P71

Tailoring AAV vectors for gene therapy of inner ear disorders by directed evolution

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Around 1.5 billion people worldwide suffer from some form of hearing loss (HL) with 25 % of these cases being disabling HL. Current treatments—such as hearing aids and cochlear implants—have significant limitations. Both rely on the presence of some residual hearing, do not slow the progression of hearing loss, and cannot restore natural hearing. Cochlear implants also require invasive surgery, and both devices demand ongoing maintenance. In contrast, through delivery of a therapeutically active nucleic acid to the affected cells in the cochlea, gene therapy treatment is expected to restore natural hearing, recover the damaged cells of the cochlea or even prevent the onset of the HL when supplied ahead of time.

Our aim is to optimize the adeno-associated virus (AAV) vector system for inner ear directed gene therapy through high throughput screening of AAV peptide display libraries. Different AAV peptide display libraries were generated and screened. All capsid variants contained 7 amino acid peptide inserts in variable region VIII as possible receptor binding ligands.

We conducted the *in vivo* screens in the inner ear of adult mice (n = 4, per library), using an administration route that demands overcoming robust biological barriers, to develop variants functional via non-surgical administration. Despite this challenge, candidates accumulated after two *in vivo* selection rounds. Twenty top candidates were cloned and produced as vectors packaging the dTomato transgene. Individual administration into the adult mouse ear showed distinct expression patterns in the mouse cochlea, with some variants specific for outer hair cells, inner hair cells (IHCs) or the stria vascularis – all promising targets for many different forms of genetic or age-related HL. The rAAV2 variants specifically transduced the nerve fibres and spiral ganglion neurones of the mouse cochlea, with some variants also entering and expressing in IHCs.

The best individual performers in the mouse ear were further produced with a GFP-barcode transgene where the barcode is unique for the variant. We confirmed the functionality of this challenging administration route in a larger animal model (sus scorfa domesticus) with all the rAAV1 variants accumulating in the pig cochlea and one top variant, Var4, also expressing in the cochlea.

Therefore, these inner ear optimised variants and in particular, Var4, have a strong translational potential for future inner ear gene therapy.

P72

Development of inner ear AAV delivery system using magnetic field

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Question

Recent advancements in gene therapy have driven extensive research into organ-specific drug delivery, including targeted delivery to the inner ear for hearing loss treatment. Unlike most other organs, the inner ear is anatomically and functionally isolated due to the presence of the blood–labyrinth barrier and its tightly regulated fluid compartments. This isolation limits systemic access and immune response, posing unique challenges for therapeutic delivery. Can drug carriers such as adeno-associated virus (AAV) be efficiently and safely delivered to the inner ear using a magnetic field-mediated system via the round window membrane (RWM), thereby avoiding the risks of direct cochlear injection while enabling gene expression in target cells?

Methods

Newborn C57BL/6N mice were anesthetized, and a mixture of AAV vectors encoding a fluorescent reporter gene captured by SPIONs (superparamagnetic iron oxide nanoparticles) was applied onto the RWM via post-auricular incision. A local magnetic field was applied externally to facilitate targeted delivery into the cochlea. Three weeks after administration, auditory function was assessed using auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE) tests. Cochlear tissues were collected and immunofluorescence staining was performed to evaluate the transduction efficiency and pattern of SPION-captured AAV. Five mice were included in each of the experimental and control groups to allow statistical comparison.

Results

Our results confirm that local magnetic delivery successfully mediated gene expression in P5 mouse cochlear of P5 mice with AAV-PHP.eB, whereas negative control mice, to which no magnetic field was applied showed no signal in immunofluorescence. Compared with direct injection, the overall transduction pattern of AAV-PHP.eB delivered via magnetic targeting closely resembled that of direct cochlear injection, particularly in neuronal populations. ABR and DPOAE measurements indicated no significant threshold elevation, suggesting that the local magnetic delivery did not adversely affect auditory function.

Conclusions

Magnetic field-guided delivery of AAV via the round window membrane is a safe and efficient method for inner ear gene transfer. This non-invasive approach avoids the risk of cochlear damage from direct injection and may serve as a versatile platform for delivering gene-editing tools and other therapeutics targeting hereditary hearing loss.

P73

A novel polyplex-loaded injectable thermogel for targeted gene delivery to the inner ear

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Gene therapy has emerged as a promising approach for treating inner ear disorders, such as hearing loss and balance impairments, which require precise and efficient delivery mechanisms. This study introduces a dual-component delivery system combining RH-PAMAM G2 dendrimer-based polyplexes with hexanoyl glycol chitosan (HGC) thermogel to enhance gene delivery to the inner ear. The RH-PAMAM G2 dendrimers, modified with histidine and arginine, demonstrated high DNA binding affinity, low cytotoxicity, and effective cellular uptake, facilitating stable plasmid DNA (pDNA) transfection in HEI-OC1 auditory cells. Encapsulating these polyplexes within the HGC thermogel, an injectable and thermosensitive hydrogel, resulted in a supportive matrix that protects against premature clearance and provides sustained gene release upon intratympanic administration. In vivo studies in a mouse model confirmed substantial gene expression in the cochlear tissues, with widespread distribution in regions including the spiral ganglion and organ of Corti, compared to polyplexes alone. The HGC thermogel also exhibited favorable biocompatibility, with no observed inflammation or adverse effects in the middle ear tissues. This novel polyplex-HGC thermogel system demonstrates potential as a safe, efficient injectable gene delivery platform, offering a significant advance in gene therapy for inner ear disorders.

P74

Mucoadhesive nanoparticle-mediated inner ear drug delivery

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Background: Drugs that float freely within the middle ear without coming into contact with the middle ear mucosa cannot contribute to inner ear drug delivery. Therefore, if drugs inserted into the middle ear can adhere well to the middle ear mucosa, it is expected that the efficiency of drug delivery can be significantly improved. In this experiment, we assessed the suitability of mucoadhesive nanoparticles as inner ear drug delivery vehicle in comparison to non-adhesive nanoparticles.

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

Results: The in vitro toxicity analysis was conducted using HEI-OC1 cells, and PVA/PLGA nanoparticles showed no cytotoxicity up to 10mg/ml, while DOPA/PVA/PLGA nanoparticles did not exhibit toxicity up to 5mg/ml. For in vivo toxicity evaluation, both types of nanoparticles were injected into the cochlea at a concentration of 5mg/ml, and hearing was assessed at the end of the second week. In both groups, there was no apparent hearing damage compared to the saline control group. Subsequently, lipophilic coumarin was encapsulated within the nanoparticles and administered to the cochlea. Cochleae were collected 1 hour, 3 hours, and 6 hours after administration, crushed in 100% methanol to create lysates, and fluorescence intensity was analyzed. At all three time points, DOPA/PVA/PLGA nanoparticles exhibited superior fluorescence intensity. Subsequently, dexamethasone was encapsulated within the nanoparticles in the same manner and delivered. DOPA/PVA/PLGA nanoparticles demonstrated superior dexamethasone delivery compared to PVA/PLGA nanoparticles or dexamethasone sodium phosphate.

Conclusions: This study demonstrated that mucoadhesive nanoparticles coated with DOPA, specifically DOPA/PVA/PLGA nanoparticles, exhibited excellent biocompatibility and enhanced inner ear drug delivery compared to non-adhesive nanoparticles. These findings suggest that mucoadhesive nanoparticles hold promise as a potential strategy to improve drug delivery to the inner ear by promoting adhesion to the middle ear mucosa.

P75

Personalized inner ear drug delivery: Development of dexamethasone-eluting round window niche implants

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Introduction: Cochlear implantation (CI) can cause insertion trauma and inflammatory responses, impairing residual hearing and affecting implant outcomes. Systemic pharmacotherapy is often used to manage the immune response after CI. However, the inner ear's unique anatomy and blood-labyrinth barrier limit the penetration of systemic medications, making it challenging to achieve therapeutic concentrations in the inner ear. As a result, systemic pharmacotherapy may not be effective in minimizing or preventing the immune response that occurs after CI. We developed a novel approach using additive manufacturing to create individualized, dexamethasone-releasing round window niche implants (RNIs) for localized, sustained drug delivery to the inner ear.

Method: Our preclinical development workflow involved designing patient-specific RNIs using digital volume tomography (DVT) and in-house software, followed by additive manufacturing using a 3D-Bioplotter. We tested the precision and accuracy of the printing process, and the DEX release was analyzed over time. The RNI was also tested for biocompatibility and bio-efficacy in vitro, and its effects on electrode insertion trauma were investigated in an animal model.

