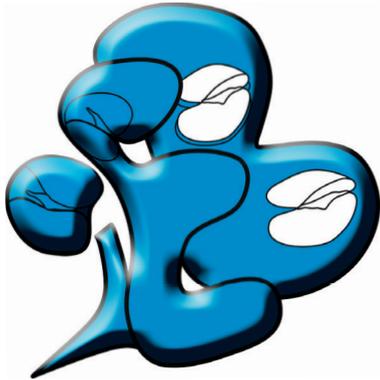


2017 Hannover

54th Workshop on Inner Ear Biology and Symposium



Interventions in the ear:

From inner ear biology to advanced therapy of hearing loss

13th - 16th September 2017



Hannover Medical School
Germany

Venue:

Hannover Medical School
Carl-Neuberg-Str. 1
D-30625 Hannover

Germany

Sponsors

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Association for Research in Otolaryngology

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Dear Participants,

We take over the relay and continue the tradition of the meeting that has the longest history in auditory research.

The first Inner Ear Biology meeting took place in Nov. 6-7, 1964. It was organized by Prof. S. Rauch and Prof. A. Meyer zu Gottesberge in Düsseldorf, Germany. This was 7 years before the first Society of Neuroscience meeting and 12 years before the first meeting of the Association for Research in Otolaryngology took place.

In 2017, we again want to provide a platform for an excellent scientific conference. Due to the strong cochlear implant program here in Hannover we want to bridge basic research into clinical application and demonstrate you also research programs in the field of auditory rehabilitation. The whole research and development chain has been built up over the last 30 years with basic research, translation into medical products and clinical research for the use of new products in patients. The Hannover team welcomes you at the research facilities and let you also enjoy Hannover and its surroundings. In the oral presentations, we tried to cover all essential topics of inner ear research. The meeting will include the Symposium "Interventions in the ear: From inner ear biology to the therapy of hearing loss". Some of the submissions were organized into special sessions on "Hearing and light" and "Bioinspired signal processing". There will be in addition an ARO-sponsored symposium entitled "Developmental consequences of hearing loss".

We organize this IEB workshop also in memory of our colleague Günther Reuter who passed away recently. He did not only contribute to the auditory science and built up the Laboratories of Experimental Otolology in Hannover, but was also an excellent mentor of many our students.

We welcome you in Hannover!



A handwritten signature in black ink, appearing to read "T. Lenarz".

Prof. Prof. h. c. Dr. med. Thomas Lenarz
Chairman, Department of Otorhinolaryngology,
Hannover Medical School



A handwritten signature in blue ink, appearing to read "A. Kral".

Prof. Dr. Dr. Andrej Kral
Chaired Professor of Auditory Neuroscience,
Hannover Medical School

Organizing committee



Prof. Prof. h.c. Dr. Thomas Lenarz



Prof. Dr. Dr. Andrej Kral



Prof. Dr. Hannes Maier



Prof. Dr. Ing. Waldo Nogueira



Dr. Gerrit Paasche



Dr. Verena Scheper



PD Dr. Athanasia Warnecke



Venue: MHH, Building J2

Hosts:

Prof. Prof. h.c. Dr. med. Thomas Lenarz
Prof. Dr. Dr. med. Andrej Kral

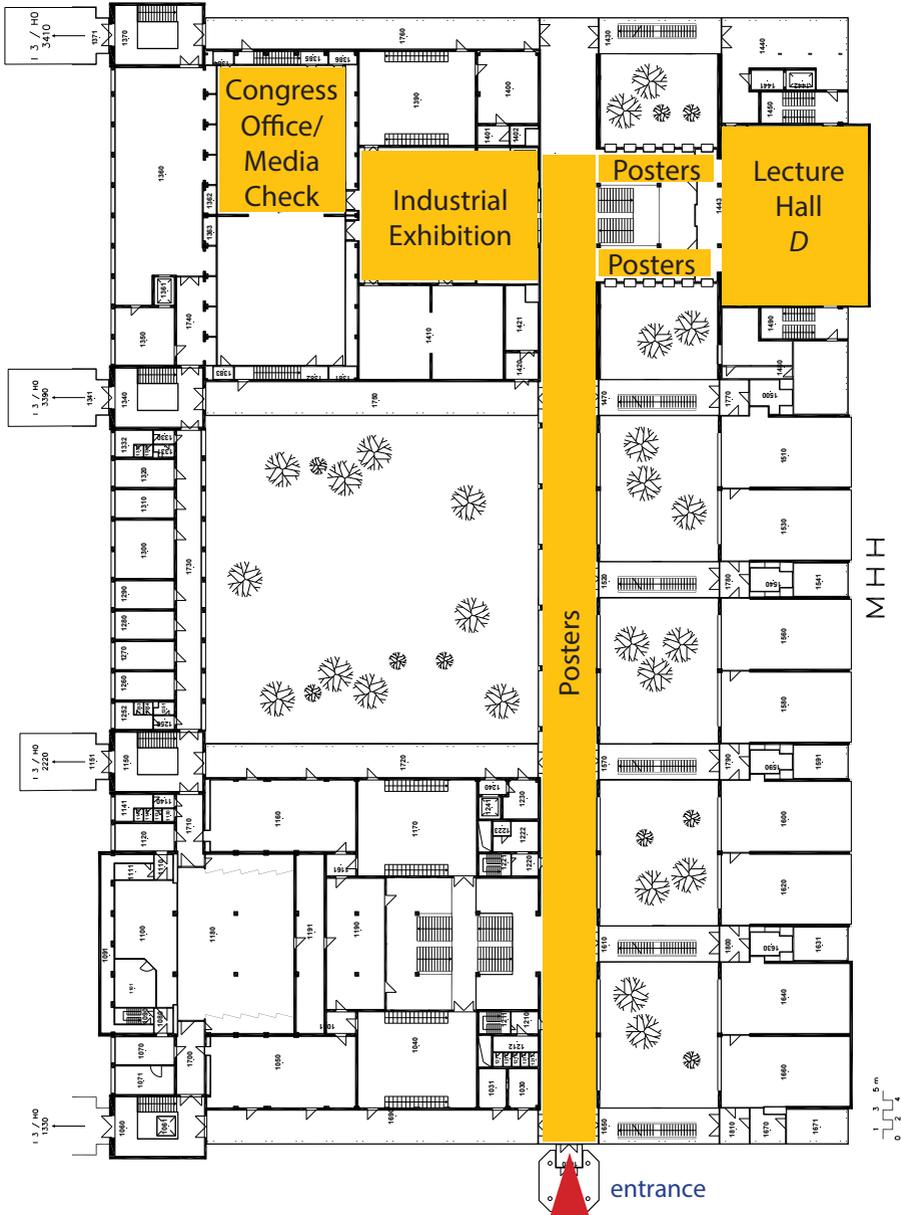
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www.ieb2017.com



**Building J2
Floor H0**



<- direction „Stadtfelddamm“

Carl-Neuberg-Str.

direction „Karl-Wiechert-Allee“ and Main entrance ->

Poster presentations

- Posters will be on display during the entire workshop
- Poster size: A0 (portrait configuration)
- Presentation at poster board only
- Presenting author is responsible for setting up and removing the poster
- Presenting authors are requested to be at their poster(s) according to the designated schedule (see workshop program). In addition, it would be recommended they leave a note at their posters indicating their availability when visiting other posters.
- Fixation material will be provided
- The IEB Workshop is not responsible for posters and materials left after the workshop.

Oral presentations

- Time for presentation: 12 minutes with an additional 3 minutes for discussion (except ARO sponsored session and symposium)
- Adherence to the allocated speaking time will be strongly enforced; session chairpersons will be urged to monitor time management strictly
- Authors are requested to submit the presentation on USB-stick or CD at the Media Check at least 2 hours before their session starts
- Presentations will be on PC (NOT Mac)
- Formats: Powerpoint 2003 or newer
- The use of own laptops will not be allowed.

Internet access

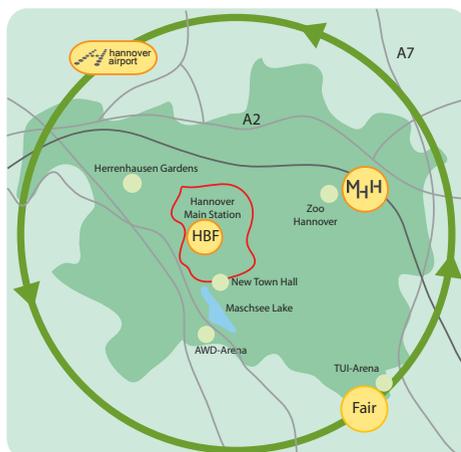
- Internet access will be available (further information on site).

Few organizational notes:

- Hannover's taxi drivers do not accept credit cards!
- Currency in Germany is € (Euro). A cash machine is located at MHH main entrance
- Germany Time: Central European Summer Time (CEST) = GMT + 2
- Power supply: In Germany the power sockets are of type F. This socket also works with plug C and plug E.



Socket type F



By bus, tram and rail through Hannover and the Hannover Region easy with public transport.

The Red Thread is painted on the pavement, and weaves its way through the inner city joining up 36 prime attractions.

More information: www.ieb2017.com

MHH – Leading medical center in Germany

The Department of Otorhinolaryngology at Hannover Medical School (MHH) is internationally renowned for hosting the world's largest cochlear implant (CI) programme to treat severely hearing impaired patients. To date, more than 8,000 people have received a CI here. One of our priority areas, therefore, is provision of hearing systems – from the development of the devices themselves to lifelong support for our patients. Together, the German Hearing Center Hannover and the scientific laboratories form the joint platform for these activities.

Other priority areas include hearing-aid fitting (and improving this process), the early identification of hearing loss in children, diagnosis and treatment of inner-ear diseases including tinnitus, skull base surgery including treatment of acoustic neuroma, tumour surgery using modern laser surgical and endoscopic techniques, diseases of the nose and sinuses, covering allergology, environmental medicine and plastic/reconstructive techniques.

Our Department has six wards with a total of 90 beds. Each year we treat around 25,000 outpatients and just under 6,000 inpatients. A highly motivated and specialised team is available, with more than 200 staff – including 30 doctors, 20 nurses, nine education professionals and speech therapists, 20 technical staff and 30 scientists.

Our case numbers: more than 500 cochlear implants, 85 middle-ear implants and 100 acoustic neuromas in 2016.

A nationwide first at MHH's ENT Department: in autumn 2011, for the first time in Germany (and only the fourth time in the world) a patient was successfully implanted with MED-EL's new 'bone bridge' system. In 2015 we were the first in Germany to implant successfully the first Oticon Medical CI.



Hearing4all: You can hear the future

Goal of the Cluster of Excellence Hearing4all of the German Research Society (DFG) is to improve hearing for all persons concerned in all situations, at all times. It includes scientists at the University of Oldenburg, the Hannover Medical School and the Leibniz University in Hannover.

This aim is of great importance, given that 18 percent of the German population – in particular, more than 50 percent of those above 65 years of age - has a hearing impairment requiring treatment. With better individual hearing diagnostics, individualised hearing aids have become possible. Using such individual support facilitates communication for the hearing impaired in an essential way – be it at work, in traffic or at home. To reach this aim, innovative concepts for hearing aid and hearing implant processing are being developed, helping not only the severely hearing impaired, but everybody by putting an individualised „hearing aid“ into every smartphone, TV set, and stereo.

We are working on improving audiological diagnostics, and developing improved rehabilitative strategies based on that. Our work focusses on fundamental, model-based research for diagnostics and auditory profiling of human hearing, be it normal hearing or even severely impaired. Using a model-based approach enables us to individualize treatment of hearing loss with adequate hearing instruments, adapting to individual needs and to the acoustic environment.



The Cluster of Excellence at the



Discover Hannover

Welcome to one of the most important international trade fair locations in the world and a modern pulsating city with lots to discover! A modern state capital with groundbreaking architecture and a model infrastructure, surrounded by idyllic little towns and villages – this is the Hannover location with all its delightful contrasts.

Hannover's strengths as a business location are its innovative companies, its international flagship fairs and its economic stability. The academic world, business and government pool their resources to put pep into the economy of the city that was home to the all-round genius Leibniz. The "Germany 2020" study by the Zukunftsinstitut, a Frankfurt-based institute for prognostics, presents Hannover as "soundly based and future-driven", with "creative entrepreneurs, excellent conditions for education and research and a talent for keeping up with decisive trends". Museums such as the Wilhelm Busch Museum, home to Max and Moritz, theatres where world-famous stars appear and a celebrated State Opera offer outstanding cultural experiences. Equally attractive are the Herrenhausen Gardens, the maritime atmosphere of the Maschsee Lake, the great diversity of sporting events and open-air concerts, and the many fairs and popular festivals. Passers-by linger in the picturesque Old Town, and the exotic landscapes of the Adventure Zoo enchant the whole family. Keen shoppers can roam through one of Germany's largest pedestrian zones or enjoy the idyllic atmosphere of the Region's half-timbered towns. All round the city, recreational areas such as Lake Steinhude or the Deister Hills offer a wide diversity of leisure activities.



„Red Thread“ Hannover

The Red Thread is painted on the pavement, is 4200 metres long, and weaves its way through the inner city joining up 36 prime attractions. This is a floorline visitors' guide of a different kind. All you have to do is follow the Red Thread! This „do it yourself“ city tour is accompanied by an informative brochure which describes all of the interesting buildings and monuments you meet along the way, and is also full of interesting historical background. Furthermore the brochure describes an „ExtraTour“ which is a 45 minutes refreshing detour to the banks of Lake Maschsee.



Useful numbers

Congress office

Regina Müller

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Program

Program overview

Wednesday, September 13th

- 13:00 – 13:30 Welcome
- 13:30 – 15:15 Special session „Hearing and light“
- 15:15 – 16:00 Symposium Advanced Bionics
- 16:00 – 17:45 Scientific session: „Development and genetics“
- 19:00 Welcome reception at Hannover Zoo

Thursday, September 14th

- 08:00 – 10:00 ARO symposium: „Developmental consequences of hearing loss“
- 10:30 – 12:30 Scientific session: „Inner ear biology I“
- 12:30 – 13:30 Symposium MED-EL
- 13:30 – 15:30 Scientific session: „Auditory prostheses“
- 16:30 – 18:00 Scientific session: „Protection and regeneration“
- 18:00 – 19:00 Visit of the laboratories and facilities at the Institute of AudioNeuroTechnology (VIANNA) and German Hearing Center
- 19:30 Science meets art: Fingerfood at Wilhelm Busch Museum

Friday, September 15th

- 08:00 – 10:30 Live surgery of auditory implants and transmission
- 11:00 – 13:00 Scientific session: „Bioinspired signal processing“
- 14:00 – 15:15 Scientific session: „Therapeutic interventions“
- 15:15 – 16:00 Business meeting
- 16:30 – 18:15 Scientific session: „Inner ear biology II“
- 19:00 Congress dinner at Herrenhausen Palace

Saturday, September 16th

- 08:00 – 17:30 Symposium: „Interventions in the ear: From inner ear biology to advanced therapy of hearing loss“

Workshop on Inner Ear Biology

10:00 Registration

13:00 Welcome

Thomas Lenarz and Andrej Kral, Chairs of IEB 2017

Christopher Baum, President of Hannover Medical School

Hearing and light

Moderators: Hannes Maier & Tobias Moser

13:30 O-1 **Optical cochlea implants**

Claus-Peter Richter

13:45 O-2 **Towards the optical cochlear implant: optogenetic stimulation of the auditory nerve**

Tobias Moser

14:00 O-3 **Intracochlear pressure generated by pulsed laser emission**

Peter Baumhoff, Nicole Kallweit, Andrej Kral*

14:15 O-4 **A model based sound coding strategy for laser stimulation in cochlear implant users with residual hearing**

Waldo Nogueira, Torben Fiedler, Peter Baumhoff, Darshan Shah, Andrej Kral, Andreas Büchner, Hannes Maier, Benjamin Krüger

14:30 O-5 **The optoacoustic stimulation of the peripheral hearing organ**

*Gentiana I. Wenzel**

14:45 O-6 **Optogenetic stimulation on adult cochlea of guinea pig**

Ning Yu, Chen Liu, Qing-qing Jiang, Da-xiong Ding, Shi-ming Yang, Ping LV, Shu Fang*

15:00 O-7 **Cochlear optogenetics drives avoidance behavior in normal hearing and deaf gerbils**

Alexander Dieter, Christian Wrobel, Daniel Keppeler, Gerhard Hoch, Marcus Jeschke, Tobias Moser*

15:30 Coffee Break & **Symposium Advanced Bionics**

Development and genetics

Moderators: Anke Lesinski-Schiedat & Jose Manuel Juiz Gomez

- 16:00 O-8 **Conditional Sox2 deletion defines residual inner ear development**
Martina Dvorakova, Romana Bohuslavova, Bernd Fritzscht, Israt Jahan, Tetyana Chumak, Josef Syka, Gabriela Pavlinkova*
- 16:15 O-9 **Molecular characterization and prospective isolation of human fetal inner ear prosensory domain progenitor cells**
Marta Roccio, Michael Perny, Megan Ealy, Hans Ruedi Widmer, Stefan Heller, Pascal Senn*
- 16:30 O-10 **How impaired SGN axon bifurcation affects sound processing in the auditory brainstem**
Steffen Wolter, Dorit Möhrle, Dennis Zelle, Marlies Knipper, Peter Pilz, Hannes Schmidt, Lukas Rüttiger*
- 16:45 O-11 **Deciphering gene expression dynamics of vestibular hair-bundle morphogenesis from single-cell snapshot data using CellTrail maps**
Daniel C. Ellwanger, Mirko Scheibinger, Matthew R. Avenarius, Rachel A. Dumont, Peter G. Barr-Gillespie, Stefan Heller*
- 17:00 O-12 **Pluripotent stem cells in disease modelling of GJB2 related hearing loss**
*Ichiro Fukunaga, Ayumi Fujimoto, Kaori Hatakeyama, Osamu Minowa, Katsuhisa Ikeda, Kazusaku Kamiya**
- 17:15 O-13 **Trends in genetic diagnostics of hereditary hearing loss**
Ronald Pennings, Celia Zazo Seco, Mieke Wesdorp, Ilse Feenstra, Hannie Kremer, Lies Hoefsloot, Margit Schraders, Helger Yntema*
- 17:30 O-14 **FRMPD4 is associated with X-linked non-syndromic hearing loss**
Barbara Vona, Daniel Liedtke, Kristen Rak, Radoslaw Katana, Lukas Jürgens, PingkalaiR Senthilan, Indrajit Nanda, Cordula Neuner, Michaela AH Hofrichter, Linda Schnapp, Jörg Schröder, Ulrich Zechner, Stefan Herms, Per Hoffmann, Tobias Müller, Marcus Dittrich, Oliver Bartsch, Peter M Krawitz, Eva Klopocki, Wafaa Shehata-Dieler, Martin C Göpfert, Thomas Haaf*
- 18:45 Bus from MHH to Hannover Zoo
- 19:00 Welcome reception, Hannover Zoo

ARO-sponsored symposium



Association for Research in Otolaryngology

Developmental consequences of hearing loss

Moderators: Andrej Kral & Hinrich Staecker

- 08:00 O-15 **Cellular and molecular studies of a critical period for afferent-dependent neuron survival in the cochlear nucleus**
Edwin W Rubel
- 08:30 O-16 **Restoring cortical inhibition improves perception following early hearing loss**
Dan Sanes
- 09:00 O-17 **Prediction and monitoring of cochlear implant outcome using functional near infrared spectroscopy**
Douglas Hartley
- 09:30 O-18 **Effects of early hearing experience on functional activation and connectivity in primary and higher-order cortical field**
Yusuf Prasadhyia
- 10:00 Coffee Break & Poster viewing (P1 - P23)

Inner ear biology I

Moderators: Gerrit Paasche & Huib Versnel

- 10:30 O-19 **A rescuable auditory synaptopathy in mice lacking CLARIN-1**
Didier Dulon, Samantha Papal, Alice Emptoz, Mateo Cortese, Said Safieddine, Aziz El-Amraoui, Christine Petit*

- 10:45 O-20 **Autonomous and evoked Ca²⁺ signals in inner hair cells and coupling to Ca²⁺ waves in the developing mouse cochlea**
*Tobias Eckrich, Kerstin Blum, Ivan Milenkovic, Stefan Münkner, Jutta Engel**
- 11:00 O-21 **The development and maintenance of hair cell stereocilia rootlets by isoform specific functions of TRIOBP**
Tatsuya Katsuno, Inna A. Belyantseva, Ronald S. Petralia, Ya-Xian Wang, Keisuke Ohta, Kazuya Ono, Makoto Ikeya, Gavin P. Riordan, Joseph Duda, Elizabeth Wilson, Tracy Fitzgerald, Atteeq U. Rehman, Ayesha Imtiaz, Jyuichi Ito, Thomas B. Friedman, Shin-ichiro Kitajiri
- 11:15 O-22 **Angulin proteins ILDR1 and ILDR2 regulate alternative pre-mRNA splicing through binding to splicing factors TRA2A, TRA2B, or SRSF1**
*Yueyue Liu, Hongyun Nie, Chengcheng Liu, Xiaoyan Zhai, Qing Sang, Yanfei Wang, Deli Shi, Lei Wang, Zhigang Xu**
- 11:30 O-23 **Abnormal actin elongation activity of a novel hearing-loss Dia1 mutant revealed by single-molecule speckle microscopy**
Takushi Miyoshi, Yuzuru Ninoyu, Naoki Watanabe, Shin-ichiro Kitajiri, Takehiko Ueyama*
- 11:45 O-24 **On Structure and function in outer hair cells**
Einat Shapira, Eitan Kimmel, Remy Pujol*
- 12:00 O-25 **Measurement of cochlear partition volume compliance using a microfluidic chamber system**
Jong-Hoon Nam, Jessica Huhnke, Daniel Marnell, Jonathan Becker
- 12:30 Lunch Break with packed lunch boxes & **Symposium MED-EL**

Auditory prostheses

Moderators: Thomas Lenarz & Lawrence Lustig

- 13:30 O-26 **New cochlear implant map based on subjective pitch match between acoustic and electrical stimuli in unilateral or highly asymmetric deafness**
*Walter Di Nardo, Roberta Anzivino, Anna Rita Fetoni, Gaetano Paludetti**

- 13:45 O-27 **From the cochlea to the brain: how temporal processing is impacted by infection and training?**
Aline Castro, Denise Gonçalves, Fabrice Giraudet, Luciana Resende, Paul Avan*
- 14:00 O-28 **Clinical evidence of binaural hearing in children with unilateral hearing loss**
Martijn Agterberg, Ad Snik, John Van Opstal
- 14:15 O-29 **The decision between direct acoustical cochlear stimulation and cochlear implantation: A retrospective analysis of results**
Eugen Kludt, Andreas Büchner, Thomas Lenarz, Hannes Maier*
- 14:30 O-30 **Spiral ganglion neurons cultured on advanced micro-electrode arrays**
Damir Kovacic, Viktorija Radotic, Dries Braeken*
- 14:45 O-31 **Future directions for cochlear implantation - restoring the spiral ganglion population with stem cells**
Leila Abbas, Daniela Cacciabue-Rivolta, Daniel Smyth, Wolfram Dueck, Marcelo Rivolta*
- 15:00 O-32 **Three-dimensional force profile during cochlear implantation depends on individual geometry and insertion trauma**
Ersin Avci, Tim Nauwelaers, Volkmar Hamacher, Andrej Kral*
- 15:15 O-33 **Anodal transcranial direct current stimulation modulates auditory cortex structural plasticity in a model of noise-induced hearing loss (from central auditory prostheses)**
Fabiola Paciello, Maria Vittoria Podda, Rolando Rolesi, Sara Cocco, Diana Troiani, Anna Rita Fetoni, Claudio Grassi, Gaetano Paludetti*
- 15:30 Coffee Break & Poster viewing (P24 - P46)
- 16:00 Poster viewing (P47 - P69)

Protection and regeneration

Moderators: Athanasia Warnecke & Edwin Rubel

- 16:30 O-34 **Lgr5-positive cells act as hair cell progenitors in the cochlea**
Albert Edge
- 16:45 O-35 **Exploring new approaches to reduce cisplatin ototoxicity**
*Eric Bielefeld**
- 17:00 O-36 **Olfactory stem cell derived hair cell progenitor**
Louise Straatman, Ronak Rahmanian, Anat Yanai, Cathy Garnis, Brian Westerberg, Kevin Gregory-Evans
- 17:15 O-37 **Mesenchymal stem cells for prevention of ototoxicity induced by cisplatin**
Laura Astolfi, Edi Simoni, Filippo Valente, Erica Gentilin, Valeria Franceschini, Alessandro Martini*
- 17:30 O-38 **Autophagy protects auditory hair cells against neomycin-induced damage**
*Zuhong He, Lingna Guo, Qiaojun Fang, Xia Gao, Renjie Chai**
- 17:45 O-39 **Netrin1 mediates the protection of cochlear hair cells by IGF1 through its canonical receptor, UNC5B**
Norio Yamamoto, Kouhei Yamahara, Takayuki Nakagawa, Juichi Ito, Koichi Omori*
- 18:00 **Visit of the laboratories and facilities at the Institute of AudioNeuro-Technology (VIANNA) and German Hearing Center (DHZ)**
- 19:00 Bus to Wilhelm Busch German Museum of Caricature and Critical Graphic Art
- 19:30 Science meets Art: Fingerfood at Wilhelm Busch Museum

Live surgery

- 08:00 **Live surgery of auditory implants and transmission from the operating theatre of the ORL-department at Hannover Medical School into the lecture hall**
- 10:30 Coffee Break & Poster viewing (P70 - P91)

Bioinspired signal processing

Moderator: Waldo Nogueira & Hubert Lim

- 11:00 O-40 **Roles of the contralateral medial olivocochlear efferent reflex demonstrated with cochlear implants**
Enrique Lopez-Poveda
- 11:15 O-41 **Bio-inspired sound coding strategies for cochlear implants based on temporal fine structure**
Peter Nopp
- 11:30 O-42 **Simple physiological model of spike-conducting axons and its application to auditory nerves**
Go Ashida
- 11:45 O-43 **Optogenetic stimulation of the cochlea — first models**
Werner Hemmert
- 12:00 O-44 **Modelling the brainstem response to cochlear implant stimulation**
Ray Meddis
- 12:15 O-45 **Neuronal network simulations in the cochlear nucleus and its implications for CI and ABI stimulation**
Andreas Bahmer
- 12:30 O-46 **Model-guided development of a noninvasive approach to evaluate cochlear Synaptopathy**
Viacheslav Vasilkov, Sarah Verhulst

- 12:45 O-47 **A predictive model of blast induced hearing loss**
Allen F Ryan, Alex Fantozzi, Elena Cardenez, Eduardo Chavez, Arwa Kurabi
- 13:00 Lunch Break with packed lunches
- 13:30 Poster viewing (P92 - P115)

Therapeutic interventions

Moderators: Verena Scheper & Marlies Knipper

- 14:00 O-48 **Functional appraisal of the auditory nerve following clinically feasible gelfoam treatment with various neurotrophic compounds in deafened guinea pigs**
Henk A. Vink, Dyan Ramekers, Hans G.X.M. Thomeer, Sjaak F.L. Klis, Huib Versnel*
- 14:15 O-49 **Beneficial effect of ProteinY* on hearing loss during experimental pneumococcal meningitis**
Silvia Erni, Michael Perny, Rolf Jan Rutten, Pascal Senn, Denis Grandgirard, Stephen L. Leib, Marta Roccio*
- 14:30 O-50 **Bone morphogenetic protein 4 promotes the survival and preserves the structure of flow-sorted Bhlhb5+ cochlear spiral ganglion neurons in vitro**
Shan Sun, Muhammad Waqas, Huawei Li, Renjie Chai*
- 14:45 O-51 **Time course of oxidative stress and apoptosis in the auditory receptor after Kanamycin treatment in the rat**
*Alejandro Gibaja-Casado, Juan C Alvarado, Jose M Juiz**
- 15:00 O-52 **Platinum-induced hidden hearing loss**
Marion Souchal, Fabrice Giraudet, Paul Avan*
- 15:15 Business Meeting
- 16:00 Coffee Break

Inner ear biology II

Moderators: Andrej Kral & Agnieszka Szczepek

- 16:30 O-53 **NO-sensitive guanylate cyclase isoforms NO-GC1 and NO-GC2 contribute to noiseinduced inner hair cell synaptopathy**
Dorit Möhrle, Katrin Reimann, Steffen Wolter, Markus Wolters, Ksenya Varakina, Evanthia Mergia, Nicole Eichert, Hyun-Soon Geisler, Peter Sandner, Peter Ruth, Andreas Friebe, Robert Feil, Ulrike Zimmermann, Doris Koesling, Marlies Knipper, Lukas Rüttiger*
- 16:45 O-54 **The synaptic ribbon is critical for sound encoding at high rates and with temporal precision**
Philippe Jean, David Lopez de la Morena, Susann Michanski, Lina Maria Jaime Tóbon, Rituparna Chakrabarti, Maria Magdalena Picher, Jakob Neef, SangYong Jung, Mehmet Gültas, Stephan Maxeiner, Andreas Neef, Carolin Wichmann, Nicola Strenzke, Chad Grabner, Tobias Moser*
- 17:00 O-55 **Impaired sound encoding in PSD-95 knockout mice**
Gulnara Yamanbaeva, Sangyong Jung, Man Ho Wong, Nicola Strenzke*
- 17:15 O-56 **The role of IGF1-related pathways in the aging of the ear**
Desislava Skerleva, Hiroe Ohnishi, Tomoko Kita, Tatsuya Katsuno, Stefan Stoyanov, Norio Yamamoto, Juichi Ito, Koichi Omori, Takayuki Nakagawa*
- 17:30 O-57 **Age-related changes in cochlear nuclei microglia and macrophages in the rat**
Paola Perin, Roberto Pizzala*
- 17:45 O-58 **Subgroups of meniere's patients with different pathomorphological and clinical traits as identified by temporal bone MR-imaging**
Andreas Eckhard, David Bächinger, Catrin Brühlmann, Tim Honegger, Vincent Wettstein, Bernhard Schuknecht, Alexander Huber, Arianne Monge Naldi*
- 18:00 O-59 **The effects of vestibular galvanic stimulation improving balance control of patients with myelopathy**
Ludimila Labanca, Tatiana R Silva, Fabrice Giraudet, Paul Avan, Denise U Gonçalves*
- 18:30 Bus to Herrenhausen Palace
- 19:00 Congress Dinner at Herrenhausen Palace

Symposium

Interventions in the ear: From inner ear biology to the therapy of hearing loss

- 08:00 **Welcome**
- 08:30 S-1 **Cochlear gene therapy for Usher III**
Lawrence Lustig
- 09:00 S-2 **Direct cortical effects of deafness genes: an insight into the contrasted results of hearing restoration**
Christine Petit
- 09:30 S-3 **The microanatomy and ultrastructure of the human cochlea – functional aspects with special reference to cochlear implantation**
Helge Rask-Andersen
- 10:00 S-4 **Temporary neurotrophic drug delivery to the inner ear for long-term effects**
Dyan Ramekers
- 10:30 Coffee Break
- 11:00 S-5 **Translating hearing health discoveries: challenges and opportunities**
Anne Schilder
- 11:30 S-6 **Use of single cell gene expression data for reconstruction of spatial and temporal aspects of the developing, mature, and regenerating inner ear**
Stefan Heller
- 12:00 S-7 **Developing molecular therapeutics for human inner ear disease**
Hinrich Staecker

- 12:30 S-8 **Pendrin and inner ear development**
Philine Wangemann
- 13:00 Lunch Break with packed lunches
- 14:00 S-9 **The use of electroporation therapy to improve the electro-neural interface of the cochlear implant**
Jim Patrick
- 14:30 S-10 **Multiple uses with potential clinical significance**
Douglas Fitzpatrick
- 15:00 S-11 **Hearing devices bypassing the middle ear, stimulating the cochlea acoustically; a classification for patients with conductive hearing impairment**
Ad Snik
- 15:30 Coffee Break
- 16:00 S-12 **Biomechanical characteristics of hearing**
Alex Huber
- 16:30 S-13 **Restoring hearing with a new auditory midbrain implant**
Hubert Lim
- 17:00 S-14 **Optogenetic studies of evoked potentials and behavior using a novel auditory brainstem implant based on light**
Daniel Lee
- 17:30 Farewell



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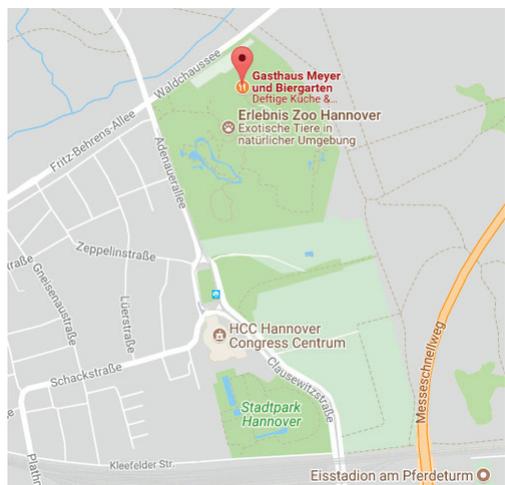
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Congress Dinner will be on Friday, September 15th, 2017 at Herrenhausen Palace.