Results: The results showed that the RNI was precisely printable, with a weight of 1.019 ± 0.021 mg (mean ± SD) after 20 prints. The accuracy of the printing process was also acceptable, with most samples showing a difference of less than 0.1 mm between the planned and printed sample. The DEX release was detectable over the full experimental period of 29 days, with a burst within the first 24 hours. The biocompatibility of the 3D-printed material samples was evaluated via MTT test, and the bio-efficacy of the released DEX was analyzed by performing a TNFα-reduction assay. The RNI was implanted into the round window niche of guinea pigs to evaluate its effects on electrode insertion trauma. The results showed that the RNI containing 1% DEX had anti-inflammatory potential concerning fibrosis inhibition in vivo.

Conclusion: Based on these encouraging results, we aim to transfer this approach to the preoperative setting of CI, reducing insertion trauma, minimizing inflammatory responses, and improving postoperative outcomes. A clinical study on this approach is currently in the application process.

P76

Local delivery of steroids to inner ear via medical device INCAT (the Inner Ear Catheter) in partial deafness patients during cochlear implantation – Preliminary results

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Background: The administration of steroids to preserve residual hearing during cochlear implantation has been described, although the results are mixed. Nevertheless, according to current knowledge, steroids may have an important role in reducing post implantation fibrosis and loss of hearing due to electrode insertion trauma and progressive effects of inflammation. Aim: The aim of the study was to assess separately the effectiveness and safety of three different algorithms of using steroids and INCAT (a medical device) Medel® in partial deafness patients who underwent cochlear implantation and secondly - the assessment of the impact of the depth of the catheter (INCAT) on hearing preservation after cochlear implantation.

Method: Ten patients underwent a cochlear implantation with an inner ear catheter. Steroid administration followed three different algorithms: 1) methylprednisolone 62.5 mg/ml in solution – 3 patients; 2) methylprednisolone 40 mg/ml in suspension – 4 patients 3) dexamethasone 4mg/ml in solution – 3 patients. Pure tone audiometry (0.125–8 kHz) was performed preoperatively and at the cochlear implant activation (one month after surgery). Hearing preservation was assessed according to the HEARRING group formula. Impedance measurements were taken at two days and one month after surgery.

Results: Patients treated with methylprednisolone 40 mg/ml in suspension showed the best hearing preservation, with 50% achieving complete preservation and 50% partial preservation. This group also had the lowest impedance changes (ranging from 1.06 to 2.11 kΩ). A shorter INCAT insertion depth appeared to be more favorable than a longer one. The smallest changes in the hearing thresholds were observed in the second group (methylprednisolone 40 mg/ml in suspension, Depo-Medrol). Hearing preservation (HP) in all patients at the CI activation was as follows: complete hearing preservation (HP) was observed in 2 patients (20%), partial HP in 5 patients (50%), and minimal HP in 3 patients (30%). No patients experienced total hearing loss at the time of CI activation.

Conclusion: All these considerations suggest that patients treated with methylprednisolone 40 mg/ml in suspension had better outcomes compared to others. The generalizability of the results is limited due to the small sample size and the inability to control for potential confounding variables, e.g. the length of the electrode array.

P77

Spontaneous cellular senescence is mediated by p16 upregulation in human vestibular schwannomas

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Background: Vestibular schwannomas (VS) are rare age-associated tumors from Schwann cells of VIII cranial nerve. They exhibit a complex etiology and cause neurological symptoms, including hearing loss (1). Currently, there are no approved pharmacological treatments for VS; management is limited to surgery and/or radiotherapy (2). HEI-193 VS cell line has shown a propensity to undergo cellular senescence upon exposure to senogenic agents (3). In this study, we characterize molecular features of spontaneous cellular senescence in human VS tumors, and explore the heterogeneous response of VS cells to senogenic-senolytic treatment.

Methods: RNA sequencing (RNA-seq), senescence-associated β-galactosidase (SA-β-GAL) activity analysis, reverse transcription quantitative PCR (RT-qPCR), western blotting, immunofluorescence and cell viability assays were employed to analyse human VS and healthy peripheral nerve (PN) tissue samples and primary cultures, as well as the immortalised human VS cell line HEI-193.

Results: RNA-seq of human VS showed regulation of the expression of genes associated with cellular senescence. SA-β-GAL+ cells were found in VS tissue, but not in PN samples. Primary VS cultures exhibited traits of cellular senescence, including elevated SA-β-GAL activity, increased protein levels of the tumor suppressor p16 and upregulation of senescence-associated secretory phenotype (SASP) components: *IL8, IL1B, CCL2* and *MMP3*. Notably, there was substantial variability in the degree of spontaneous senescence among primary VS cultures, which was also reflected in bleomycin response: cultures with fewer SA-β-GAL+ cells responded better than those with higher cellular senescence levels. Poor response to bleomycin was linked to impaired activation of the p53/p21 pathway. Conversely, VS cultures with low baseline senescence showed strong responses to combined treatment with bleomycin (senogenic) and navitoclax (senolytic).

Conclusion: Our findings indicate that spontaneous cellular senescence in VS is driven by p16 upregulation and is associated with heterogeneous responses after a senogenic-senolytic treatment. It suggests that assessing senescence markers may be critical for tailoring effective therapeutic strategies in VS patients.

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- (1) Lassaletta L, et al. (2019) Front Neurol; 18:10:978
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P78

Investigating the effects of the microenvironment on differentiation of inner ear organoids from mouse embryonic stem cells

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Hearing loss is one of the most common forms of sensory impairment in humans, affecting more than 5% of the world's population. To date, our understanding of the mechanisms of hearing loss and the generation of therapies have been hampered by the lack of human in vitro models. Previous work showed that it is possible to generate inner ear organoids (IEOs) containing functional hair cells from mouse or human pluripotent stem cells through three-dimensional (3D) culture systems. However, their translation into an *in vitro* model for drug screening or developmental modelling is limited by low differentiation yield, lack of reproducibility and standardisation of the differentiation protocol.

This study aims to explore microenvironments for IEOs and otic neuron differentiation from mouse embryonic stem cells (mESCs) that could ultimately be combined with engineering devices for translational research. IEOs containing hair cells, supporting cells and neurons were generated with a Atoh1/nGFP mESC line (provided by S. Heller). The microenvironment was explored by culturing mESC aggregates in microwells or in 3D MatrigelTM, collagen or a combination of both. Results show that microwells allow the control of mESC aggregates size but affect otic differentiation. Maintenance in gel droplet domes sustained the formation of IEOs but also supported the formation of ganglion-like neural structures in the surrounding matrix. Characterisation by immunostaining, electrophysiology and single nuclei RNA sequencing confirmed the presence of a population of functional neurons, often observed in clusters.

This work provides a step forward the characterisation of microenvironment suitable for development of IEOs combined with functional, presumptive otic neurons. Further work on this model is required to better understand otic neuron development in the context of IEOs and the production of stem-cell based *in vitro* models for investigating the mechanisms underlying sensory neural hearing loss

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Presentation and comparison of highly sensitive techniques for human perilymph proteomics

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Background:

The cause for sensorineural hearing loss lies usually in pathological changes into the inner ear, resulting in functional damage of sensitive cellular structures into the cochlea and leading to cochlear implantation. The various types of cochlear hearing loss are triggered by e.g. diseases, toxic substances, genetic predisposition, aging or inflammatory processes and lead to molecular changes in the composition of the inner ear fluid perilymph. We suggest that the proteins composition of the perilymph depicts a molecular fingerprint of the different pathologic processes for individual patients. The use of highly sensitive analytical techniques like mass spectrometry (proteomics) and multiplex protein arrays offers the possibility for detailed analysis of protein composition in low volume human perilymph samples in the µl range.

Methods:

The collection of human perilymph was developed using modified micro glass capillaries for liquid biopsy during inner ear surgeries like cochlear implantations and vestibular schwannoma surgeries. Our in-depth shot-gun proteomics approach is based on liquid chromatography coupled to mass spectrometry (LC-MS). Additionally, up to 48 inflammatory proteins can be identified by different types of antibody based multiplex arrays. [1, 2]

Results:

The proteomics approach enables the detection of a multitude of proteins in parallel in human perilymph. The use of different types of multiplex platforms for detection of inflammatory proteins was evaluated for perilymph samples of few microliters (0.5 to 12 µl, mean volume 2µl) by downsizing the recommended sample volume. It was shown that a parallel perilymph analysis by LC-MS and multiplex arrays is possible in a perilymph sample upon a volume of 3µl.