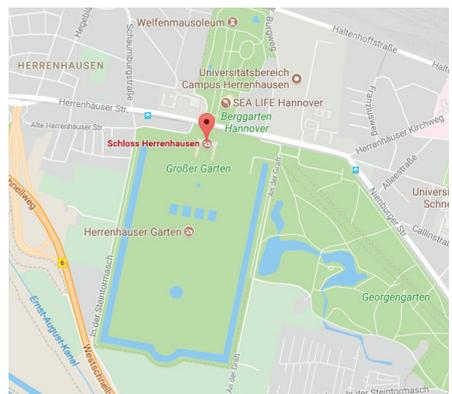
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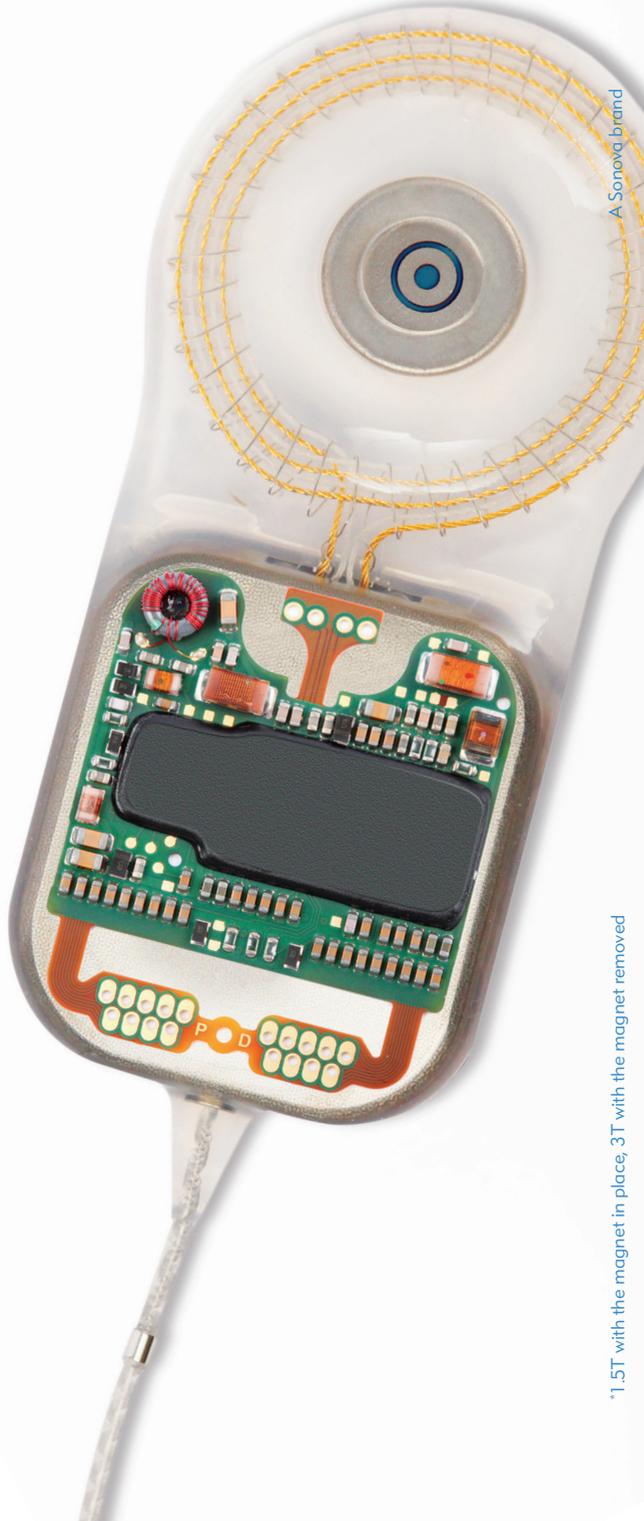
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Abstracts

O-1 Optical cochlea implants

Claus-Peter Richter*Northwestern University*

Photonic stimulation has been proposed for neural stimulation in cochlear implants. Two general approaches have been explored, the optogenetic approach and stimulation with infrared light, also called infrared neural stimulation (INS). Increased spatial selectivity with optical stimulation can result in more independent channels to transfer information to auditory neurons. To translate the idea to a clinical feasible device, several constraints of optical stimulation must be addressed.

The power required for optical stimulation is larger than that for electrical stimulation, a factor of about 10 for optogenetics and a factor of about 100 for INS. This requires the careful configuration of current sources that control the light sources for optical stimulation. Power consumption can be minimized by optimally selecting and placing the optical sources or by positioning the light delivery system (LDS). Distance and orientation of the LDS is critical to target the neurons. Another important aspect in designing the LDS is its size and physical property. It can be an array of miniature light sources, a bundle of optical fibers, or a bundle of waveguides. Likewise, a combined opto-electrical hybrid approach must be considered. Both, optogenetics and INS are limited by the pulse repetition rate. For INS, the rate-limiting factor is the heat deposition. With each radiation pulse heat is delivered to the tissue and can lead to a neat heating with subsequent tissue damage. At present, sustained pulse repetition rates for optical stimulation are typically below 250 pulses per second (pps). A novel coding strategy must be implemented that accommodates low average pulse repetition rates without giving up the performance with contemporary coding strategies.

The talk will list and discuss in detail some of the parameters required for a safe human optical cochlear implant prototype.

Funded with federal funds from the NIDCD, R01 DC011855.

O-2 Towards the optical cochlear implant: optogenetic stimulation of the auditory nerve

Tobias Moser (for the Göttingen Cochlear Optogenetics Program)

Institute for Auditory Neuroscience, University of Göttingen Medical Center, Göttingen, Germany

Auditory Neuroscience Group, German Primate Center, Göttingen, Germany

Auditory Neuroscience Group, Max-Planck-Institute of Experimental Medicine, Göttingen, Germany

When hearing fails, speech comprehension can be restored by auditory prostheses. However, sound coding with current prostheses, based on electrical stimulation of auditory neurons, has limited frequency resolution due to broad current spread. We aim to improve frequency and intensity resolution of cochlear implant coding by establishing spatially confined optical stimulation of spiral ganglion neurons (SGNs). We have established optogenetic stimulation of the auditory pathway in rodents using virus-mediated expression of channelrhodopsins to render SGNs light-sensitive. Optogenetic stimulation of spiral ganglion neurons activated the auditory pathway, as demonstrated by recordings of single neuron and neuronal population responses at various stages of the auditory system. Fast opsins enabled SGN firing at near physiological rates (hundreds per second). We approximated the spatial spread of cochlear excitation by recording local field potentials in the inferior colliculus in response to suprathreshold optical and electrical stimuli, which suggested a better frequency resolution for optogenetic than for electrical stimulation. Towards characterizing the percept induced by cochlear optogenetics we studied activation of neurons in primary auditory cortex and performed a behavioral response in virus-injected gerbils. Behavioral thresholds of light amplitude were found to be below physiological thresholds ($< 2\text{mW}$, close to the threshold of the neurons in auditory cortex) and thresholds of light pulse duration were as short as 0.1ms . This study demonstrates that stimulation of channelrhodopsin-expressing spiral ganglion neurons with blue light creates both a stable physiological response and a robust auditory percept over several weeks. In summary, optogenetic stimulation of the auditory nerve is feasible and bears substantial potential for future application in research and hearing restoration.

O-3 Intracochlear pressure generated by pulsed laser emission

Peter Baumhoff^{1,*}, Nicole Kallweit², Andrej Kral¹

1 Hannover Medical School

2 Laserzentrum Hannover e.V.

** Presenting author*

Intracochlear stimulation by pulsed laser emission has been shown to evoke responses in hearing cochleae. The stimulation mechanism is hypothesized to be based on an optoacoustic effect. To test this hypothesis we characterized laser induced intracochlear responses by a combination of neuronal and pressure recordings in-vivo.

We recorded cochlear neural responses to pulsed laser stimulation in 23 anaesthetized guinea pigs. The hearing condition was quantified via auditory brainstem responses (ABRs) and compound action potentials (CAPs). A tunable optical parametric oscillator (Ekspla NT342A) and an infrared neural stimulator (Capella R-1850) were used for optical stimulation in the Scala tympani. During laser stimulation, multi-unit activity (MUA) was recorded along the tonotopic axis of the Inferior colliculus (IC) and was compared to pressure changes recorded at the cartilaginous external meatus.

Strong MUA evoked by intracochlear laser emission was observed in IC units with best frequencies (BFs) between 5 kHz and 10 kHz, irrespective of laser wavelengths. However, the response strengths were highest for pulses in the near-infrared range (NIR). BF regions with the lowest acoustic response thresholds typically had also the lowest thresholds for laser stimulation. The pressure recordings at the external meatus pointed to intracochlear peak pressure levels near 2 Pa (~100 dB SPL). Maximum levels and spectral content corresponded well to the MUA response characteristics. Deafening completely eliminated the laser responses.

Our results indicate that intra-cochlear laser stimulation generates a substantial pressure wave in the cochlea induced by fast pressure changes. Thus, the observed neuronal responses could be explained by an optoacoustic stimulation mechanism. Together with a dominant activation below 10 kHz, our results can be taken as proof-of-principle for potential applications of optoacoustic NIR laser technology in implantable hearing aids.

Supported by DFG (EXC 1077), EU ACTION (FP7) and MedEl Company, Innsbruck, Austria

O-4 A model based sound coding strategy for laser stimulation in cochlear implant users with residual hearing

Waldo Nogueira, Benjamin Krüger

Hannover Medical School, Cluster of Excellence "Hearing4all", Hannover, Germany

Introduction

Electric acoustic stimulation (EAS) provides large improvements in speech understanding when compared to electric stimulation alone. However, some EAS users do not use acoustic amplification through a hearing aid because of comfort issues. A way to circumvent this problem is to substitute the hearing aid by intra-cochlear acoustic amplification through laser stimulation, also known as optoacoustic stimulation (OAS). Studies have demonstrated that pulsatile laser stimulation can elicit a pulse train of sound pressure waves. Hence, this principle can be used to transmit the speech envelope through amplitude modulation of laser pulse trains. However, the acoustic pulses generated by intra-cochlear laser stimulation are not ideal and this may impact the efficiency of this technique to transmit high quality speech sounds. The purpose of this study is to model these laser generated acoustic pulse trains and assess whether these pulses compromise speech intelligibility and quality of a sound coding strategy for laser stimulation in EAS users.

Method

Five MED-EL Flex20 electrode users with ipsilateral residual hearing in the low frequencies participated in the study. Speech intelligibility and quality was compared between a conventional and a laser based sound coding strategy, termed EOAS. Note that the EOAS strategy delivers acoustic stimulation and no laser light was delivered to the cochlea. The EOAS sound coding strategy splits the audio signal into a high pass filtered and a low pass filtered component. The high pass filtered signal is transmitted directly to the recipient's cochlear implant speech processor. In conventional EAS, the low pass filtered signal is amplified by a hearing aid. In the EOAS strategy, the low pass filtered signal was processed by two models of pulsatile laser stimulation generating acoustic pulse train. The first model (model 1) resembles an ideal acoustic pulse train and the second model (model 2) is based on an in vitro measurement of real laser stimulation in a human temporal bone. These models account for different distortions potentially introduced by laser stimulation. The conventional EAS and the two model based sound coding strategies were compared in speech intelligibility, based on the adaptive OLSA speech test, and sound quality (naturalness, pitch and clarity of speech and music), based on a subjective questionnaire.

Results and Conclusions

Subjects with relatively good residual hearing obtained a clear benefit (5.58 dB in speech reception threshold; SRT) from the hearing aid with respect to the electric stimulation alone. The same benefit was observed for the EOAS strategy, 5.67 dB SRT and 5.94 dB SRT for model 1 and model 2 respectively. No significant differences in speech and music quality ratings were found between all sound coding strategies. These findings suggest that intra-cochlear laser stimulation has the potential to be used in future EAS devices to substitute the conventional hearing aid.

This work was supported by the DFG Cluster of Excellence EXC 1077/1 "Hearing4all" and the FP-7 EU-ACTION project.

O-5 The optoacoustic stimulation of the peripheral hearing organ**Gentiana I. Wenzel^{1,*}***¹ University of Saarland*** Presenting author*

The hearing performance with conventional hearing aids and cochlear implants is dramatically reduced in noisy environments and for sounds more complex than speech e. g. music, partially due to the lack of localized sensorineural activation across different frequency regions with these devices as well as due to poor sound quality, acoustic feedback, occlusion effect and chronic inflammation of the outer ear canal. Light is a source of energy that can be very exactly focused thus offering perspectives for optimal activation of the auditory system. Depending on the laser wavelength, pulse duration, and intensity, it is possible to induce a brief and localized thermal expansion of tissue that results in an acoustic transient within the so-called stress confinement regime, the optoacoustic effect. Studies investigating the optoacoustic stimulation of sectors along the peripheral auditory pathway from the cochlea to the tympanic membrane will be presented. Pulsed laser irradiation induces optoacoustic waves within the cochlea and localized vibrations of the basilar membrane in close proximity to the optoacoustic wave's source. The vibration magnitude can be modulated by adjusting the laser pulse energy. Laser light applied at the tympanic membrane (TM) induces vibrations that activate the hearing system as well. To develop a useful auditory prosthesis, controlled modulation of the incoming optical signal for a precise activation of the peripheral hearing organ is mandatory. Using a novel optical pulse amplitude modulation strategy, the frequency specific activation of the peripheral hearing system was possible. Overall, the data demonstrates that the peripheral hearing organ can be effectively and consistently activated through optoacoustic stimulation. Further studies are however needed before a new generation of optoacoustic-based auditory prostheses can enter clinical studies.