Conclusion:

The analysis of human perilymph by mass spectrometry gives us a comprehensive and differentiated picture of the perilymph proteome. By antibody-based multiplex arrays inflammatory proteins, which are not traceable in mass spectrometric analysis, can be quantified. The combination of these methods depicts an extension of proteome analysis and provides detailed information about the state of the inner ear because proteins directly control and regulate most of the body's biological functions. This precious information could be used as a personalized diagnostic tool for CI patients.

[1] Schmitt et al. J. Proteome R. 2017

[2] Warnecke et al. Front. Neurol. 2019

P80

A novel sampling method for perilymph in a large animal model

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Introduction: The cochlea is a small and highly delicate sensory organ in which even minor biopsies can lead to irreversible damage and functional impairment. One promising alternative for studying cochlear physiology is the analysis of perilymph fluid, which directly interfaces with critical auditory structures – including hair cells, spiral ganglion neurons, the auditory nerve, and the cochlear spiral ligament. Various techniques exist for perilymph collection, such as sampling via the cochlear apex, the round window membrane, or the semicircular canals. However, these approaches are typically irreversible and are limited to terminal procedures in animal models or are performed in humans only during surgeries that inherently compromise hearing.

Methods: A novel, minimally invasive device for perilymph sampling, which is currently under development, was evaluated in a pilot study involving three pigs. The animals underwent surgical procedures under general anesthesia, during which the cochleae were exposed to allow device application. Cochlin, an inner ear-specific protein, was quantitatively assessed in the sampled perilymph using an ELISA assay (Human Cochlin DuoSet ELISA, R&D Systems). Auditory brainstem responses (ABRs) were recorded preoperatively and one week postoperatively to assess potential changes in auditory function. Following the post-surgical assessment, the cochleae were extracted and examined for procedural trauma using high-resolution micro-computed tomography.

Results: The novel device has successfully enabled the clear extraction of perilymph from the cochlea. ELISA confirmed the presence of cochlin in the collected fluid, verifying the sample integrity. Auditory function remained largely stable over the one-week observation period, with only minor fluctuations (10-15 dB) in ABR thresholds. Imaging analysis is ongoing and will be completed shortly to further evaluate the potential structural effects associated with the procedure.

Conclusion: The novel sampling device is well suited for minimally invasive perilymph extraction in the pig. Unlike conventional techniques, which typically permit only a single sampling due to their invasive nature, this novel approach holds the potential for repeated sampling from the same subject. The potential for low-trauma and repeatable access to perilymph introduces new opportunities for diagnostic or monitoring applications in humans, without necessitating hearing loss or extensive surgical intervention.

P81

Early transcriptomic heterogeneity in C57BL/6N cochleae due to *Cdh23^{ahl}*

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The mouse continues to be an essential model organism for our understanding of the development, function, and maintenance of the mammalian auditory system. However, issues relating to reproducibility of studies across different laboratories have been a cause of concern, and this is likely to continue as we move into a new era of therapeutic interventional studies.

Cadherin23 (*Cdh23*) is an integral component of tip links, which are essential for mechanical gating of the transducer channels in response to sound-induced deflection of the stereocilia bundle. In mouse, the hypomorphic *Cdh23^{ahl}* (*Cdh23$^{c.753A}$*) allele is present in >20 different inbred strains. This includes the commonly utilised C57BL/6N and C57BL/6J strains, predisposing these mice to an "accelerated age-related" hearing loss starting at around 3-months of age. Although the association between Cadherin23 and tip-links is well established, mice homozygous for the *Cdh23^{ahl}* allele display a wide range of cochlear pathologies, some of which occur before hair-cells begin to degenerate. Hearing loss induced by the *Cdh23^{ahl}* allele can also be potentiated through genetic interaction with mutations in other genes, resulting in an early onset hearing loss. Thus, we hypothesised that subclinical molecular changes occur within the auditory system of mice homozygous for the *Cdh23^{ahl}* allele prior to the onset of hearing loss in these mice.

To investigate this, we have utlised bulk RNA-sequencing to assess the cochlear transcriptomes of C57BL/6N (C57BL/6N-*Cdh23^{ahl}*) mice compared with a highly-genetically controlled, co-isogenic C57BL/6N-*Cdh23$^{753A>G}$* strain in which *Cdh23* has been "corrected" using CRISPR/Cas9-mediated homology-directed repair. For each strain, cochlear transcriptomes were generated utilising male (n=4) and female (n=4) mice, at both P16 and at P30. Using this approach, we demonstrate that the *Cdh23^{ahl}* allele induces molecular changes in the inner ear as early as postnatal day 16. Furthermore, by 1 month of age, cochlear transcriptomes of *Cdh23^{ahl}* mice exhibit greater heterogeneity compared to those of C57BL/6N-*Cdh23$^{753A>G}$* with more than a third of the upregulated genes at P30 contain binding motifs associated with a single transcription factor family.

These findings give an insight into the mechanisms of age-related hearing loss, and critically, demonstrate that the *Cdh23^{ahl}* allele is not physiologically benign during these early time points, and may influence phenotypic expressivity in genetically modified mouse models more broadly than previously assumed.

P82

Revealing heterogeneity and damage response in the adult human utricle

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The human utricle is a vestibular organ responsible for maintaining balance, a function that commonly deteriorates with age. With the aging population projected to double to 2 billion by 2050 and no current pharmaceutical or biological treatments available, balance disorders are a significant unmet medical need. The utricle consists of sensory and non-sensory cells, which are closely related. Non-sensory cells have a limited capacity to regenerate sensory cells in a damaged balance organ and, therefore, are a relevant therapeutic target.

We present the cellular and transcriptional landscape of the adult human utricle and its response to damage. Performing bulk and single-cell RNA sequencing (scRNA-seq) of patient-derived utricles, we discovered six transcriptionally distinct non-sensory cell types, including a novel supporting cell-like cell population, demonstrating the cellular heterogeneity of the adult human utricle. In addition, we conducted cross-species analysis and identified human-specific genes, including novel hair cell markers.

To assess damage response, we applied an aminoglycoside damage paradigm and performed bulk and scRNA-seq on gentamicin-treated and control samples. After 24 hours of damage, we determined the early transcriptional changes of the utricle. Our findings demonstrate that this organ has the capacity to respond to ototoxic damage within 24 hours and potentially initiate a regenerative response via an early-responding supporting cell population. We validated candidate genes via immunohistochemistry and RNAscope *in situ* hybridization.

This study represents a major step forward in inner ear regenerative medicine. Our results will serve as a foundation for preclinical studies, paving the way for therapeutic strategies to promote balance recovery.

Protection, Repair, Regeneration

P83

Progenitor cells in the adult human express LGR5 and have the potential to produce MYO7A+ cells in vitro

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Background: In the embryonic cochlea, supporting cells give rise to sensory hair cells. In adult mice, supporting cells retain expression of progenitor markers such as Lgr5 and Sox2, yet spontaneous hair cell regeneration does not occur. Most previous studies have focused on neonatal mouse cochlear explants, which limit translational relevance due to species and developmental differences, as hearing loss primarily affects adults. For translational purposes this study assessed the regenerative potential of the adult human inner ear.

Methods: Sensory epithelium of the cochlea and vestibular organ was collected from ten adult patients undergoing surgery for skull-base tumors after informed consent or from bodies donated to science. Tissues were collected in medium or PBS and processed for in-situ immunofluorescence or for generation of organoids. For in-situ immunofluorescence tissues were fixed, permeabilized and incubated with antibodies. For the generation of organoids, tissues were digested with thermolysin and single cells were filtered, mixed with Matrigel and 3D drops were made. Cells were grown on expansion medium for 20-30 days and differentiation medium for 3 days. Organoids were fixed, permeabilized and processed for immunofluorescence and whole-mounted for imaging in a confocal microscope.

Results: Cochlea and vestibular organ epithelium from adult patients express LGR5. Supporting cells from sensory epithelium from human inner ear demonstrated the capacity to proliferate and form organoids in vitro. Vestibular-organ-derived organoids were generated from 10/10 patients. Cochlea-derived organoids were generated in 6/10 patients. After differentiation, MYO7A+ hair cell-like cells were generated in these organoids.

Conclusions: These results indicate that progenitor cells with regenerative potential are present in the human and mouse adult cochlea and vestibular organ. When activated through targeted manipulation of signaling pathways, these cells can proliferate and give rise to hair cell-like cells. This highlights their promise as therapeutic target cells for future therapies to restore hearing in adults.