O-6 Optogenetic stimulation on adult cochlea of guinea pig**Ning Yu¹, Chen Liu^{1,*}, Qing-qing Jiang¹, Da-xiong Ding², Shi-ming Yang¹, Ping LV², Shu Fang²***1 Institute of Otorhinolaryngology Head Neck Surgery, Chinese PLA General Hospital**2 Department of Otolaryngology, Affiliated Hospital of North Sichuan Medical College*** Presenting author*

It is reported that the optogenetic stimulation can induced reaction on the transgenic mice, SGNs and outer hair cells. Optical stimulation is proposed as an alternative to electrical stimulation. Hence, in there, we designed a method based on optogenetics to stimulate SGNs with 470nm blue laser on the adult guinea pig and cultured basilar membrane by using virus - mediated expression of the channelrhodopsin 2 (ChR2). The expression of ChR2 in the cochlea of Guinea pigs and basilar membrane were verified by immunohistochemistry and RT-PCR. One fiber laser was used to stimulate ChR2-expressing SGNs through the round window. The light induced reactions were recorded by using patch clamp in vitro and TDT auditory workstation. The Compound Action Potentials (CAPs) irritated by 470 nm blue light showed non-liner characteristic clearly in vivo. ChR2 can be transfected into adult animal cochlea, which may show the possibility of the optical cochlea stimulation in the future.

*Keywords**Optogenetics; Cochlea; Spiral ganglion neurons; Tissue culture; Virus transfection; Compound Action Potential (CAP)*

O-7 Cochlear optogenetics drives avoidance behavior in normal hearing and deaf gerbils

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3 Institute for Auditory Neuroscience, University Medical Center Göttingen; Auditory Neuroscience and Optogenetics, German Primate Center, Göttingen

4 Institute for Auditory Neuroscience, Collaborative Research Center 889, Auditory Neuroscience and Optogenetics, German Primate Center; Göttingen

** Presenting author*

Electrical stimulation of spiral ganglion neurons (SGNs) via cochlear implants (CI) represents the state of the art means of hearing restoration in profoundly hearing impaired, enabling speech recognition in most users. However, the electric current spreads in the saline environment of the cochlea, which in turn leads to activation of large subsets of SGNs, limiting the number of independently usable stimulation channels. This inherent limitation of CIs could be overcome by using optogenetic stimulation. Here, SGNs are genetically modified to express channelrhodopsin2 and subsequently stimulated with light. Focusing light to activate small subsets of SGNs would then allow to increase frequency resolution of artificial sound encoding.

Here, channelrhodopsin-2 variant CatCh was injected into the spiral ganglion of adult gerbils, leading to light sensitivity of SGNs and an optical fiber was implanted into the cochlea. Using a shuttlebox paradigm animals were trained on a detection task in which they learned to avoid mild electrodermal stimuli to the feet upon perception of a stimulus (blue laser pulse delivered through the optical fiber) via locomotion. A different set of animals was trained acoustically and deafened afterwards in which we restored avoidance behavior cued by optogenetic stimulation but not acoustic stimulation.

We demonstrate that optogenetic stimulation of SGNs drives avoidance behavior with thresholds as low as 2.5 mW (for 1 ms pulses) and 0.1 ms (at 25 mW) in normal hearing animals. Furthermore, optogenetic stimulation could restore avoidance behavior in acoustically pre-trained animals in a gerbil model of ototoxic deafness. Finally, avoidance behavior that was trained using optogenetic stimulation was transferred to acoustic stimulation and vice versa, suggesting similarity of percepts.

In conclusion, this study demonstrates that stimulation of channelrhodopsin-expressing SGNs with blue light creates a percept strong enough to cue behavior in both normal hearing and deaf animals at least for several weeks.

O-8 Conditional Sox2 deletion defines residual inner ear development

Martina Dvorakova^{1,*}, Romana Bohuslavova², Bernd Fritzsich³, Israt Jahan³, Tetyana Chumak⁴, Josef Syka⁴, Gabriela Pavlinkova²

1 Institute of Biotechnology, CAS; Faculty of Science, Charles University, Czechia

2 Institute of Biotechnology, CAS, Czechia

3 University of Iowa, Iowa, USA

4 Institute of Experimental Medicine, CAS, Czechia

** Presenting author*

During inner ear development, transcription factor Sox2 is expressed in neurosensory precursors of the neurosensory domain. These precursors will later develop as main functional cells of the inner ear. These functional cells are hair cells, supporting cells and neurons. The expression pattern of Sox2 indicates its role in the specification of all neurosensory cells.

To unravel the precise function of Sox2 and its interactions with other factors during inner ear development, we used Cre recombinase system under the control of Islet1 gene to conditionally delete Sox2 gene. By Is1-Cre system we achieved delayed and incomplete deletion of Sox2 in neurosensory cells. The inner ear was analyzed in several developmental stages by immunohistochemistry and electron microscopy. We labelled the main structures such as supporting cells, hair cells and neurons by specific antibodies. The overall morphology of the inner ear was reconstructed as a 3D structure.

Hemizygous Sox2 CKO mice did not show any abnormalities. However, Sox2 CKO mutant mice did not survive postnatally and the inner ear morphology was severely affected. Decreased Sox2 expression was obvious in Sox2 CKO inner ear. All regions bearing sensory epithelium were reduced or completely absent and had a variable number of developing hair cells and supporting cells. Early forming neurons were developed normally, whereas late differentiating neurons did not form in delayed deletion of Sox2 by Is1-Cre. Additionally, formed neurons subsequently died by apoptosis due to missing target hair cells.

Our results demonstrate the necessity of Sox2 for the proper sensory development and indicate that Sox2 is necessary not only for the formation, but also for the survival of neurons in the inner ear. In addition, these results open questions for subsequent studies based on Sox2 impairment in the inner ear.

O-9 Molecular characterization and prospective isolation of human fetal inner ear prosensory domain progenitor cells

Marta Roccio^{1,*}, Michael Perny¹, Megan Ealy², Hans Ruedi Widmer¹, Stefan Heller³, Pascal Senn⁴

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⁴ University of Bern & University hospital Geneva

* Presenting author

Mechanosensitive hair cells located in the cochlea are essential for the detection of sound. Their loss is irreversible in humans and a major cause of permanent hearing loss. Unraveling the mechanisms of human inner ear development and hair cell specification might enable establishment of novel therapeutic directions such as the development of human cell-based assays for screening of otoprotective and otoregenerative compounds.

Hair cells occur in sensory epithelia that are derived from prosensory domains. Our current knowledge of the developmental mechanisms and timing leading to hair cell formation relies on molecular studies performed in mice.

Here we have analyzed the development of the human fetal inner ear between week 8 and week 12 postconception, when hair cells become specified. We have analyzed gene and protein expression of the developing cochlear duct, spiral ganglion and utricle.

In addition, 3D organoid culture methods have been developed for expansion of flow-cytometrically sorted EPCAM+ human cochlear duct cells. Organoids conserve protein expression and localization typical of their *in vivo* counterparts, namely epithelial marker expression (CD49F, EPCAM, ECadherin, b-Catenin), apical basal polarity (ZO-1), cochlear duct markers (SOX9) and prosensory markers (SOX2; CD271), and can be induced to differentiate to hair cells expressing the markers MYO7A and apical hair bundles.

Importantly, we have identified a surface marker combination, based on the co-expression of EPCAM and the neurotrophin receptor CD271 that can be used for isolation of prosensory hair cell progenitors. The organoids derived from the EPCAM+/CD271+ population differentiate with high efficiency to MYO7A+ hair cells displaying apical F-actin-rich bundles, in contrast with the lack of hair cell differentiation from the remaining cochlear duct population (EPCAM+/CD271-).

Our data provides beyond state-of-the-art insight into the development of the human cochlea with particular emphasis on new methods for selection and expansion of inner ear /hair cell progenitors *in vitro*.

O-10 How impaired SGN axon bifurcation affects sound processing in the auditory brainstem**Steffen Wolter^{1,*}, Dorit Möhrle¹, Dennis Zelle¹, Marlies Knipper¹, Peter Pilz¹, Hannes Schmidt¹, Lukas Rüttiger¹**¹ University of Tübingen

* Presenting author

cGMP signaling triggered by the binding of C-type natriuretic peptide (CNP) to its receptor guanylyl cyclase B (GC-B; Npr2) has been linked by genetic evidence to a remarkable variety of physiological functions like skeletal bone growth, female fertility, cardiac growth, fat metabolism and gastrointestinal function. For the nervous system it has been recently demonstrated that the CNP/GCB/ cGMP/cGMP-dependent protein kinase type I (cGKI) signaling pathway is essential for sensory axon branching at the dorsal root entry zone of the spinal cord during embryonic development [1]. Also auditory nerve fibers (ANF) - that differ in their discharge rate and sound sensitivity - bifurcate at the border of the embryonic hindbrain and consequently extend daughter branches that finally innervate the anteroventral, posteroventral, and dorsal cochlear nuclei (aVCN, pVCN, DCN). The absence of GC-B impairs axonal bifurcation and results in a blurred tonotopic organization of central auditory circuits in mice. [2]. Here, we describe the hearing function of adult GC-B knockout mice and their wildtype littermates in detail, using evoked auditory brainstem response (ABR), distortion product otoacoustic emission (DPOAE) and auditory steady state response (ASSR). Histological correlates of the auditory phenotype were verified by applying immunohistochemistry on fixed sections of cochlea and high-resolution fluorescence microscopy. We discuss the results in the context of previous findings.

O-11 Deciphering gene expression dynamics of vestibular hair-bundle morphogenesis from single-cell snapshot data using CellTrail maps**Daniel C. Ellwanger^{1,*}, Mirko Scheibinger¹, Matthew R. Avenarius², Rachel A. Dumont², Peter G. Barr-Gillespie², Stefan Heller¹***1 Stanford University School of Medicine, Stanford, CA 94305, USA**2 Oregon Health & Science University, Portland, Oregon 97239, USA*** Presenting author*

Key players in the function of the inner ear are cohorts of mechanosensing organelles, called stereociliary bundles, protruding from the apical surface of sensory hair cells. Bundle growth and maturation involves an orchestration of distinct sequential and overlapping cellular processes whose temporal program of gene expression remains to be elucidated. The recent advance of high-throughput single-cell technologies facilitates the generation of -omic readouts from thousands of samples captured at a wide range of cellular maturation stages during tissue development at high resolution. However, valuable cell-specific temporal and spatial information cannot be observed or preserved during sample preparation, and remains hidden in high dimensional cellular expression profiles. In this work, we present CellTrail, a novel unsupervised algorithm for the de novo chronological ordering, visualization and analysis of single-cell expression data obtained from a single snapshot of a developing tissue. We applied CellTrail to a 183-dimensional RT-qPCR gene expression panel of 1,008 cells collected from the developing utricle of the chicken inner ear to elucidate the expression dynamics during bundle growth. CellTrail identified two sensory cell subtype-associated trajectories towards hair bundle formation, composed of transient and quiescent cellular states. Examination of mature functional cellular features verified the predicted cell separation and ordering. Leveraging trajectory information enabled us, for the first time, to infer and compare expression dynamics during maturation of spatially distinct vestibular hair cell types. Here, we discovered an unexpected potentially novel hair cell type II subtype located in the extrastriola. In situ hybridizations and immunohistochemistry combined with quantitative measurements of bundle lengths validated the identified spatial and temporal expression patterns on mRNA and protein level, respectively. We demonstrate CellTrail's generalization to single-cell RNA-Seq data by analyzing measurements from the neonatal mouse utricle and performed a comparative assessment to show the superiority of our algorithm to recent trajectory reconstruction approaches.

O-12 Pluripotent stem cells in disease modelling of GJB2 related hearing loss**Ichiro Fukunaga¹, Ayumi Fujimoto¹, Kaori Hatakeyama¹, Osamu Minowa², Katsuhisa Ikeda¹, Kazusaku Kamiya^{1,*}**¹ Juntendo University² RIKEN

* Presenting author

Mutation of the Gap Junction Beta 2 gene (GJB2) is the most frequent cause of hereditary deafness worldwide and accounts for up to 50% of non-syndromic sensorineural hearing loss cases in some populations. GJB2 encodes connexin (CX) 26, a component in cochlear gap junction. We have demonstrated that the drastic disruption of gap junction plaque (GJP) macromolecular complex composed of CX26 and CX30 are critical pathogenesis starting before hearing onset (Kamiya, J Clin Invest, 124(4):1598–1607, 2014). Therefore, cochlear CX26-gap junction plaque (GJP)-forming cells such as cochlear supporting cells are thought to be the most important therapeutic target for the treatment of hereditary deafness. The differentiation of pluripotent stem cells such as induced pluripotent stem (iPS) cells into cochlear CX26-GJP-forming cells had not been reported. To develop the effective therapy for GJB2 associated hearing loss, restoration of GJP macromolecular complex using iPS cells are expected to rescue the hearing function of GJB2 related hearing loss.

In this study, we developed a novel strategy to differentiate induced pluripotent stem cells into functional CX26-GJP-forming cells that exhibit spontaneous ATP- and hemichannel-mediated Ca²⁺ transients typical of the developing cochlea. Furthermore, these cells from CX26-deficient mice recapitulated the drastic disruption of GJPs, the primary pathology of GJB2-related hearing loss (Fukunaga, Stem Cell Reports, 7(6), 1023-1036, 2016). These in vitro models should be useful for establishing inner-ear cell therapies and drug screening that target GJB2-related hearing loss.

O-13 Trends in genetic diagnostics of hereditary hearing loss**Ronald Pennings^{1,*}, Celia Zazo Seco¹, Mieke Wesdorp¹, Ilse Feenstra¹, Hannie Kremer¹, Lies Hoefsloot¹, Margit Schraders¹, Helger Yntema¹**¹ Radboudumc

* Presenting author

Introduction

Over the past decades, many deafness genes have been identified to cause hereditary hearing impairment (HI). It therefore, has become possible to screen for these genes in the out-patient clinic. The importance of genetic screening of HI is that patients can be counseled about the cause and prognosis of their hearing loss and effects of rehabilitation. Hearing impairment is genetically heterogeneous and testing of several single HI-related genes is laborious and expensive. This study evaluates the diagnostic utility of whole exome sequencing (WES) targeting a panel of HI-related genes.

Methods

Two hundred index patients, mostly of Dutch origin, with presumed hereditary HI underwent WES followed by targeted analysis of an HI gene panel of approximately 100 genes. 206 additional patients underwent single gene testing guided by phenotype analyses.

Results

We found causative variants underlying the HI in 67 of 200 patients (33.5%). Eight of these patients have a large homozygous deletion involving a known HI gene, which could only be identified by copy number variation detection. Variants of uncertain significance were found in 11 patients (5.5%). In the remaining 122 cases no potentially causative variants were detected (61%). The diagnostic yield of single gene testing in the 206 additional patients was 7.6%.

Conclusion

The diagnostic yield for HI using WES targeting a HI gene panel is higher (33.5%) than targeted sequencing of single genes (7.6%). In our patient cohort, causative variants in GJB2, USH2A, MYO15A, STRC, and in MYO6 were the leading causes for autosomal recessive and dominant HI, respectively. Segregation analysis of variants of uncertain significance will further increase the diagnostic yield of WES. A practical workflow for genetic testing of hereditary HI for screening in the out-patient clinic will be presented.

O-14 FRMPD4 is associated with X-linked non-syndromic hearing loss

Barbara Vona^{1,*}, Daniel Liedtke¹, Kristen Rak², Radoslaw Katana³, Lukas Jürgens⁴, Pingkalai R Senthilan⁵, Indrajit Nanda¹, Cordula Neuner¹, Michaela AH Hofrichter¹, Linda Schnapp¹, Jörg Schröder¹, Ulrich Zechner⁶, Stefan Herms⁷, Per Hoffmann⁷, Tobias Müller⁸, Marcus Dittrich¹, Oliver Bartsch⁶, Peter M Krawitz⁹, Eva Klopocki¹, Wafaa Shehata-Dieler², Martin C Göpfert³, Thomas Haaf¹

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2 Comprehensive Hearing Center, Department of Otorhinolaryngology, University Hospitals, Würzburg, Germany

3 Department of Cellular Neurobiology, University of Göttingen, Göttingen, Germany

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8 Department of Bioinformatics, Julius Maximilians University, Würzburg, Germany

9 Institute for Medical and Human Genetics, Charité Universitätsmedizin Berlin, Berlin, Germany

** Presenting author*

Although many genes have been implicated in non-syndromic hearing loss (NSHL), diagnostic rates of approximately 50% among patients suggest many genes are pending identification. NSHL is the most common sensory deficit that demonstrates classic genetic heterogeneity with roughly 1% of coding genes in the genome anticipated to be involved in NSHL. Autosomal recessive (75-80%), dominant (15-20%), and X-linked (1-4%) forms of NSHL can be observed.

Whole exome sequencing of a German family revealed a novel missense variant in the gene FERM and PDZ domains-containing protein 4 (FRMPD4) on chromosome Xp22.2. This gene was first described as a regulator of dendritic spine morphogenesis. Previous array-based screening among families with X-linked intellectual disability (XLID) showed duplication of Xp22.2, partially including FRMPD4, implicating the gene in XLID. Interestingly, point mutations in FRMPD4 have been associated with XLID, a phenotype not observed in our family.

Mouse expression localizes Frmpd4 to spiral ganglion neuron peripheral dendrites. frmpd4 zebrafish mutants were studied for innervation and structural defects in the otic vesicle and posterior lateral line (PLL) neuromasts. PLL neuromasts are observed with reduced axonal outgrowth that is also reduced in the PLL nerve. Abnormal innervation is also apparent in the otic vesicle. Neuromast labeling marked a reduction of otic vesicle and PLL neuromasts in mutant zebrafish. Scanning electron microscopy revealed an absence of kinocilia in PLL neuromasts of frmpd4^{-/-} zebrafish. Furthermore, mutants show delayed acoustically evoked behavioural responses indicating hearing impairment. Investigation of transgenic Drosophila mutants exhibited a mild auditory phenotype. Our results associate FRMPD4 with X-linked NSHL and suggest mutations are correlated with pleiotropic effects

O-15 Cellular and molecular studies of a critical period for afferent-dependent neuron survival in the cochlear nucleus

Edwin W Rubel

O-16 Restoring cortical inhibition improves perception following early hearing loss

Dan Sanes

O-17 Prediction and monitoring of cochlear implant outcome using functional near infrared spectroscopy

Douglas Hartley

O-18 Effects of early hearing experience on functional activation and connectivity in primary and higher-order cortical field**Prasandhya Yusuf^{1,*}, Peter Hubka¹, Jochen Tillein², Andrej Kral¹**¹ Hannover Medical School² J.W. Goethe University

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Cochlear stimulation activates auditory cortex through thalamic inputs, while the auditory cortical areas subsequently interact via corticocortical connections. These activations are presented in local field potentials (LFPs) in the form of evoked (phase-locked) and induced (non-phased-locked) cortical responses, respectively. Here we investigated an effect of auditory developmental experience on evoked and induced responses in the primary auditory cortex (A1) and posterior auditory field (PAF). We evaluated time-frequency representations (TFR) of bipolar derivation LFPs recorded simultaneously from A1 and PAF in hearing controls (HCs) and congenitally deaf cats (CDCs). Responses elicited by acoustic stimulation in HCs and cochlear implant electric stimulation in HCs and CDCs were compared to investigate the effect of stimulation mode (acoustic vs. electric) and developmental experience (hearing vs. deaf). Evoked and induced TFR power were calculated using the wavelet analysis, while coupling strength between A1 and PAF was estimated using the weighted phase-lag index. Moreover, we checked the directionality of the coupling using the non-parametric reversed Granger testing.

The evoked responses appeared mainly at early latency (<100ms) while induced responses appeared more abundant at long latencies (>100ms), corresponding to their assumed role in thalamocortical vs. corticocortical processing, respectively. In HCs, electric stimulation resulted in reduced induced activity compared to acoustic stimulation, indicating the effect of the stimulation mode on the induced responses. The comparison of electrically elicited responses between HC and CDC showed no significant effect of deafness on A1 evoked responses, but a near loss of A1 and PAF induced responses in CDCs, particularly at longer latencies. Furthermore, the coupling between two recorded fields were decreased in CDCs, potentially in the top-down (PAF-A1) directed-connectivity. This finding supports the concept that developmental hearing experience is essential for integration of thalamocortical and corticocortical activations in the auditory cortex.

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O-19 A rescuable auditory synaptopathy in mice lacking CLARIN-1**Didier Dulon^{1,*}, Samantha Papal², Alice Emptoz², Mateo Cortese², Said Safieddine², Aziz El-Amraoui², Christine Petit²**¹ University of Bordeaux² Institut Pasteur

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Clarin-1, a tetraspan-like membrane protein defective in Usher syndrome type IIIA (USH3a) associating progressive deafness and blindness, is essential for the hair-bundle morphogenesis of auditory hair cells. Here we addressed a possible role of clarin-1 at the inner hair cell (IHC) ribbon synapses by characterizing two *Clrn1* mouse mutants, a constitutive knock-out (*Clrn1^{ex4-/-}*) and a postnatal, hair cell-specific conditional knockout (*Clrn1^{ex4fl/fl}Myo15^{-cre+/-}*) mice. Whereas *Clrn1^{ex4-/-}* mice were profoundly deaf, *Clrn1^{ex4fl/fl}Myo15^{-cre+/-}* mice displayed progressive hearing threshold elevation which develops with normal otoacoustic-emissions and hair bundle morphology, thus indicating an auditory neuropathy. Both mutant IHCs displayed reduced exocytotic Ca²⁺-efficiency due to a spatial disorganization of Ca²⁺ channels, and a loss of afferent dendrites associated with an abnormal enlarged distribution of postsynaptic AMPA-receptors. Protein-protein interactions suggested that clarin-1 interacts with the synaptic Cav1.3 Ca²⁺ channel complex through the Cavβ2 auxiliary subunit and the PDZ domain-containing protein, harmonin (defective in Usher syndrome type 1C). Cochlear gene therapy strategy in newborn mutant mice, through AAV-mediated transfer of the clarin-encoding cDNA into hair cells, prevented the synaptic defects and reduced hearing loss at adult age. Our results reveal clarin-1 as a key organizer of the IHC ribbon synapses, and suggest new perspectives for USH3a patients' hearing treatment.

This work was supported by the Fondation Agir Pour l'Audition.

O-20 Autonomous and evoked Ca²⁺ signals in inner hair cells and coupling to Ca²⁺ waves in the developing mouse cochlea**Tobias Eckrich¹, Kerstin Blum¹, Ivan Milenkovic², Stefan Münkner¹, Jutta Engel^{1,*}**¹ Saarland University² University of Leipzig

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During final differentiation of the cochlea before the onset of hearing, IHCs generate Ca²⁺ action potentials in the absence of sound whereas supporting cells (ISCs) of the great epithelial ridge (Kölliker's organ) generate intercellular ATP-mediated Ca²⁺ waves. There is a debate as to whether Ca²⁺ action potentials of IHCs are independent of Ca²⁺ waves or not. Using the Ca²⁺ indicator Fluo 8-AM and a confocal laser scanning microscope (Zeiss LSM 710) we performed Ca²⁺ imaging in acutely dissected inner ear explants at postnatal day 4-5. Using small fields of view and scan rates of ≥ 30 Hz, we were able to resolve fast Ca²⁺ transients in IHCs simultaneously with the slower Ca²⁺ waves in adjacent supporting cells. Three signal types of IHCs were observed, single fast Ca²⁺ transients (fCaT), minibursts of 2 to 5 fCaTs, and bursts of 6 to ~ 70 fCaTs. Mean length of a fCaT was 860 ms, with a time-to-peak of 16 ms. The overall frequency of fCaTs was 0.32 ± 0.11 Hz, but increased to 4 – 10 Hz within bursts. The occurrence of fCaTs in IHCs critically depended on the presence of extracellular Ca²⁺ and of Cav1.3 Ca²⁺ channels, indicating that fCaTs reflected Ca²⁺ action potentials in IHCs. ATP superfused from the strial side caused both fCaTs in IHCs and Ca²⁺ waves in ISCs, the onset of which was delayed by ~ 0.97 s. In summary, IHCs were able to generate fCaTs and minibursts autonomously without Ca²⁺ wave activity in adjacent ISCs whereas ISC Ca²⁺ waves were very likely to trigger bursts and minibursts in nearby IHCs. The mechanisms behind the Ca²⁺ transients and waves remain to be elucidated.

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O-21 The development and maintenance of hair cell stereocilia rootlets by isoform specific functions of TRIOBP

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Mutations of *TRIOBP*, which encodes several alternative splice isoforms, are associated with human nonsyndromic deafness DFNB28. Recently, a mutation and a SNP only affecting the longest *TRIOBP* isoform (*TRIOBP-5*) were implicated in moderate deafness and age-related hearing loss, respectively. A mouse model that has a simultaneous deficiency for two *Triobp* splice isoforms was engineered by deleting exon 8 (*Triobp* ^{Δ ex8/ Δ ex8}), which encodes sequence present in both *TRIOBP-4* and *TRIOBP-5* isoforms. *Triobp* ^{Δ ex8/ Δ ex8} mouse hair cells fail to develop stereocilia rootlets, which are densely packed F-actin bundles that penetrate the stereocilia core and an apical F-actin meshwork of the hair cell cuticular plate. Moreover, purified *TRIOBP-4* protein can tightly bundle F-actin in vitro, suggesting that the F-actin bundling activity of *TRIOBP-4* is necessary for the in vivo formation of stereocilia rootlets. But the exact function of the *TRIOBP-5* splice isoform in the auditory system is unknown. We examined the localization of *TRIOBP-4* and *TRIOBP-5* proteins and show that *TRIOBP-4* is localized largely to the rootlet portion within the stereocilia core. On the other hand, *TRIOBP-5* is localized exclusively to the rootlet portion within the cuticular plate. To explore the functional difference between the *TRIOBP-4* and *TRIOBP-5* isoforms, we generated a *TRIOBP-5* isoform-specific knockout mouse (*Triobp-5*^{-/-}) by deleting exons 9 and 10, unique to this isoform. Although *Triobp* ^{Δ ex8/ Δ ex8} mice have profound congenital deafness, *Triobp-5*^{-/-} mice show progressive hearing loss, despite the presence of the intact *TRIOBP-4* isoform. SEM analyses of *Triobp-5*^{-/-} reveal degeneration of initially normal appearing stereocilia. Reconstruction of images from serial section TEM shows rootlets in *Triobp-5*^{-/-} hair cells, but they are thin and dysmorphic already at P14-P16 and then disappear with age. Our data argue that the function of *TRIOBP-5* is to reinforce and maintain stereocilia rootlets, while the function of *TRIOBP-4* is crucial for their initial development.

O-22 Angulin proteins ILDR1 and ILDR2 regulate alternative pre-mRNA splicing through binding to splicing factors TRA2A, TRA2B, or SRSF1**Yueyue Liu¹, Hongyun Nie¹, Chengcheng Liu¹, Xiaoyan Zhai¹, Qing Sang², Yanfei Wang¹, Deli Shi¹, Lei Wang², Zhigang Xu^{1,*}***1 Shandong University**2 Fudan University*** Presenting author*

Angulin proteins are a group of evolutionally conserved type I transmembrane proteins that contain an extracellular Ig-like domain. In mammals, three angulin proteins have been identified, namely immunoglobulin-like domain containing receptor 1 (ILDR1), immunoglobulin-like domain containing receptor 2 (ILDR2), and lipolysis-stimulated lipoprotein receptor (LSR). All three proteins have been shown to localize at tight junctions (TJs) and are important for TJ formation. Mutations in ILDR1 gene have been shown to cause non-syndromic hearing loss (NSHL). In the present work, we show that ILDR1 binds to splicing factors TRA2A, TRA2B, and SRSF1, and translocates into the nuclei when the splicing factors are present. Moreover, ILDR1 affects alternative splicing of Tubulin delta 1 (TUBD1), IQ motif containing B1 (IQCB1), and Protocadherin 19 (Pcdh19). Further investigation show that ILDR2, but not LSR, also binds to the splicing factors and regulates alternative splicing. When endogenous ILDR1 and ILDR2 expression is knockdown with siRNAs in cultured cells, alternative splicing of TUBD1 and IQCB1 is affected. In conclusion, we show here that angulin proteins ILDR1 and ILDR2 are involved in alternative pre-mRNA splicing via binding to splicing factors TRA2A, TRA2B, or SRSF1.

O-23 Abnormal actin elongation activity of a novel hearing-loss Dia1 mutant revealed by single-molecule speckle microscopy

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Background

Dia1 belongs to the Formin homology protein family. Our previous live-cell single-molecule speckle (SiMS) microscopy revealed that the FH1-FH2 domains of Dia1 processively elongates actin filaments (Higashida et al., Science, 2004). The activity of FH1-FH2 domain is suppressed by autoinhibitory interaction between the DID and DAD domains in Dia1. Dia1 is encoded by *Diaph1*, and linked to autosomal dominant nonsyndromic hearing loss, DFNA1 in human. Recently, we found a novel *Diaph1* mutation, which is associated with autosomal dominant hearing loss. The mutation (c.3610C>T) alters the 1204th Arginine to a stop codon (R1204X) in the C-terminal DAD domain. To evaluate the effect of this mutation, we observed actin elongation of Dia1 molecules using live-cell single-molecule imaging, and compared the emergence of activated molecules between wild-type (WT), R1204X and M1190D (a constitutively active form).

Method

For transfected *Xenopus laevis* XTC cells expressing a low amount of GFP-tagged Dia1(WT), Dia1(R1204X), and Dia1(M1190D), time-lapse images were acquired every 300msec. Fluorescent speckles showing directional movements for continuous 3 frames were counted as activated molecules. For quantification, the numbers of activated molecules were normalized by fluorescent intensity of cells. Statistical analysis was performed by one-way ANOVA and post-hoc Bonferroni's test.

Results

Few activated molecules were observed in cells expressing GFP-Dia1(WT) (0.29/10 μ m², n = 5). In contrast, activated molecules were frequent in cells expressing GFP-Dia1(R1204X) and GFPDia1(M1190D) (6- and 18-fold increase, normalized by expression levels, p = 0.0288 and p < 0.0001, n = 6). Frequency of activated molecules were also different between R1204X and M1190D (p = 0.0002).

Conclusion

Using SiMS microscopy, we proved that the novel mutant of Dia1, Dia1(R1204X), is constitutively active. Difference between R1204X and M1190D suggests the moderately disrupted autoinhibition of R1204X. Combined with pull-down assays and transgenic mice, we revealed that disrupted autoinhibition is the pathology of this hearing loss.

O-24 On Structure and function in outer hair cells**Einat Shapira^{1,*}, Eitan Kimmel¹, Remy Pujol²**¹ Technion² University of Montpellier

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Questions regarding the identity of the mammalian cochlear amplifier and its mechanism have intrigued scientists for decades. It is common to assume that when sound reaches the inner ear it initially induces vibrations of the basilar membrane (BM); only then, outer hair cells (OHCs) detect and amplify these vibrations. We challenge this notion, claiming that in a passive element like the BM, the induced distortion at low sound pressure levels would be too small compared to the noise and, therefore, cannot be detected or magnified. Moreover, this concept has difficulties in explaining a significant characteristic of mammalian hearing—amplification of high frequencies. Based on the morphology of the mammalian cochlea, and in particular of OHCs, we suggest that sound induced motility of OHCs could result from a synchronized action of hundreds of thousands of actuators in OHC's lateral wall. They were denoted by us as nanometric acoustic motile sensors (NAMSs). We show that a mechanism of stochastic resonance in the NAMSs can account for the major features of mammalian hearing: wide dynamic range, attributed to amplification of low sound pressure level (SPLs) and compressive nonlinearity at higher SPLs; sharp frequency selectivity; generation of spontaneous otoacoustic emissions; and the ability to process relatively high frequencies. Another unique, unexplained feature of mammalian OHCs is that their length (L), which span between $10\ \mu\text{m}$ and $80\ \mu\text{m}$, is inversely correlated with the logarithm of the frequency coding (f) of the cochlea, which varies between 10 Hz and 105 Hz. This is often named “the xylophone”. Surprisingly, we were able to derive from the NAMS model, an explanation for the L - f relationship for the OHCs of all mammals by combining fundamentals of statistical mechanics with the energy balance of a NAMS ensemble in a single OHC.