P84

Epigenetic regulation of EDNRB2, a key factor for fate determination during hair cell regeneration in chick auditory epithelia

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Question: Our previous studies have demonstrated that JAK-STAT signaling plays a key role in the initiation of hair cell regeneration processes (Matsunaga 2020), and EDNRB2 regulates fate determination of reprogrammed supporting cells during hair cell regeneration in chick basilar papilla explants (Matsunaga 2023; Takeuchi under review). This study aimed to elucidate the epigenetic regulation of EDNRB2 during hair cell regeneration in chick basilar papillae through an integrated analysis of RNA and ATAC sequencing.

Methods: We employed an explant culture model of chick basilar papillae, in which the direct conversion of supporting cells into hair cells plays a predominant role in hair cell regeneration, as previously demonstrated in our studies (Matsunaga 2020; Matsunaga 2023; Takeuchi under review). We prepared three time-point samples before hair cell damage by streptomycin, and 24 h and 48 h after exposure to streptomycin. We then performed an integrated analysis of RNA and ATAC sequencing using these samples. Based on this data, motif-enrichment screening was performed using FIMO.

Results: An integrated analysis of RNA and ATAC sequencing identified 80366 unique peaks and 24888 genes. According to the location of core peaks and of transcription starting sites of identified genes, we identified 634460 pairs of peaks and genes. Based on the correlation between depths of peaks and gene expression levels, 21211 pairs of peaks and genes were extracted. In this database, three enhancer candidate peaks for EDNRB2 were found. A motif enrichment screening of these candidate peaks indicated 98 transcription factors as candidates that regulate chromatin accessibility of the EDNRB2 gene. Among 98 transcription factors, 18 transcription factors were extracted based on their expression levels and alteration patterns, which include transcription factors associated with JAK-STAT signaling.

Conclusions: An integrated analysis of RNA and ATAC sequencing suggested the importance of JAK-STAT-EDNRB axis in direct conversion of supporting cells into hair cells in chick basilar papillae.

P85

***In vitro* expansion and differentiation of Lgr5 positive supporting cells from normal hearing and deafened adult and aged mice**

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Background and Aims: Over 400 million people globally experience hearing loss requiring medical intervention, typically hearing aids or cochlear implants. While these devices improve quality of life, they do not fully restore natural hearing. A major cause of sensorineural hearing loss is irreversible damage to cochlear hair cells (HCs). Unlike mammals, non-mammalian species can regenerate HCs from supporting cells (SCs) even during adulthood. Studies in neonatal mice have shown Lgr5+ SCs can generate new HCs. Our research translates this regenerative potential to adults. We investigated the regenerative capacity of adult mouse-derived Lgr5+ SCs by studying cochlear organoid proliferation and differentiation. We model sensorineural hearing loss in mice using kanamycin to induce HC loss.

Methods: Mature adult (P60) and aged (P250) Lgr5GFP transgenic (C57BL/6) mice were used. Cochleae were harvested after confirming normal hearing auditory brainstem responses (ABRs). P60 Lgr5GFP transgenic (C57BL/6) mice were deafened with a combination of kanamycin and furosemide. Seven days after deafening, ABRs were performed and cochleae were harvested if threshold shifts were above 40 dB. Harvested cochleae were dissociated into single cells and cultured in Matrigel to generate organoids using three distinct media. Two media were used from published protocols (McLean et al., 2017; Xia et al., 2023), and a third was developed in our lab (unpublished). Characterization of the organoids was performed by immunofluorescence.

Results: Organoids were successfully cultured from Lgr5GFP-positive supporting cells (SCs) of both normal-hearing and deafened adult mice. Although the number of organoids obtained from deafened mice was a third compared to normal-hearing mice, their morphology remained consistent. McLean media led to necrotic cores during differentiation, and Xia media caused unwanted neurite formation. To address this, we developed optimized media for both organoid expansion and differentiation. Our optimized media supported organoid expansion to sizes comparable with established protocols. Importantly, both Xia media and our optimized media allowed for the differentiation of myosin 7A positive cells from organoids derived from both normal-hearing and deafened mouse tissue.

Conclusions: Organoid numbers from deafened mouse tissue are lower than normal hearing, yet morphology and regenerative capacity remain similar. Optimization of the culture media led to healthy expansion and directed differentiation of the organoids into HC-like cells.

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Functional cochlear hair cells in adult mouse cochlea explants

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Background: Functionally innervated cochlea sensory hair cells (HCs) are essential for hearing. Irreparable damage to HCs in adults, caused by excessive noise, aging and/or ototoxic medication, is one of the leading causes of sensorineural hearing loss. However, the majority of research, thus far, has focused on the young, still developing, mouse cochlea. Here, we evaluated to the survival of HCs in adult mouse cochlea explants.

Methods: Mouse cochleas were harvested from adult (postnatal day 30-60) Lgr5-eGFP-IRES-creERT2 mice. The sensory epithelium was dissected out in ice-cold PBS and the tectorial membrane carefully removed, to expose the HCs. Each section was placed on a Matrigel-coated coverslip and allowed to attach for 30 minutes before being covered with basal medium and incubated for up to 14 days. At set time points sections were fixed and immunofluorescence staining was performed to analyse morphology of the cells, then imaged in a confocal microscope. Functionality and viability of HCs in cultured explants will be analysed by the live neuron marker FM1-43fx and whole-cell patch-clamp recordings.

Results: Sensory epithelium explants were successfully incubated from adult mouse inner ear tissue for up to 14 days. HCs, as well as LGR5-positive supporting cells and spiral ganglion neurons, were still visible after 14 days in medium. Viability of the spiral ganglion neurons was observed by neurite out-growth of the cells.

Conclusions: Our results show that the adult mouse sensory epithelium can be incubated long-term. The different cells (HCs, supporting cells and spiral ganglion neurons), vital for hearing, are all still present and have healthy morphology after 2 weeks in vitro. We will also show preliminary data indicating the functionality of these cells. This will provide us with a more relevant translational model with which to study future **regenerative therapies for restoring hearing in adults**.

P87

OtoNeurons in a dish: Generating spiral ganglion neurons from human induced pluripotent stem cells

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The human inner ear comprises several sensory cell types that play an essential role in sound detection and balance. According to the World Health Organization (WHO), by 2050, nearly 1 in 10 people are expected to experience some form of hearing impairment due to hereditary genetic factors, age-related sensorineural degeneration, or ototoxic medications. Although cochlear implants and hearing aids partially improve hearing abilities, no existing treatment has yet been found to fully restore inner ear function. This limitation stems from the low regenerative capacity of the hair cells and spiral ganglion neurons. Human induced pluripotent stem cell (hiPSC)-derived inner ear organoids (IEOs) recapitulate important aspects of human inner ear development. These three-dimensional structures contain functional hair cells that form synaptic connections with mature neurons and are a more physiologically relevant *in vitro* model. However, their use in high-throughput studies is limited due to off-target cell induction, inter- and intra-experimental variability in the efficiency and structure of the organoids, and intensive labor that hampers the scalability of the experiments. To this end, we are generating OtoNeurons from hiPSC in a high-throughput two-dimensional system to investigate their role in physiological and pathological conditions. Using immunocytochemistry, we unraveled the timing of neuroblast delamination from otic vesicles in hiPSC-derived IEOs, allowing us to determine the optimal time point for isolating otic vesicle-like cells. Immunostaining, flow cytometry and scRNA-seq data from the Inner Ear Organoid Atlas (IODA) revealed a specific surface protein with expression limited to otic vesicle-like cells which was used as a marker to purify otic cells from other off-target populations. Ongoing work focuses on optimizing cell density seeding, materials for microstructured substrates, and identifying important small molecules and growth factors to drive OtoNeuron differentiation. Results are evaluated via gene and protein expression to assess otic neuronal identity. This approach offers a simplified scalable platform to study human otic neurogenesis and holds strong translational potential for high-throughput drug discovery of otoprotective agents and regenerative studies.