O-25 Measurement of cochlear partition volume compliance using a microfluidic chamber system**Jong-Hoon Nam, Jessica Huhnke, Daniel Marnell, Jonathan Becker**

The mammalian cochlea is a spiral shaped cavity filled with fluid divided by the cochlear partition. The geometrical and mechanical properties of the cochlear partition vary monotonically along the length of the cochlea. There has been much effort to correlate the mechanical properties of the cochlear partition with cochlear function. Unfortunately, the experimental data of cochlear partition stiffness measured at the middle to the apical turns of the cochlea are rare because of difficulty in accessing those locations. Furthermore, existing stiffness measurements using microprobes suffer ambiguity in interpreting results due to high nonlinearity in force-displacement relationship. We developed a microfluidic chamber system that enabled us to apply calibrated hydrostatic pressures to cochlear partitions isolated from the gerbil cochlea. Deformations of the cochlear partition due to hydrostatic pressures were analyzed through image correlations. Unlike microprobe measurements, the pressure-displacement relationship was highly linear within physiological pressure ranges (< 2 Pa). For locations 8.5 mm from the base of the gerbil cochlea, the compliance at the center of the cochlear partition was 85 nm/Pa. To further analyze the measurement results, we used a 3-D finite element model. Using the model, the compliance due to pressure was converted to a point stiffness of 77 mN/m, and possible experimental uncertainties were analyzed.

Key words: organ of Corti / outer hair cell / mechanics

O-26 New cochlear implant map based on subjective pitch match between acoustic and electrical stimuli in unilateral or highly asymmetric deafness

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Introduction

Previous works reported that interaural mismatch between the conventional frequency bands allocation and real pitch perception may be the cause of discomfort in patients with CI and contralateral residual hearing. The objective of the study was to test the effectiveness of a customized re-allocated map, obtained on the basis of personal interaural pitch sensations, in order to reduce the gap between the two hearing systems and to improve the naturalness of music sounds.

Methods

Four Med-El implanted patients with unilateral or highly asymmetric deafness were asked to find the optimal match between the electric stimulus and the acoustic one, elicited simultaneously in the contralateral ear by means of MultiFrequency audiometry (1/12 octave frequency resolution between each acoustic stimulus). The procedure was repeated for three times in separated sessions. On the basis of individual pitch matching results, the frequency map was individually adapted by manual frequency reallocation using Maestro6.0 software. A music test battery was designed to assess frequency discrimination and melodic recognition. A subjective rating about the new map in specific everyday life settings was also collected through the submission of SSQ Questionnaire, while a global self-assessment of popular songs was measured on the basis of a Visual Analogic Scale (0-10).

Results

The average mismatch reported was 1.75-1.25 octaves lower than the conventional map. Music test results showed the same ability in melody recognition and pitch discrimination in comparison with the conventional one. On the contrary, misalignment correction provided a significant improvement in pleasantness and naturalness of musical message.

Conclusion

Our data suggest that lowering the pitch-place mismatch with frequency range redistribution in a customized map could be a reliable procedure to reduce the discomfort due to signal overlap, restoring the balance between the two hearing systems and improving music perception in CI patients with contralateral residual hearing.

O-27 From the cochlea to the brain: how temporal processing is impacted by infection and training?**Aline Castro^{1*}, Denise Gonçalves¹, Fabrice Giraudet², Luciana Resende¹, Paul Avan²***1 Universidade Federal de Minas Gerais**2 Université d'Auvergne Clermont-Ferrand*** Presenting author*

Infectious diseases are often associated with higher prevalence of hearing loss and hearing complaints. From the cochlea to the brain, the auditory pathway can be affected by viruses and other aggressors. Thus, infections may be related to sensory and neural deficits in the auditory mechanisms. Time processing is the key to understanding speech. It comprises the precision and speed that sounds are perceived in time. This analytical process begins at the cochlea and progressively increases its complexity up to the cortical level. Human HTLV-I T-cell lymphotropic virus is associated with a progressive neurological disease. Previous studies have shown that the response of P300 to the auditory stimulus is altered by this virus. However, it is not yet clear if auditory processing skills are altered by a process failure from the bottom up or if there is a modification modified by top-down mechanisms (such as memory and attention). Considering that both hypotheses are true, HTLV-I could affect auditory discrimination and temporal processing of the cochlea to the cortex. We are conducting a longitudinal cohort study of patients infected with HTLV-I. Our preliminary data showed that the temporal resolution, measured by Gaps in Noise Test (GIN), had lower results than other tests among these patients. P300 latency was also delayed compared to normal controls and literature. Some studies with humans and rats showed a correlation with the potential response related to the event and the performance of temporal resolution. In addition, there is evidence of enhancement of P300 latency and temporal processing due to auditory training. Thus, we believe that the HTLV-I population may benefit from an acoustic stimulation intervention to improve auditory abilities and cognitive function. The future of our research is to test our hypothesis using time discrimination training and the potential of type P3 in the animal model.

O-28 Clinical evidence of binaural hearing in children with unilateral hearing loss**Martijn Agterberg¹, Ad Snik¹, John Van Opstal²***1 Radboudumc Nijmegen**2 Donders Institute for Neuroscience*

Direct cochlear stimulation with a middle-ear implant can theoretically restore binaural hearing in children with unilateral conductive hearing loss. In patients we rely on psychophysical measurements to demonstrate accurate binaural hearing. One of the most accurate methods to demonstrate binaural hearing abilities is a method that investigates sound localization in the horizontal plane (Snik et al., 2015). Unfortunately, sound localization tests differ remarkably from clinic to clinic, ranging from setups with speakers positioned 60 degrees apart (Lovett et al. 2009), 45 degrees apart (Schoonhoven et al., 2016), 30 degrees apart (Heyning et al., 2016; Bosman et al., 2011; Priwin et al., 2007), 15 degrees apart (Van Deun et al., 2010), 10 degrees apart (Grieco-calub and Litovsky, 2012), to setups where stimuli can be presented from every possible position (Agterberg et al., 2011; Kuhnle et al., 2013). Furthermore, the setups differ in the stimulus duration, pointing method, bandwidth of the stimuli, visibility of the speakers and amount of roving of the sound level. These differences result in conflicting outcomes and in results that are difficult to compare. The present study investigates binaural hearing (i.e. the accurate neural processing at the level of the brainstem) in patients with middle ear implants. The proposed psychophysical tests are built in a mobile lab, so the same method can be applied when children are tested in different clinics.

O-29 The decision between direct acoustical cochlear stimulation and cochlear implantation: A retrospective analysis of results**Eugen Kludt^{1,*}, Andreas Büchner¹, Thomas Lenarz¹, Hannes Maier¹***1 Hannover Medical School*** Presenting author*

The new implantable hearing system Codacs™ was designed to close the treatment gap between active middle ear implants and cochlear implants in cases of severe-to-profound mixed hearing loss. We compare the outcome of patients with the Codacs device to patients which had a cochlear reserve within the current Codacs indication criteria but received a CI due to no alternative treatment at the time of implantation. In a retrospective study, we compared the clinical outcome of 66 patients with the Codacs implant to 54 CI patients with comparable pre-operative bone conduction (BC) thresholds that were potential candidates for both categories of devices. Word recognition scores (WRS) were determined using the Freiburg monosyllables in quiet at 65 dB SPL speech level and speech intelligibility in noise with the HSM sentences test at 65 dB SPL (+10 dB SNR). The gross average of WRS outcome in CI patients was 60% and was not dependent on the preoperative BC thresholds. Codacs patients with a BC PTA better than 60 dB HL had a significantly better WRS of 80 % mean. In Codacs patients with a BC PTA worse than 60 dB, WRS score was not significantly different from the CI patient group. The average score in the HSM sentence test at +10 dB SNR was 79% in the whole Codacs cohort. Even the performance of Codacs patient subgroup with BC thresholds worse than 69 dB HL was on average 54% significantly better than the speech intelligibility in noise of the entire CI patient cohort: 30% mean. Our results indicate for patients with sufficient cochlear reserve that speech intelligibility in noise with the Codacs™ hearing implant is significantly better than with a CI. In quiet, the advantage of acoustical amplification was significant only for patients with pre-operative BC PTA above 60 dB HL.

O-30 Spiral ganglion neurons cultured on advanced micro-electrode arrays**Damir Kovacic^{1,*}, Viktorija Radotic², Dries Braeken³***1 Faculty of Science, University of Split**2 University of Split, School of Medicine**3 imec*** Presenting author*

One of the strategies to improve the cochlear implant technology is to increase the selectivity of the electrical stimulation of the auditory nerve. We are proposing a novel neuro-electronic interface based on micro-nail-shaped electrode array embedded on silicon-based integrated circuits. Herein we test the in vitro interaction of these substrates with rat spiral ganglion neurons (SGN) [1]. Silicon substrates with micro-nails of different dimensions and spacing were fabricated using standard CMOS-based post-processing [2]. SGN were extracted from P5 rat pups and cultured in vitro on poly-L-ornithine coated silicon substrates in Neurobasal-A with B27 and GDNF. Glass coverslips were used as controls. Cell cultures were fixed and stained with Tuj1 (neurons) and DAPI (cell nuclei). Tuj1+SGNs grew successfully on micro-electrode arrays, as on control surfaces (139 ± 43 and 181 ± 53 neurons respectively). The micro-nails allowed excellent neurite outgrowth and induced intimate interactions between cell and silicon. After 7 days in vitro, 21% of SGN on micro-electrode arrays showed neurites longer than $100 \mu\text{m}$, similarly as SGN on control surfaces (29%). Nail shaped electrodes also promoted axonal guidance, as proved previously with other types of neurons [3] Neurites were oriented preferentially along 30° , 90° or 60° in nail structures with spacing between $1 \mu\text{m}$ and $2.4 \mu\text{m}$, following the underlying geometry of the silicon surface. Micro-nails support in vitro SGN growth and interaction with neurite outgrowth. Moreover, the neuronal outgrowth followed geometrical features of the surface, indicating these engineered surfaces could be used for directed neuronal growth and differentiation. Altogether, these results indicate micro-electrode arrays are a promising technology for future auditory neuro-electronic interfaces.

O-31 Future directions for cochlear implantation – restoring the spiral ganglion population with stem cells**Leila Abbas^{1,*}, Daniela Cacciabue-Rivolta¹, Daniel Smyth², Wolfram Dueck², Marcelo Rivolta¹**¹ University of Sheffield² Cochlear Technology Centre

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The cochlear implant has been seen as the success story of neural prostheses, restoring hearing to many for whom this sense was absent. However, in order for a favourable outcome to be achieved, the patient must have at least the semblance of a functioning auditory nerve, in order to 'connect' the inner ear to the brain – sadly for many people, this is not currently a therapeutic option.

A gerbil model has been developed with a two-pronged sensorineural hearing loss – auditory neuropathy is induced with topical ouabain and subsequently, hair cells are destroyed by kanamycin/furosemide treatment. To recapitulate cochlear implantation, a fully-implantable rodent stimulator¹ is used, in which the electrode is activated by a magnetic field. Using a three-dimensional field with waking animals allows us to establish a functional threshold for each animal – they show characteristic behavioural changes when the magnetic stimulus is applied. In anaesthetised animals, eABR measurements are taken – the input-output function generated in the brainstem by varying the levels of magnetic stimulation applied to the device can be used as a quantitative measure of electrode function.

Current work in our group has focussed on rebuilding of the auditory nerve using human otic neural progenitors (hONPs) derived from embryonic stem cells. The current cell line in use, H14-NopSox2-GFP, expresses a GFP construct which reports the expression of SOX2 under the control of specific otic/nasal placode enhancers, allowing the purification of hONPs in vitro prior to transplantation. A paradigm has been established in which the doubly-deafened animals simultaneously receive an implant and a cell transplant; their progress is monitored over the following three months to look for behavioural changes in the chronic stimulator and eABR responses. Histology suggests that the transplanted cells survive and differentiate in the implanted animals, with neural fibres tracking towards the implant.

O-32 Three-dimensional force profile during cochlear implantation depends on individual geometry and insertion trauma

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Objectives

To preserve the acoustic hearing, cochlear implantation has to be as atraumatic as possible. Therefore, understanding the impact of the cochlear geometry on insertion forces and intracochlear trauma might help to adapt and improve the electrode insertion and reduce the probability of intracochlear trauma.

Design

The study was conducted on 10 fresh-frozen human temporal bones. The inner ear was removed from the temporal bone. The bony capsule covering the scala vestibuli was removed and the dissected inner ear was mounted on the three-dimensional (3D) force measurement system (Agilent technologies, Nano UTM, Santa Clare, CA). A lateral wall electrode array was inserted, and the forces were recorded in three dimensions with a sensitivity of 2 μ N. Afterwards, the bones were scanned using a Skyscan 1173 micro-computed tomography (micro-CT). The obtained 3D force profiles were correlated with the videos of the insertions recorded through the microscope, and the micro-CT images.

Results

A correlation was found between intracochlear force profiles measured in three different directions with intracochlear trauma detected with micro-CT imaging. The angle of insertion and the cochlear geometry had a significant impact on the electrode array insertion forces and possible insertion trauma. Intracochlear trauma occurred frequently within the first 180° from the round window, where buckling of the proximal part of the electrode carrier inside the cochlea, and rupturing of the spiral ligament was observed.

Conclusions

The combination of the 3D force measurement system and micro-CT can be used to characterize the mechanical behavior of a CI electrode array and some forms of insertion trauma. Intracochlear trauma does not always correlate with higher force amplitudes, but rather with an abrupt change of force directions.

Author keywords: cochlear anatomy / insertion trauma / micro-CT / force profile / electrode array

O-33 Anodal transcranial direct current stimulation modulates auditory cortex structural plasticity in a model of noise-induced hearing loss (from central auditory prostheses)

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Introduction

Transcranial direct current stimulation (tDCS) is emerging as non-invasive tool capable of modulating cortical function by affecting neuronal excitability and synaptic plasticity. Our previous study demonstrated that chronic noise exposure causes alterations of cortical synaptic organization consisting in the reduction of apical and basal spine density in pyramidal neurons of layers 2/3 and 5/6 in the auditory cortices. Here we investigated whether tDCS can counteract these cortical damage by affecting structural plasticity.

Methods

Rats were exposed to noise (100 dB, 60 min/day/10 days), subjected to anodal tDCS (350 μ A for 20/2 days) over the left auditory cortex (AC) starting from 24h after the end of noise paradigm, and sacrificed 21 days later for morphological and electrophysiological analyses.

Results

Noise decreased spine density in layer 2/3 AC pyramidal neurons. tDCS counteracted the reduced spine number caused by noise both in apical and basal arborizations. Western blot analyses indicated an increase of synaptic plasticity-related proteins (Bdnf and Synaptophysin) in AC after tDCS. Field recordings were performed from AC slices to assess tDCS impact on basal synaptic transmission at layer 2/3 horizontal connections. Comparison of the input-output (I-O) curves showed that after noise the amplitude of field excitatory post-synaptic potentials was smaller than in controls. Interestingly, responses to current pulses were increased in Noise-tDCS group compared to noiseexposed rats and I-O curves of Noise-tDCS rats were similar to controls, suggesting that tDCS counteracted the effects of noise on synaptic function.