P88

The clinical effect of steroids for hearing preservation in cochlear implantation: Conclusions based on three cochlear implant systems and two administration regimes

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The main aim of this study was to assess the clinical effect of steroids (dexamethasone and prednisone) on hearing preservation in patients who underwent cochlear implantation with different cochlear implant systems (Oticon®, Advanced Bionics®, Med-El®). 147 adult patients met the inclusion criteria and were enrolled to the study and divided into three groups depending on the brand of cochlear implant they received and participated in all follow-up visits regularly. They were also randomly divided into three subgroups depending on the steroid administration regime: (1) intravenous dexamethasone (0.1 mg/kg body weight twice a day for three days); (2) combined intravenous and oral steroids (dexamethasone 0.1 mg/kg body weight twice a day plus prednisone 1 mg/kg weight once a day); and (3) no steroids (control group). The results were measured by pure tone audiometry (PTA) at three time points: (i) before implantation, (ii) at processor activation, and (iii) 12 months after activation. A hearing preservation (HP) figure was also calculated by comparing the preoperative results and the results after 12 months. Further measures collected were electrode impedance and hearing threshold in the non-operated ear. The highest HP measures (partial and complete) were obtained in the subgroups who were given steroids. Of the 102 patients given steroids, HP was partial or complete in 63 of them (62%). In comparison, partial or complete HP was achieved in only 15 patients out of 45 (33%) who were not given steroids. There were differences between the three cochlear implant groups, with the Med-El and Advanced Bionics groups performing better than the Oticon group (45% and 43% of the former two groups achieved partial or complete HP compared to 20% in the latter). Hearing thresholds in the non-operated ear were stable over 12 months. Generally, impedance was slightly lower in the 12 month follow-up in comparison with the activation period, with the exception of the Oticon group. (4) Conclusions: Pharmacological treatment with steroids in patients undergoing cochlear implantation helps to preserve residual hearing. (Skarżyńska MB, Kołodziejak A, Gos E, Walkowiak A, Lorens A, Pastuszak A, Plichta Ł, Skarżyński PH. The Clinical Effect of Steroids for Hearing Preservation in Cochlear Implantation: Conclusions Based on Three Cochlear Implant Systems and Two Administration Regimes. Pharmaceuticals (Basel). 2022 Sep 22;15(10):1176. doi: 10.3390/ph15101176. PMID: 36297289; PMCID: PMC9609478.)

P89

Perilymph and cochlear tissue distribution of the novel drug AC102 after intratympanic drug delivery in a large animal model

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Question: The unmet need of clinically approved and effective pharmacotherapeutics and difficulties in efficient cochlear drug delivery continue to challenge researchers and clinicians alike. AC102 is a promising candidate drug, demonstrating preclinical efficacy in treating noise-induced hearing loss, and tinnitus and alleviating cochlear implantation trauma, while also currently being under clinical investigation for sudden idiopathic sensorineural hearing loss. AC102's small and lipophilic structure suggests that it can quickly diffuse into the cochlea and evenly distribute within its tissue and along the cochlea's turns. To confirm this hypothesis, we applied AC102 intratympanically in pigs followed by apical perilymph (PL) sampling and cochlear tissue collection to quantify its distribution.

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

Results: One hour after intratympanic application, AC102 was evenly distributed along the cochlea with a mean PL concentration of 40.67±15.62 ng/mL, which increased to 95.09±84.18 ng/mL at 4 hours after administration, before continuously decreasing to 0.31±0.09 ng/mL after 24 hours. At all timepoints, AC102 levels in all cochlear tissues were considerably higher with levels in sensory tissue being up to 100-fold and those in neural tissue being about 30-fold higher compared to PL levels. Conversely, AC102 in systemic fluids (CSF and plasma) remained consistently at lower compared to PL.

Conclusions: Owing to its small structure and high lipophilicity, AC102 possesses the ability to quickly penetrate into the cochlea and evenly distribute along the cochlear length, thus also rapidly reaching the cochlear apex. Moreover, AC102 swiftly enters and accumulates within different cochlear sensory and neural tissues, thereby reaching the actual targets of inner ear pharmacotherapy. Given the porcine cochlea's similarity in size and physiology to that of humans, it is reasonable to expect comparable results in humans supporting the potential for efficient local drug delivery of AC102 via intratympanic administration.

P90

PROHEAR – A Phase 2a randomized double-blind, placebo-controlled and split-body clinical trial testing ACOU085 as an otoprotectant testicular cancer patients receiving cisplatin

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Background: Cisplatin is a widely used chemotherapeutic agent causing dose-dependent permanent ototoxic hearing loss in 70% or more (Cheung et al., 2022) of an estimated half a million annual patients globally (Dillard et al., 2022), highlighting prevention of cisplatin-induced ototoxicity as a strong, unmet medical need. The Kv7.4 voltage-gated potassium channels expressed in the outer hair cells (OHCs) of the cochlea maintain resting membrane potentials by mediating potassium efflux, thereby supporting functional hearing and OHC survival. We report on the currently ongoing PROHEAR clinical Phase 2a trial which has reached more than 50% enrolment, and in which the otoprotective potential of the novel, proprietary Kv7.4 activator ACOU085 (Bimokalner) against cisplatin-induced ototoxicity and hearing loss is tested based on promising data from several preclinical models.

Methods: Testicular cancer patients 18-45 years old with planned cisplatin treatment (cumulative dose ≥300 mg/m2 administered in three chemotherapeutic cycles) and normal hearing in both ears according to current WHO criteria are randomly allocated to treatment intra-individually with ACOU085 (right/left ear) and placebo (right/left ear) following confirmed eligibility and written informed consent. Full audiological assessments including Air/Bone PTA, speech understanding tests in quiet/noise and DPOAE recordings are performed at visits 1-5 (days 1/22/43/64/150) ahead of each chemotherapy cycle (visits 1-3), at the end of cycle 3 (visit 4) and after a 3-month follow-up (visit 5) along with standard clinical assessments.

After audiological evaluation on visits 1-3, the patients receive ACOU085/placebo via transtympanic injection in either ear using a proprietary, thermoreversible extended-release formulation.

Using the unique split-body trial-design, intra-individual changes of audiometric variables for ACOU085 and placebo treated ears between visits 2-5 and visit 1 (baseline) are compared. Meaningful differences between ACOU085 and placebo treated ears is considered evidence supporting a clinically relevant otoprotective effect of ACOU085 against cisplatin-induced ototoxicity and preservation of hearing in cancer patients.

Results: Preliminary, blinded interim results from the first enrolled patients completing 3 treatment cycles during chemotherapy indicate a high (>90%) incidence of ototoxicity according to ASHA criteria with PTA changes (up to 35+ dB) at high and extended high frequencies (6-16 kHz).

Conclusions: The preliminary results of the clinical trial indicate more prevalent and severe cisplatin-induced hearing loss than generally expected for adult patients, perhaps revealed by the use of extended high-frequency audiometry. With continued enrollment of up to 40 planned participants in the PROHEAR Phase 2a clinical trial, we will provide a detailed presentation of the background and trial design, along with updated interim observations from the study.

Cochlear Implants

P91

Electrical stability, inner ear biocompatibility, and anti-adhesive effect of an alginate coating for cochlear implants

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Introduction: Despite best efforts for improvements, implantation of the cochlear implant (CI) induces trauma in the inner ear and by this triggers an inflammatory cascade resulting in, i.a., loss of residual hearing and fibrotic encapsulation of the electrode array. A coating of the array that smoothens the insertion and inhibits cell adhesion may reduce the inner ear trauma and prevent cell degradation and encapsulation. Reduction of insertion forces has been determined in vitro for a CI coating with alginate hydrogel, making it a promising candidate for this application. Testing of its chemical and electrical stability and interaction with inner ear tissue is addressed in the here presented study.

Methods: For stability testing, alginate (Alginatec®) was applied as barium cross-linked hydrogel coating to CI electrode arrays. The arrays were immersed in artificial perilymph and the CIs electrically stimulated (radio music, 16 h daily) with normal or supra-threshold settings for two months. Once a week, impedances were measured, coating integrity was microscopically documented, and medium was changed. In another set of experiments, the alginate gel was tested in co-culture with freshly prepared rodent inner ear tissue regarding ototoxicity, neurotoxicity (gel beads, 7d), and cell adhesion (adherent gel layer, 48h).

Results: The hydrogel coating did not negatively affect the current flow. A temporary increase of the impedances from 1.8 kΩ to 2.5 kΩ (average impedances over all contacts and CIs) was detected directly after coating but within one week it decreased to 1.7 kΩ and finally to 1.2 kΩ. The alginate coating of the arrays did not degrade under electrical stimulation in artificial perilymph. However, in some cases a loosening was seen in the basal area, where handling during experiments induced the highest mechanical impact. The week-long co-culture with alginate beads did neither reduce the number of inner hair cells, outer hair cells, or neurons. Cell adhesion and growth was present on the uncoated well area, while the alginate gel layer was completely free of cells in most cases.

Conclusions: The results proved an overall good robustness of the alginate gel in inner ear simulating conditions and under CI-associated electrical stimulation, which remained unaffected by the coating. In vitro biocompatibility of alginate with cochlear hair cells and neurons could be demonstrated, while cell growth was not supported by the alginate surface. These findings encourage further investigations of alginate as coating material for CIs.