Conclusion

Our findings provide novel evidence that anodal tDCS affects structural plasticity in the AC and counteracts the detrimental effects of sensory deafferentation. These results widen the horizons on brain areas whose plasticity is targeted by tDCS and open the way to the possibility to exploit tDCS to treat diseases thought to be related to altered plasticity like tinnitus.

O-34 Lgr5-positive cells act as hair cell progenitors in the cochlea**Albert Edge¹***¹ Harvard Medical School*

Wnt signaling is required for the differentiation of hair cells during embryogenesis. Wnt stimulated proliferation of otic progenitors and induced expression of transcription factor, *Atoh1*, which converted the progenitors to hair cells. *Lgr5*, a downstream target of the Wnt pathway, and a protein that marks intestinal epithelial stem cells, was expressed in cells that gave rise to hair cells based on lineage tracing. *Lgr5* was expressed in a specific subset of supporting cells that differentiated to hair cells at a higher rate than the other (*Sox2*-positive) supporting cells. *Lgr5*-positive cells proliferated as organoids after stimulation of Wnt signaling in vitro. The *Lgr5*-positive cells could be differentiated to hair cells by a combination of Notch inhibition and Wnt stimulation. Over 11,500 hair cells could be obtained from a single mouse cochlea, as compared to less than 200 in the absence of induction. The newly generated hair cells had bundles and molecular machinery for transduction, synapse formation, and specialized hair cell activity. Following ototoxic damage in neonatal ears, supporting cells divided and transdifferentiated to hair cells. Division and transdifferentiation were blocked by inhibition of Wnt signaling. Spontaneous regeneration in response to Wnt in the newborn inner ear specifically targeted the *Lgr5*-expressing cells. These data suggest that *Lgr5*-positive cells act as hair cell progenitors in the cochlea and that Wnt signaling stimulates proliferation and transdifferentiation of these cells to hair cells.

Supported

O-35 Exploring new approaches to reduce cisplatin ototoxicity**Eric Bielefeld^{1,*}***¹ Speech and Hearing Science, The Ohio State University, Columbus, OH, USA*** Presenting author*

Ototoxicity is a significant side effect of cisplatin chemotherapy. Cisplatin can trigger apoptosis of the hair cells, stria vascularis, and spiral ganglion neurons, all of which are crucial for cochlear signal transduction. The audiometric result is a sensorineural hearing loss that progresses from the high to mid frequencies with increasing cumulative dose. Numerous pharmaceutical approaches have been tested to reduce cisplatin ototoxicity, but carry the risk of potentially interfering with cisplatin's anticancer effect. Our lab is exploring two approaches to reducing cisplatin ototoxicity. The first is through chronotolerance. Chronotolerance is the differential effect of a physical insult depending on the time of exposure within the 24-hour light/dark cycle. In rodents, cisplatin's nephrotoxicity is more severe during light-hour exposures, but noise-induced hearing loss is more severe during dark-hour exposures. Thus, the best predictors of chronotolerance for cisplatin's ototoxicity are in conflict from another. We exposed rats to cisplatin at two hours after light onset or two hours after light offset, and found that the dark-hour exposures led to less threshold shift and hair cell loss. For the second approach to preventing cisplatin ototoxicity, our lab has investigated endoplasmic reticulum stress and unfolded protein response (UPR) pathways as underlying triggers for cisplatin-induced apoptosis in the cochlea. Specifically, we tested CHOP-deficient mice. CHOP is a key signaling molecule in the UPR pathway for the induction of apoptosis. Many CHOP knockout mice (-/-) had severe hearing impairment at baseline, indicating a possible role for the UPR in auditory development. Heterozygous CHOP mice (+/-) had better baseline hearing sensitivity than -/- mice, and also showed lower susceptibility to cisplatin ototoxicity than C57Bl6/J control mice (+/+). This is an initial indication that endoplasmic reticulum stress and the UPR could play significant roles in cisplatin-induced apoptosis in the cochlea, and represent possible targets for future pharmaceutical intervention.

O-36 Olfactory stem cell derived hair cell progenitor

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Introduction:

Olfactory stem cells are a potential source for regeneration of neuronal cells. In this project it was evaluated whether olfactory stem cells can be used to regenerate hair cell progenitors.

Materials and Methods:

Stem cells were isolated from the olfactory epithelium from the nose of the rat. Subsequently the cells were differentiated into hair cell progenitors using modified previously described protocols for cochlear hair cells differentiation. Different markers for differentiation of the olfactory stem cells into hair cell progenitors were evaluated using RT-PCR (including CD44, Nestin, P27KIP1, Jagged-1, Myo-7a).

Results:

The quantitative RT-PCR expression profile showed down-regulation of stem cell markers and up-regulation of genes associated with cell differentiation towards cochlear haircells.

Conclusion:

The preliminary results of this study showed that olfactory stem cells can potentially be used as a source for hair cell progenitors.

O-37 Mesenchymal stem cells for prevention of ototoxicity induced by cisplatin**Laura Astolfi^{1,*}, Edi Simoni¹, Filippo Valente², Erica Gentilin¹, Valeria Franceschini³, Alessandro Martini¹***1 Bioacoustics Research Laboratory, Department of Neurosciences, University of Padua, Italy**2 Ear Science institute Australia, Perth, Australia**3 Department of Biological, Geological and Environmental Sciences, University of Bologna, Italy.***Presenting author*

In the mammalian cochlea, mesenchymal stem cells lose their properties shortly after embryo development, therefore only an exogenous transplantation of stem cells could induce regeneration of tissues damaged by acoustic traumas or exposure to ototoxic agents. Several studies deal with the use of antioxidants or anti-inflammatory molecules to prevent ototoxicity induced by cisplatin (Cpt), an antineoplastic agent commonly employed in clinical treatments of solid tumors, but a complete protection from hearing loss caused by Cpt has never been reported. Our study concerns the use of mesenchymal stem cells to prevent ototoxicity caused by exposure to Cpt. Human mesenchymal stem cells (HMSC) were isolated from adipose tissues from healthy donors undergoing plastic surgery and injected in a rat animal model. Anaesthetized rats were pretreated with an intratympanic (IT) bilateral injection of HMSC and with an intraperitoneal injection (IP) of Cpt (4.6 mg/Kg) for two consecutive days, to a final cumulative dose of 14 mg/Kg. The auditory threshold was monitored before and after treatment by Auditory Brain Response. Four days after treatment all animals were painlessly sacrificed for histological analyses. The results show that the cumulative Cpt dose caused a significant hearing loss with cochlear damage, including loss of hair cells in the basal region. In controls treated only with normal saline an inflammatory response, but not hypoacusia, was observed near the round window, and the same effect was observed in controls treated only with HMSC. The IT pretreatment with HMSC before exposure to Cpt significantly reduced hearing loss caused by Cpt, as confirmed by a lower histological damage of hair cells. These data show for the first time that stem cells may be used to prevent Cpt-induced damage. Further studies are required on the biochemical and molecular mechanisms of action/protection exerted by these cells against ototoxic damage.

O-38 Autophagy protects auditory hair cells against neomycin-induced damage**Zuhong He¹, Lingna Guo¹, Qiaojun Fang¹, Xia Gao², Renjie Chai^{1*}***1 Southeast University**2 Affiliated Drum Tower Hospital of Nanjing University Medical School*** Presenting author*

Aminoglycosides are toxic to sensory hair cells (HCs) by inducing the production of reactive oxygen species (ROS) that in turn activate apoptotic pathways. Autophagy is an essential and highly conserved self-digestion pathway that plays important roles in the maintenance of cellular function and viability under stress. However, the role of autophagy in aminoglycoside-induced HC injury is unknown. In this study, we systematically investigated the role of autophagy in aminoglycoside-induced HC damage. First we found that autophagy activity was significantly increased, including enhanced autophagosome-lysosome fusion, in both cochlear HCs and HEI-OC-1 cells after neomycin/gentamicin injury. This suggested that autophagy might be correlated with aminoglycoside-induced cell death. We then used rapamycin, an autophagy activator, to increase the autophagy activity and found that the ROS levels, apoptosis, and cell death were significantly decreased after neomycin/gentamicin injury. In contrast, treatment with the autophagy inhibitor 3-methyladenine (3-MA) or knockdown of autophagy-related proteins (ATGs), including ATG5, BECN1 (Beclin1), and ATG7, resulted in reduced autophagy activity and significantly increased ROS levels, apoptosis, and cell death after neomycin/gentamicin injury. Lastly, after neomycin injury, the antioxidant N-acetylcysteine (NAC) could successfully prevent the increased apoptosis and HC loss induced by 3-MA treatment or ATG knockdown, suggesting that autophagy protects against neomycin-induced HC damage by inhibiting oxidative stress. We also found that the dysfunctional mitochondria were not eliminated by autophagy (mitophagy) in HEI-OC-1 cells after neomycin treatment, suggesting that autophagy might not directly target the damaged mitochondria for degradation.

This study demonstrates that moderate ROS levels can promote autophagy in order to recycle damaged cellular constituents and maintain cellular homeostasis, while the induction of autophagy can inhibit apoptosis and protect the HCs by suppressing ROS accumulation after neomycin/gentamicin injury. Our results suggest that autophagy might be a new therapeutic target for the prevention of aminoglycoside-induced HC death.

O-39 Netrin1 mediates the protection of cochlear hair cells by IGF1 through its canonical receptor, UNC5B**Norio Yamamoto^{1,*}, Kouhei Yamahara¹, Takayuki Nakagawa¹, Juichi Ito², Koichi Omori¹***1 Kyoto University**2 Shiga Medical Center Research Institute*** Presenting author*

Sensorineural hearing loss (SNHL) is mainly caused by the damage of cochlear hair cells (HCs). As HCs and supporting cells (SCs) that exist around HCs do not proliferate in postnatal mammals, the loss of HCs and SCs is irreversible, emphasizing the importance of preserving their numbers to prevent SNHL. It is shown that insulin-like growth factor 1 (IGF1) is instrumental in the treatment of SNHL [1]. Our previous study indicates that IGF1 protects HCs against aminoglycoside by activating IGF1 receptor and its major downstream pathways in SCs [2], which results in the upregulation of the expression of the Netrin1-encoding gene (*Ntn1*) [3].

To determine if NTN1 acts as a downstream molecule of IGF1 to protect cochlear HCs against inner ear damage, we performed several experiments. First, we demonstrated that NTN1, similar to IGF1, promoted HC survival. Secondly, we found that NTN1 blocking antibodies attenuated IGF1-induced HC protection from aminoglycoside, indicating that NTN1 is the effector molecule of IGF1 signaling during HC protection.

To elucidate the responsible receptors among six canonical NTN1 receptors that mediated the effect of cochlear HC protection, the localization of canonical NTN1 receptors were tested using in situ hybridization. UNC5B is the only receptor that was expressed in the organ of Corti through whole cochleae. Addition of blocking antibodies of UNC5B attenuated the cochlear HC protection by IGF1 or NTN1, indicating that UNC5B is the NTN1 receptor involved in the HC protection.

Apoptosis was significantly suppressed by NTN1 when HCs were protected from aminoglycoside although proliferation of SCs was not affected by NTN1.

These results provide new insights into the mechanisms underlying IGF1 protection of cochlear HCs, suggesting a possibility of using NTN1 as a new treatment for SNHL.

O-40 Roles of the contralateral medial olivocochlear efferent reflex demonstrated with cochlear implants

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The medial olivocochlear reflex (MOCR) is thought to facilitate the intelligibility of speech in noisy environments by reducing the adaptation of auditory nerve fibers to the background noise, a monaural, neural effect. Here, I will use a binaural cochlear-implant sound coding strategy inspired by the contralateral MOCR (Lopez-Poveda et al. 2016 *Ear & Hear* 37(3):e138-e148) to argue that in natural, binaural hearing, the contralateral MOCR probably facilitates speech-in-noise intelligibility by additional mechanisms. These include the enhancement of amplitude modulation cues in speech, noise reduction in the ear with the better signal-to-noise ratio, the enhancement of interaural level differences at the times and frequencies when speech features occur, and the spatial segregation of concomitant speech sources positioned in different spatial locations.

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O-41 Bio-inspired sound coding strategies for cochlear implants based on temporal fine structure

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A main component of a cochlear implant system is the algorithm by which the stimulation data are derived from the sound signal. These algorithms are usually referred to as sound coding strategies. These sound coding strategies are based – at least to some extent – on the physiology of the cochlea in normal hearing. The one aspect that can be found in all cochlear implants today is the tonotopical organization of the cochlea. Other aspects that are represented at least in the some cochlear implant systems are the coding of the temporal fine structure in the low frequencies as well as the frequency-dependent phase delays introduced by the travelling wave mechanics in the cochlea. While the importance of some of these aspects is generally accepted in cochlear implants, the relevance of other aspects is still under discussion. In this presentation, the current status of this discussion will be reviewed.

O-42 Simple physiological model of spike-conducting axons and its application to auditory nerves

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Computational modeling has been a powerful tool in analyzing, simulating, and predicting the complex behaviors of nonlinear systems. In cochlear implant (CI) studies, for example, computational models have been used to characterize the interface between the implanted electrodes and auditory nerves to evaluate the performance of the device. In order to precisely simulate the electrical activity of a sensory nerve, however, a number of parameters need to be tuned or optimized, which include various types of ionic conductances existing on its membrane. Since the amount of available experimental data is limited in most cases, it is often difficult (if not impossible) to justify the selection of model parameters. Moreover, optimizing a large number of parameters generally requires enormous computational resources. Thus it is ideal to have a simple model that still captures the fundamental characteristics of the targeted system with a small number of unknown parameters. Here we propose a novel type of spike-conducting axon model that is based on the exponential integrate-and-fire (EIF) model of a single compartment neuron. By modifying the EIF mechanisms of generating action potentials, our model can replicate both spike initiation and repolarization with fewer parameters than the conventional Hodgkin-Huxley (HH) models that are widely used for simulating axonal spike propagation. Our simulation results show that morphological parameters (such as the axonal diameter and internodal length) affect the conduction velocity of the EIF-based model similarly to that of the HH-type model. As an application of our new approach, the central axon of an auditory nerve is modeled. We show that, by tuning a few parameters, distinct spiking activity of high and low frequency nerves can be simulated. These simulation results are largely comparable to known experimental data from animal studies. Relevance of the model to CI research will also be discussed.

O-43 Optogenetic stimulation of the cochlea — first models

Werner Hemmert*Technische Universität München*

We evaluate the potential of optogenetic methods for the stimulation of the auditory nerve and assess the feasibility of optogenetic cochlear implants (CIs). We provide an overview of all critical steps like opsin targeting strategies, how opsins work, how their function can be modeled and included in neuronal models and the properties of light sources available for optical stimulation. From these foundations, quantitative estimates for the number of independent stimulation channels and the temporal precision of optogenetic stimulation of the auditory nerve are derived and compared with state-of-the-art electrical CIs. We conclude that optogenetic CIs have the potential to increase the number of independent stimulation channels by up to one order of magnitude to about 100, but only if light sources are able to deliver confined illumination patterns independently and parallelly. Already now, opsin variants like ChETA and Chronos enable driving of the auditory nerve up to rates of 200 spikes/s, close to the physiological value of their maximum sustained firing rate. Apart from requiring 10 times more energy than electrical stimulation, optical CIs still face major hurdles concerning the safety of gene transfection and optrode array implantation, for example, before becoming an option to replace electrical CIs.