P92

Electrocochleography and electrically evoked compound action potentials to assess cochlear health in cochlear implant users

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Hearing performance of cochlear implant (CI) users relies on the condition of the auditory nerve. The nerve gradually degenerates following loss of hair cells and supporting cells, hence we hypothesize that residual presence of these cells is beneficial for neural health. Electrically evoked compound action potentials (eCAPs) can be used to assess the condition of the nerve. In particular, differential eCAP measures are strongly correlated with survival of the spiral ganglion cells (SGCs; Ramekers et al., 2014). The presence of residual hair cells can be assessed with intracochlear electrocochleography (ECochG; e.g., Giardina et al., 2019). In an ongoing trial, we record both eCAPs and ECochG in CI recipients which allows us to examine the relationship between neural health (assessed by eCAPs) and hair cell survival (assessed by ECochG).

Fifteen adult subjects with severe sensorineural hearing loss received a CI (Flex28 arrays of MED-EL). Postoperatively, eCAPs were recorded to biphasic current pulses with varying interphase gaps (IPGs; 2.1 to 30 µs) and varying current levels, using an alternating polarity stimulus paradigm (AutoART, MED-EL). Outcome measures obtained at each array electrode include amplitude and latency at maximum current levels, and the current level halfway the amplitude growth function, level50%. Relative eCAP measures were obtained by the difference between measures at IPG of 30 and 2.1 µs. ECochG was performed to tones at 115 dB SPL at frequencies from 250 Hz to 4 kHz. Outcome measures include the amplitude of the cochlear microphonics (CM).

In all patients eCAPs could be recorded with amplitudes between 200 and 1000 µV. CM was observed in a majority of participants with amplitudes up to 100 µV, not only at low frequencies but occasionally also at 2 and 4 kHz. Correlations between eCAP amplitude (averaged across electrodes) and CM amplitudes were not significant; neither did relative eCAP outcomes significantly vary with CM amplitudes (Spearman, $p>0.2$).

The lack of correlations between eCAPs reflecting neural health and CM reflecting residual hair cells may be explained as follows. The CM mainly reflects surviving outer hair cells, while inner hair cells are more important for neurotrophic support of the SGCs. Alternatively, it is rather the supporting cells being relevant for neurotrophic support (Zilberstein et al., 2012) and hair cell survival does not mirror supporting cell survival that well. We conclude that ECochG and eCAP provide largely independent measures reflecting the health of different cochlear structures.

P93

Microphone potentials at the round window: Intraoperative recordings during cochlear implantation

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Introduction

Cochlear microphone potentials (MP) are predominantly generated by outer hair cell activation through acoustic stimulation. MPs are increasingly applied in the intraoperative diagnosis of preservation of residual hearing during cochlear implantation. One of the significant steps in cochlear implant (CI) electrode positioning is the opening of the round window membrane (RWM). To specifically assess this step's influence on the MPs, recordings pre and post RWM opening were performed with the same measurement system.

Material and Methods

Intraoperative electrocochleography (ECochG) was performed in 11 CI candidates at the University ENT Clinic in Bochum during cochlear implantation. In these candidates, we aimed at a cochlear coverage of 80%, which was of higher priority than preservation of residual hearing, i.e. these were not EAS cases. Depending on the state of residual hearing, we used low frequency pure tone stimuli with a level of 30dB above threshold at a minimum of 80dB (HL). Measurements were performed via the cochlear implant with the distal electrode contact placed at the RW before and after opening of the RWM.

Results

Recording of MPs via the CI before RWM opening was possible in 6 candidates. MP amplitudes before and after RWM opening correlate well (r = 0.80) and indicated the post RWM opening potentials being larger by a factor of 2 (+6dB) than those pre RWM opening. In 3 of the 5 cases where no MPs could be detected pre, potentials were observed post RWM opening. In 2 cases CMs were not found pre nor post RWM opening.

Conclusion

Measurement of MPs through the CI pre RW opening is feasible. The opening has a significant amplifying effect on the MP amplitudes, which could be due to reduced electrical insulation by the RW or due to changes in the cochlear pressure resp. hydrodynamics, i.e. an effective reduction of mechanical attenuation or a combination of both.

P94

Morphometry of cochlear fluid compartments using micro-CT imaging and 3D segmentation

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Dimensions of cochlear fluid compartments, such as the cross-sectional area of scala tympani, is a key anatomical parameter influencing cochlear implant placement, drug diffusion, and inner ear fluid dynamics. Understanding morphological variations in scalae compartments is essential for optimizing clinical interventions and improving computational models of cochlear mechanics and drug spread.

In this study, we performed a quantitative morphometric analysis of cochlear fluid spaces (scala tympani, vestibuli and media) using high-resolution isotropic micro-CT data with 15µm voxel size from 31 human cochleae, that were contrast enhanced and decalcified. Segmentation was carried out using Thermo Fisher's AMIRA software. The centerlines of individual compartments were computed with AMIRA's "Centerline" module and further processed via its Python interface. To ensure geometric fidelity, centerline data were smoothed and tangent vectors were calculated using a 5-point central difference method. These vectors were used to construct orthogonal planes every 175µm along the cochlear spiral, on which we measured cross-sectional areas. We plotted a precisely orientated cross sectional profile that is not achievable with serial section reconstructions.

Our results reveal substantial anatomical variability in the cross-sectional profile of the scala tympani along the tonotopic axis. These differences have direct implications for electrode array fit, insertion depth, mechanical stability, and the spatial pattern of drug delivery. Additionally, they may influence local ion homeostasis, fluid pressure transmission, and the efficacy of electrical stimulation. This data provides clinically relevant metrics for surgical planning, personalized implant design, and post-operative assessment. From a computational perspective, it delivers critical morphological input for simulations of cochlear biophysics and auditory signal processing.

By linking detailed anatomical reconstructions with practical and theoretical applications, this work bridges imaging science, cochlear implantology, and auditory neurosciences - supporting more precise therapies and refined models of hearing.

P95

In vitro investigation of electrospun PVDF-TrFE fiber mats to reduce fibroblast growth after cochlear implantation

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Introduction

Cochlear implants (CI) are implanted to restore hearing in patients with severe to profound sensorineural hearing loss. Due to the foreign body response and insertion trauma connective tissue is formed around the electrode array, which electrically insulates the implant, hampering electrical stimulation of the auditory nerve. Modification of the electrode surfaces might be a way to reduce the adhesion of connective tissue. In the current study, fiber mats consisting of the hydrophobic material poly(vinylidene fluoride-trifluoroethylene) (PVDF-TrFE) were investigated in vitro regarding their contribution to electric conductivity, biocompatibility, and growth of fibroblasts on their surfaces.

Methods

Fiber meshes of different thicknesses were cut into 2 cm rectangular samples. Using an in-house manufactured chamber with four measuring cells, the increase of impedance was measured on conventional SEM holders. In a second step, fibers were spun directly on platinum electrode contacts of a similar size as in CI electrode arrays, which were embedded in silicone. In addition, MTT tests were performed as eluate tests to investigate cytotoxic effects. The growth of NIH/3T3 fibroblasts on Ø 6 mm circular samples in a time span of seven days was examined by means of confocal laser scanning microscopy.

Results

Measured impedances for the fiber mesh samples on the SEM holders increased by 225.99 ± 107.09 Ω compared to the reference samples. This increase in impedance was dependent on the thickness of the fiber whereas immersion in physiologic saline decreases impedance in a time dependent manner. Wetting of the fibers can be accelerated by the additional use of EtOH. The only constraint in biocompatibility was found with 100% extraction solution (69 ± 4.88% cell viability). The growth of fibroblasts is reduced on the electrospun fiber meshes compared to the surrounding cell culture material.

Conclusion

A thin film of electrospun PVDF-TrFE seems to have the potential to reduce fibroblast growth on stimulating contacts of CI electrodes without increasing the electrode impedance too much. An appropriate strategy for wetting the mesh appears mandatory.

P96

Effects of cochlear implantation on proteomic- and miRNA-perilymph profiles in a large animal model

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Background: Knowledge of the processes that occur in the inner ear is scarce. In recent years, OMICS technologies have begun to shed light on the complex microenvironment of the cochlea. The cochlear perilymph surrounds nearly all cells of the inner ear. Hence, analysis of molecular expression profiles of the inner ear fluid provides an opportunity to investigate intracochlear mechanisms. In this study, we analyzed changes in protein and microRNA (miRNA) expression after cochlear implantation in a large animal model using perilymph liquid biopsies and examined expression patterns over a period of up to six months.

Methods: Minipigs and Piglets were implanted with cochlear implants (CIs) that are commonly used in humans. Prior to electrode insertion, perilymph was collected via the round window using microglass capillaries. The same animals were sampled 24 h, 7 days, and 6 months after cochlear implantation via an apical perilymph sampling approach with the CI in situ. Small RNA sequencing and characterization of miRNAs were conducted for all samples collected after 24h and 7 days post cochlear implantation. Proteomic analysis was carried out for samples with a long-term follow-up of 6 months using liquid chromatography-mass spectrometry.

Results: Matched perilymph samples from 20 animals were analyzed. Sampling of perilymph prior to cochlear implantation and up to six months after CI insertion was feasible in all animals. Collected amounts reached from 2-8µl of perilymph per sample. Distinct miRNA profiles were detected in each sample reaching from 152 to 324 identified miRNAs. Differential expression analysis after 24h (n=6) and 7 days (n=5) showed significant changes in expression patterns before and after cochlear implantation, indicating their potential as prospective biomarkers. Proteomic analysis of matched perilymph samples (n=9) after 6 months showed a clustering of protein secretion into the perilymph. While 16 proteins showed significant upregulation, a larger number of 182 differentially expressed proteins were downregulated 6 months after cochlear implantation. Among these were proteins previously described as playing a role in neuronal cell development, neurite outgrowth, and immunosuppression.

Conclusions: Our study is the first to report the effects of cochlear implantation on miRNA and proteomic perilymph profiles in a large animal model. This provides an opportunity to screen for inner ear-specific biomarkers to improve CI outcomes and can serve as a basis for enhancing the treatment of inner ear disorders in the future.

Speech Processing, Auditory Perception, Cognition

P97

The link between hearing and cognition - What can we learn from an autism mouse model?

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Autism spectrum disorder is discussed in the context of altered neural oscillations and imbalanced cortical excitation–inhibition of cortical origin. We discuss here observations that document that developmental changes in peripheral auditory processing, while preserving basic hearing function, lead to altered cortical oscillations. Local field potentials (LFPs) were recorded from auditory, visual, and prefrontal cortices and the hippocampus of BdnfPax2 KO mice. These mice develop an autism-like behavioral phenotype through deletion of BDNF in Pax2+ interneuron precursors, affecting lower brainstem functions, but not frontal brain regions directly. Evoked LFP responses to behaviorally relevant auditory stimuli were weaker in the auditory cortex of BdnfPax2 KOs, connected to maturation deficits of high-spontaneous rate auditory nerve fibers. This was correlated with enhanced spontaneous and induced LFP power, excitation–inhibition imbalance, and dendritic spine immaturity, mirroring autistic phenotypes. Thus, impairments in peripheral high-spontaneous rate fibers have the potential to alter spike synchrony and subsequently cortical processing relevant for normal communication and behavior. The finding is discussed in the context of defined sensory input during the critical developmental period to set the threshold and operation point for corticofugal plasticity responses in adulthood.

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Keywords

fast auditory processing, hearing, autism, Bdnf

P98

Central compensation of neural responses to cochlear synaptopathy can be supported by dendritic spine remodeling through elevated cGMP levels

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Hearing loss is increasingly acknowledged as a significant cause of disability and a notable risk factor for cognitive decline and dementia. Many older adults struggle to understand speech in noisy environments, often due to progressive cochlear synaptopathy, which involves the loss of synaptic connections between hair cells and auditory nerve fibers. Previous studies have indicated that cochlear synaptopathy can lead to poorer temporal auditory processing, as manifested by reduced auditory steady state responses (ASSRs). However, in certain instances, deficits in temporal processing can be alleviated by central compensatory mechanisms, such as an enhanced input/output function of auditory brainstem responses (neural gain). This is accompanied by a better hippocampal long-term potentiation (LTP) and long-term depression (LTD) adjustment and upregulation of brain-derived neurotrophic factor (BDNF). In this study, we investigated the cyclic guanosine monophosphate (cGMP) signaling pathway as a potential molecular target involved in central auditory compensation. Mice were administered either a phosphodiesterase 9A inhibitor, which raises intracellular cGMP levels, or a vehicle control. Our results indicate a higher level of cGMP, which favors structural remodeling for central auditory adaptation and points toward the importance of this pathway in compensatory processes.

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Bdnf cgmp Hearing

P99

Strategy to investigate GMP-forming guanylyl cyclase (GCs, GC-A) function for central auditory processing deficits

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Although hearing loss is not life-threatening, mid-age hearing disorders were recently shown to be a major risk factor for dementia (Livingston et al., 2017, 2024, Lancet). Indeed, the prevention of hearing loss over age has been suggested as a major action on lowering the future prevalence of dementia (Livingston et al., 2024, *Lancet*). Age-depending hearing disorders are assumed to be the result of an accumulation of acoustic injuries over lifetime (Wu et al. 2019, *Neurosc*). We recently observed that cGMP-forming transmembrane guanylyl cyclase A (GC-A) exhibits a specifically protective function for maintaining cochlear hair cell stability and central auditory processing during age-dependent or stress and trauma-induced hearing impairment (Marchetta et al. 2022, *Br.J. Pharmacol*).

Here, we introduce the generation of a mouse animal model that may be suitable for the specific analysis of GC-A effects. GC-A will be tissue-specifically deleted using a tamoxifen-inducible conditional GC-A CamkIICre mouse (in cooperation with M. Kuhn). The GC-A CamkIICre mouse will be crossed with a BDNF-live exon viewing (BLEV) reporter mouse to specifically detect activity-dependent usage of *Bdnf* exon-IV and -VI promoters through bi-cistronic co-expression of CFP and YFP (Singer et al. 2018, *Front Mol Neurosci*). BLEV-reporter mice allow the visualization of stimulus-induced activity-dependent changes of BDNF in target regions, including capillaries and vessels. GC-A will be deleted in the forebrain followed by acoustic overexposure or in aged mice to study whether GC-A driven changes occur in this brain region. The protective effect of the cognitive stimulator phosphodiesterase 9 inhibitor (PDE9i) on age-dependent hearing loss (Savitska et al. 2022, *Front Neurosci*) will be tested for its GC-A cascade specificity.

Using the BLEV reporter mouse model, we expect new insights insight into the cellular targets of presumptive GC-A induced protective mechanisms and their therapeutic potential for cognition-associated hearing disorders.

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P100

Characterizing auditory degeneration with advancing age in a mouse model of Alzheimer's disease

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Recent epidemiological studies have highlighted a strong association between hearing loss and cognitive decline (doi: 10.1212/WNL.0b013e31826e263d; doi:10.1016/S0140-6736(17)31363-6). In particular, dysfunctions in both peripheral and central auditory pathways have been linked to the onset and progression of cognitive impairment (doi:10.1111/jgs.13649; doi:10.1007/s40520-014-0266-3; doi: 10.7554/eLife.70908), as well as to an increased risk of developing neurodegenerative disorders, including Alzheimer's disease (AD) (doi: 10.1046/j.1532-5415.2002.50114.x; doi: 10.1038/nrneurol.2015.12).

Despite the growing interest in the relationship between hearing loss and cognitive deterioration, the extent to which AD may be associated with a specific vulnerability of the auditory system, especially during aging, remains poorly understood.

To address this, we conducted functional, morphological, and molecular analyses in both wild-type (WT; B6129SF2/J) and AD model (3×Tg-AD) mice at different ages (3, 6, 12, and 18 months). Our aim was to characterize age-related alterations in the auditory system, including the cochlea and auditory cortex, while also investigating key markers of AD pathogenesis.

Our findings revealed pronounced alterations, in both the cochlea and the auditory cortex of AD model mice, accompanied by progressive age-related hearing loss. Notably, there was an age-dependent increase in AD-related pathological markers, including amyloid-β, phosphorylated Tau (pTau), and cholinergic dysfunctions, in the auditory structures, suggesting a direct involvement of AD pathology in auditory system degeneration.

Taken together, the results of this study provided a full characterization of the auditory function and auditory structures in 3×Tg-AD mice, giving insight on an intriguing role of AD misfolding proteins in auditory neurodegeneration induced by AD pathology or by physiological aging.

P101

DX243 weakens acoustic trauma-induced cortical brain oscillation changes and distinct neurocochlear impairments

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A dramatic shift in societal demographics will lead to rapid growth in the number of older people with hearing deficits. The increasing risk of cognitive decline predicted to be associated with hearing loss has become a key challenge for future societies. Over age accumulating acoustic injury contributes particularly to speech discrimination deficits in background noise, a feature predicted to be linked to the high vulnerability of auditory fibers with low spontaneous discharge rate and high activation thresholds. We here investigate a new potential drug, dendrogenin, which is predicted to exhibit otoprotective function and balanced neurite outgrowth. We test the effect of dendrogenin on cortical local field potentials (LFP) and peripheral paradigms after acoustic trauma (AT) in the rat animal model. In line with previous studies, AT reduced acoustic stimuli-induced local field potentials in the auditory cortex 2 weeks after AT, while neither acoustic stimuli nor AT effected LFP in the prefrontal cortex, visual cortex and hippocampus. 2 weeks after daily injection of DX but also 6 weeks after the last DX injection a dose dependent protective effect of DX could be observed on AT induced decline of LFP spectral power. DX did not affect hearing thresholds after AT but exhibited with the same dose response as shown for its effect on LFP, a protective effect on auditory fiber functions. A significant preservation of IHCs ribbon numbers after AT, less impairment of specifically noise-sensitive auditory fiber processing (tested through Notch-noise) and a positive preserving effect on pre-postsynaptic integrity during multi-click responses with decreasing time intervals, suggest a possible selective effect of DX on the most vulnerable low-SR auditory fiber type. These findings suggest dendrogenin as a potential neuroprotective drug for the treatment and prevention of age- and noise-induced hearing loss.

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Two sound detection of fast noise burst sequences – An electrophysiologic perspective

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The fast sequence of a short and a long noise burst (NB) separated by a short gap has been shown to evoke nonlinear transition of subcortical electrophysiological responses depending on their parameters (Burkhard et al. 2019). However, it was not clear whether, and how, this phenomenon is related to the perception of the discontinuity of such sounds. The present study focused on an analysis of electrophysiological responses and auditory perception of these NB sequences.

Electroencephalographic signals (EEG) were recorded from a selected scull positions using Ag-AgCl electrodes in a group of young probands (n=14, age: range of 23-42 years) with normal hearing. Auditory responses were evoked by a sequence of two NBs separated by a short gap. Durations of the leading NB varies (5, 10, 30 ms), whereas durations of the trailing NB and gap are fixed (10 ms and 50 ms, respectively). Each NB has a 1 ms rising/falling phase. The stimuli were presented by Tucker-Davis Technology system RZ6 (TDT, Alachua, USA). Auditory perception of NB sequences with different parameters were detected by answering a question, if probands hear one or two sounds after each presentation (min. 20 presentations). EEG signals were then recorded using TDT SI4 amplifier and RZ2 system (sampling rate of 24.414 kHz) to all three NB sequence conditions and separate NBs of corresponding durations (i.e. 5, 10, 30, and 50 ms) with repetition interval of 1.3 s with 10% jitter We have recorded 300 trials for each stimulus condition.

The combination of short leading NBs (5 and 10 ms) combined with the 10 ms long gap were usually perceived as one continuous sound, whereas leading NB of 30 ms duration were consistently perceived as two sound sequence. The EEG responses yielded corresponding difference in the latency range between 150-450 ms. As expected, the individual EEG responses showed high interindividual variability. Despite of this variability differences between NB-gap-NB sequences could be observed at individual level. Present preliminary data suggest electrophysiological biomarkers of diverse neuronal activations linked to specific auditory perception of fast sound sequences in EEG. Considering the importance of fast sound sequences in speech perception and nonlinear nature used stimuli, the results could provide insights into complex auditory processing interaction underlying categorical speech perception based on fine temporal characteristics of fast sound sequences.

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P103
Stimulus onset contributions to speech comprehension

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The slowing and reduction of auditory responses in old age leads to deficits in speech processing and a loss of cognitive abilities. There is currently a controversial debate as to whether this is related to central brain atrophy or rather to a delay in neuronal processing in the periphery.

We examined young, middle-aged, and older study participants with and without hearing threshold loss using pure-tone (PT) audiometry, short-pulsed distortion-product otoacoustic emissions (pDPOAE), auditory brainstem responses (ABR), auditory steady state responses (ASSR), speech comprehension (OLSA), and syllable discrimination in quiet and noise. Amplitudes and latencies of speech EEG responses to syllables served as markers for the central auditory processing. To identify markers in the EEG specific for deficits in speech processing, OLSA speech reception thresholds (SRTs) were weighted using a normalization step (pure-tone normalized OLSA thresholds, PNOT) which enabled us to separate participants with good and bad speech reception performance independent of pure tone threshold (PTT) and age.

Poor speech comprehension could be linked with differences in cochlear amplifier performance and ABR wave latency shift. The EEG responses of these poor performing participants were characterized by a delay in the most likely thalamic-generated EEG responses to phoneme stimuli. We discuss the changes in amplitude and delays of the EEG responses in the required temporal fine structure (TFS) or temporal envelope (TENV) coding of syllables in word discrimination.

Our data suggest markers for speech comprehension performance in the central EEG responses, different in quiet and ipsilateral noise masking.

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Meaningful adult hearing health outcome measures: Health care and research applications

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The pure-tone audiogram is the clinical gold standard for adult hearing health assessment. However, the audiogram does not directly capture communication difficulties that patients report and restoring audibility does not assure that communication challenges will resolve. This is highly problematic both for the care of individual patients and from a public health perspective. With the most important patient concerns often going unmeasured in both clinical and research settings, we cannot assess whether interventions were effective either for a given patient or at the group level, within randomized, controlled clinical trials.

Recognizing this issue, several United States (U.S.) health agencies came together to work towards the development of solutions that prioritize patient experience. Specifically, the U.S. National Institutes of Health, Defense Health Agency, and Veteran's Administration commissioned a report from the National Academies of Science, Engineering, and Medicine (NASEM) on meaningful outcome measures for adult hearing health. The NASEM committee report, "Measuring Meaningful Outcomes for Adult Hearing Health Interventions", was released on May 7, 2025.

The committee came to consensus on two recommended outcome domains: understanding speech in complex listening environments and hearing-related psychosocial health. Recommended measurements include the Words-in-Noise (WIN) test and the Abbreviated Profile of Hearing Aid Benefit (APHAB) global score as measures of speech understanding, and the Revised Hearing Handicap Inventory (RHHI) as a measure of hearing-related psychosocial health.

These outcome measures are recommended in both clinical and research settings and they are recommended across indications and interventions. In other words, whether a patient or research participant has age-related hearing loss or noise-induced hearing loss; whether a patient or research participant receives a hearing aid, a pharmaceutical intervention, a biologic therapy, or auditory rehabilitation; these outcome measures are recommended for all. Because the committee was tasked with identifying domains that are important across patients, settings, and interventions, it is likely that supplementary measures will be needed. The focus of this presentation will be the recommended outcome domains and measures and considerations in their use in the assessment of investigational inner ear medicines.

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Trans-tympanic laser stimulation of the cochlea can induce auditory related neural and behavioral responses

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Cochlear implants have been widely used for the hard-of-hearing individuals, while at the same time, conventional cochlear implants have several limitations attributed to electrical stimulation (i.e., the need for surgical implantation, which can damage residual hearing caused by the complication). Infrared laser stimulation has emerged as a possible alternative to electric stimulation because laser can activate the spatially selective area of the cochlea from the outer ear. Here, we examined the feasibility of infrared laser stimulation from the outer ear through the investigation of the laser-evoked electrophysiological and behavioral response in Mongolian gerbils (*Meriones unguiculatus*). A pulsed infrared laser and a clicking sound were presented to the subjects. The amplitude of the laser-evoked cochlear response was systematically decreased following insertion of a filter, which absorbs infrared laser, into the laser path between the tympanic membrane and cochlea; however, the auditory-evoked cochlear response did not decrease. The laser-evoked response returned to around the original level after the filter was removed. This result indicates that laser irradiation could bypass the function of the middle ear and directly activate the cochlea. Subsequently, head-fixed classical conditioning was performed to quantify the laser-evoked perception. A click-train of 4000 Hz or a repetitive pulsed infrared laser irradiation to the lateral side of the cochlea from the ear canal through a tympanic membrane was presented as a conditioned stimulus for a reward. Licking behavior was recorded as a conditioned response. After the training, gerbils showed licking responses to the conditioned stimulus without paired water. In the test session, white noise was presented as a masking stimulus during the auditory and laser stimulation period. In addition, laser stimulation with various intensities was presented to the subjects trained on auditory stimulation. As a result, laser-evoked licking behavior decreased with the masking stimulus intensified as auditory-evoked licking behavior did. The laser irradiation induced the licking behavior in the auditory-trained gerbils in an intensity-dependent manner, indicating stimulus generalization from auditory to laser stimulation. These results suggest that the infrared laser irradiation of the cochlea can evoke auditory perception. These studies will be an essential step for the clinical application of earphone-type noninvasive auditory prostheses with infrared laser-based technology.