O-44 Modelling the brainstem response to cochlear implant stimulation

Ray Meddis*Department of Psychology, University of Essex*

The response of the inner ear to simple sounds is surprisingly complex (instantaneous compression, two-tone suppression, combination tones, rate saturation, adaptation and three kinds of (negative) efferent feedback, etc.) Computer models of the auditory periphery help us to grasp the implications of this complexity and to understand how peripheral pathology leads to different kinds of hearing loss. Conversely, they can help us to associate patterns of loss with different pathologies. Models can suggest and help assess new kinds of hearing aid algorithms that go beyond amplification by incorporating some of these peripheral complexities into their signal processing. However, we also need tools to help us understand how the periphery works in conjunction with signal processing in the auditory brainstem and how hearing prostheses such as hearing aids or cochlear implants can restore (or fail to restore) normal patterns of brainstem response. The talk will be illustrated using a long established model of the auditory periphery harnessed to new open-source software for simulating the response of networks of large numbers of different types of auditory brainstem neurones.

O-45 Neuronal network simulations in the cochlear nucleus and its implications for CI and ABI stimulation

Andreas Bahmer

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The cochlear nucleus (CN) is a shunting yard for the information conveyed by the auditory nerve. The main projecting cells of the CN to the ascending auditory pathway are the so-called chopper neurons. These neurons correspond anatomically to t-stellate cells. Neuroanatomical and neurophysiological evidence shows that they are interconnected and can excite each other with a constant synaptic delay. "Regular" chopper neurons impress by their constant firing (with a low coefficient of variation) and their high dynamic range in coding temporal modulations. According to the outstanding properties of these neurons our neuronal network topology resembles networks of t-stellate cells. The neural network was implemented in Matlab using leaky-integrate-fire and Hodgkin-Huxley like neuron models. Simulation results show that the outstanding properties of the chopper neurons can be matched by our proposed topology.

Temporal information transfer to the auditory nerve using cochlear implants appears as a bottle neck. In order to improve the temporal information transfer, our neuronal network model was used as a starting point. The broadband frequency processing operated by onset neurons in our model is the basis for a salient information transfer within a high dynamic range. In order to match this property broadband stimulation with cochlear implants was tested. The results show that broadband stimulation can improve rate pitch discrimination.

For auditory brainstem implants (ABI) chopper neurons are the main target cells of stimulation. Electrophysiological measurements after electrical stimulation with ABI show the same time constant which is the basis for our interconnected neuronal network model. The time constant does not increase with stimulation amplitude. This can be explained by the topology of our model.

O-46 Model-guided development of a noninvasive approach to evaluate cochlear synaptopathy

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Cochlear synaptopathy is a recently discovered hearing dysfunction that may affect humans and relate to difficulties in speech perception and supra-threshold sound processing. Animal studies suggest that cochlear synaptopathy can arise due to aging and loud noise exposure, which can cause selective loss of afferent synapses on inner hair cells and subsequent auditory-nerve fiber degradation, even without permanently affecting hearing thresholds (a phenomenon called “hidden hearing loss”). Despite an increasing focus on the auditory evoked responses as a successful approach to reveal cochlear synaptopathy, diagnosis in humans is still an open question, especially for listeners with different combinations of peripheral hearing deficits.

In the present study, we employed a computational model of auditory brainstem responses to simulate the interplay between mixed pathologies and isolate the amount of outer-hair-cell (OHC) dysfunction and auditory-nerve fiber loss from simulated auditory evoked potentials. Model predictions were compared against recorded auditory evoked potentials in listeners with normal and elevated audiometric thresholds.

The results of the study suggest that both auditory brainstem and envelope-following responses (ABR and EFR) can be affected by OHC loss and synaptopathy. Relative ABR and EFR metrics can be combined to build a peripheral hearing loss map that might quantify each aspect when different deficits are present. ABR wave-V to EFR amplitude ratio, most sensitive to the OHC loss, in combination with the magnitude slope between EFR fundamental and first harmonic, capturing synaptopathy degree, could be considered as a combination of metrics that can separate these two deficits in listeners with high-frequency sloping (or flat) audiograms.

This study was funded by the DFG Priority Program 1608.

O-47 A predictive model of blast induced hearing loss**Allen F Ryan^{1,*}, Alex Fantozzi¹, Elena Cardenez¹, Eduardo Chavez¹, Arwa Kurabi¹**¹ UCSD

* Presenting author

Blast is a significant cause of hearing loss in the military. While well studied, there is less information on the time course of hearing recovery. This is needed to predict recovery and need for possible therapeutic intervention.

We evaluated the effects of blast exposure on the hearing of 62 chinchillas, chosen for their wide frequency range, audiogram shape similar to that of humans, and an extensive database on blast-induced hearing loss. Single or multiple exposures were delivered bilaterally. Ears with tympanic membrane rupture were excluded. Pre-exposure auditory sensitivity and blast-induced hearing loss were measured via ABR immediately (2-30 min), 1, 2 and 3 hours and 1, 3, 7 and 14 days following blast.

The maximum initial hearing loss without tympanic membrane rupture was ~50 dB. Hearing losses showed little recovery in the first hour after blast, but recovered more rapidly thereafter. Recovery rate depended upon the level of initial hearing loss, with the most rapid recovery observed after the highest immediate losses. Permanent threshold shifts were observed in only a few cases. The data were used to generate a generalized predictive model for hearing loss after blast. The model was compared to human data obtained historically, and correctly predicted recovery rates. Data to assess the possibility of blast-induced synaptopathy in the chinchilla ears are currently being analyzed.

The model provides a predictive framework for blast-induced hearing loss, that compared well with human data on blast exposure of the unprotected ear. The results indicate that thresholds measured in the initial hours after exposure can be used to predict ultimate threshold recovery. The model can therefore be used to assess the need for potential therapeutic intervention.

Supported by a grant from the US Department of Defense. Significant contributions were made by Philemon Chan and Kevin Ho of L-3 Applied Technologies, Inc.

O-48 Functional appraisal of the auditory nerve following clinically feasible gelfoam treatment with various neurotrophic compounds in deafened guinea pigs

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** Presenting author*

There is growing evidence that in cochlear implant (CI) recipients the health of the auditory nerve may be compromised, which may negatively impact CI effectiveness. In animal studies, it has been established that spiral ganglion cells (SGCs) progressively degenerate in the absence of hair cells – most likely because the neurotrophic support from the organ of Corti is lost, since numerous studies have shown increased survival with neurotrophic treatment.

We aimed to improve existing neurotrophic factor delivery strategies, to develop an effective and clinically feasible treatment for CI users. To achieve this, we have compared the effects of several neurotrophic agents in ototoxically deafened guinea pigs. Neurotrophins and small-molecule TrkB agonists, and combinations thereof were delivered in a clinically feasible way by gelfoam placement on the round window membrane (Havenith et al. [2015] *Otol Neurotol* 36:705-713). Histological analysis of the SGCs was complemented with advanced electrophysiological assessment (electrically evoked compound action potentials; eCAPs), shown to be indicative of neural health (Ramekers et al. [2014] *J Assoc Res Otolaryngol* 15:187-202).

Treatment with brain-derived neurotrophic factor (BDNF) resulted in substantial SGC preservation, limited to the basal turn of the cochlea, which is consistent with previous findings. Preliminary results (four animals) of treatment with 7,8,3'-trihydroxyflavone (THF; a small molecule TrkB agonist) indicate less SGC preservation in the basal turns than with BDNF. Consistent with our previous findings, the absolute eCAP measures show no clear treatment effect. However, the differences between eCAP measures when varying the inter-phase gap show a positive effect of BDNF but not of THF treatment.

Although both BDNF- and THF-treated animals show increased basal cochlear SGC survival, only BDNF treatment indicates a positive electrophysiological effect. This suggests that increased basal survival can lead to functional preservation. In addition, while theoretically superior to BDNF, the small molecule THF seems less effective.

O-49 Beneficial effect of ProteinY* on hearing loss during experimental pneumococcal meningitis**Silvia Erni^{1,*}, Michael Perny¹, Rolf Jan Rutten², Pascal Senn³, Denis Grandgirard¹, Stephen L. Leib¹, Marta Roccio¹***1 University of Bern**2 Audion Therapeutics**3 University of Bern & University hospital Geneva*** Presenting author*

Over 5% of the world's population suffer from hearing loss. Sensorineural hearing loss is also the most common long-term deficit after pneumococcal meningitis, occurring in up to 30% of surviving patients. Treatment options for inner ear pathologies are currently limited and novel pharmaceutical treatments are highly needed. Here, we tested the putative otoprotective properties of the recombinant ProteinY* with previously reported anti-inflammatory, anti-apoptotic and neuronal protective functions, in an experimental model of pneumococcal meningitis. Pneumococcal meningitis was induced in infant rats (n=28). Animals were randomized for treatment of systemically applied ProteinY (3x 50 µg) (n=13) or vehicle (n=15). Hearing thresholds were assessed by measuring auditory brain stem responses (ABR) 1 and 3 weeks after infection and spiral ganglion neuron and hair cell density were determined by histological analyses 3 weeks after infection. We observed lower ABR thresholds in animals receiving the compound versus untreated animals, reaching significant levels 1 week post infection for clicks and pure tones at 4 kHz and 16 kHz and 3 weeks post infection at frequencies of 8 kHz and 32 kHz. Moreover, we observed a reduced percentage of animals presenting high hearing thresholds (80-100dB) in the compound treated cohort. Spiral ganglion neuron density did not differ significantly when comparing infected untreated versus ProteinY-treated animals. The same holds true for numbers of inner and outer hair cells. Nevertheless, when animals were stratified according to the severity of hearing loss, we observed statistically significant higher numbers of remaining inner and outer hair cells in the ProteinY-treated group compared to the untreated group in animals with severe hearing loss (threshold above 80 dB). In conclusion, ProteinY appears to be a promising therapeutic option with the potential to improve hearing thresholds as well as to protect hair cells in case of severe bacterial meningitis. *ProteinY (Patent currently being filed).

O-50 Bone morphogenetic protein 4 promotes the survival and preserves the structure of flow-sorted Bhlhb5+ cochlear spiral ganglion neurons in vitro**Shan Sun^{1,*}, Muhammad Waqas², Huawei Li¹, Renjie Chai²**¹*Eye and Ear, Nose, Throat Hospital, Fudan University*²*Key Laboratory for Developmental Genes and Human Disease, Ministry of Education, Institute of Life Sciences, Southeast University, Nanjing*

*Presenting author

Spiral ganglion neurons (SGNs) are the primary auditory neurons, and damage or loss of SGNs leads to sensorineural hearing loss. BMP4 is a growth factor that belongs to the transforming growth factor- β superfamily and has been shown to play a key role during the development, but little is known about its effect on postnatal cochlear SGNs in mice. In this study, we used the P3 Bhlhb5-cre/tdTomato transgenic mouse model and flow-activated cell sorting to isolate a pure population of Bhlhb5+ SGNs. We found that BMP4 significantly promoted SGN survival after 7 days of culture. We observed fewer apoptotic cells and decreased expression of pro-apoptotic marker genes after BMP4 treatment. We also found that BMP4 promoted monopolar neurite outgrowth of isolated SGNs, and BMP4 preserved the number and the length of neurites in the explant culture of the modiolus harboring the SGNs. We showed that BMP4 enhanced the neurites growth as determined by the higher average number of filopodia and the larger area of the growth cone. Finally, we found that BMP4 significantly elevated the synapse density of SGNs in explant culture. Thus, our findings suggest that BMP4 has the potential to promote the survival and preserve the structure of SGNs.

O-51 Time course of oxidative stress and apoptosis in the auditory receptor after Kanamycin treatment in the rat**Alejandro Gibaja-Casado¹, Juan C Alvarado¹, Jose M Juiz^{1,*}**¹ University of Castilla-La Mancha

* Presenting author

Aminoglycosides such as kanamycin cause hearing loss, which has been linked to unbalanced oxidative stress and cell death. Using auditory brainstem responses (ABR), quantitative real time PCR (qRT PCR) and quantitative histology, we report the time course of cellular and molecular events related to kanamycin damage to the inner ear.

Wistar rats received daily subcutaneous injections of kanamycin (400 mg/kg) for 14 days and were tested in groups at 3 and 11-15 days after the beginning of treatment. At day 3, ABRs were normal. There was strong cochlear up-regulation of JNK pathway genes (Mapk8 and Jun), as well as downregulation of the anti-oxidative enzyme Glutathione peroxidase 1 (Gpx1). At day 11, ABRs were still normal. Mapk8 returned to normal, whereas Jun remained up-regulated. Gpx1 was still downregulated. At day 15, ABRs showed elevated thresholds. The number of outer hair cells at the basal and middle turns was reduced. At this endpoint, expression of both Gpx1 and Catalase (Cat) were down-regulated and Jun was still up-regulated. There were no detectable changes in the expression of apoptosis genes, although apoptotic outer hair cells were detected by TUNEL, starting at day 11 posttreatment, at a narrow "transition zone" which progressed apically from 11 to 15 days, leaving behind outer hair cell loss.

Kanamycin treatment induces early up-regulation of the JNK pathway in the cochlea, indicative of cellular stress. Down-regulation of Gpx1, a key antioxidant enzyme, suggests early antioxidation failure, followed later by down-regulation of Cat, another crucial antioxidant enzyme. Progressive apoptosis and death of outer hair cells is detectable at day 11, when auditory thresholds are also affected, up to day 15. This sequence of events adds to our understanding antibiotic ototoxicity in the cochlea and may help to design treatments to prevent or reverse cochlear damage.

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O-52 Platinum-induced hidden hearing loss**Marion Souchal^{1,*}, Fabrice Giraudet¹, Paul Avan¹***¹Neurosensory biophysics laboratory***Presenting author*

The sensorineural hearing loss is typically described by elevated hearing thresholds associated with outer hair cells alterations. However, some patients have difficulties with speech intelligibility in context of “normal hearing”. So, recently, in addition to auditory neuropathy spectrum disorder, appeared the concept of a new pathological entity called “hidden hearing loss”. Briefly, in animal models, an acoustic trauma can induce fibers defects and especially fibers with “high thresholds” (or “slow spontaneous rates fibers”-SR). In this case, the auditory function assessed via DPOAE and ABR shows normal hearing sensitivity and cannot illustrated this SR fibers degeneration. Oxaliplatin, a platinum salt used in colorectal cancer treatment, has many side effects, including the occurrence of peripheral neuropathy. So, the present study examined the effects of oxaliplatin in the hearing function of adult CBA/J mice and in the cochlear morphology. We found no significant differences in the hearing, based on ABR and DPOAE, between the treated and the control animals. However, the histological study revealed a surprising degeneration of the ganglion spiral cells. With further electrophysiological tests, we showed an increase in wave I amplitude of ABR, associated with a decrease in the medial olivocochlear reflex, according to a controlateral suppression test. Mice treated with oxaliplatin, therefore, constitute a valuable animal model of hidden hearing loss which remains to be further characterized. This animal model could correspond to the patients with normal audiogram complaining of “I hear but I don’t understand it!”:

O-53 NO-sensitive guanylate cyclase isoforms NO-GC1 and NO-GC2 contribute to noiseinduced inner hair cell synaptopathy

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Nitric oxide (NO) activates the NO-sensitive soluble guanylate cyclase (NO-GC, sGC) and triggers intracellular signaling pathways involving cGMP [1]. For cochlear hair cells, controversial studies show that NO-mediated cascades have both protective and detrimental potential [2, 3]. Here we examine the cochlear function of mice lacking one of the two NO-sensitive guanylate cyclase isoforms (NO-GC1 KO or NO-GC2 KO). The deletion of NO-GC1 or NO-GC2 did not influence electromechanical outer hair cell (OHC) properties, as measured by distortion product otoacoustic emissions, neither before nor after noise exposure, nor were click or noise burst-evoked auditory brainstem response thresholds different from controls. Yet, inner hair cell (IHC) ribbons and auditory nerve responses showed significantly less deterioration in NO-GC1 KO and NO-GC2 KO mice after noise exposure. Consistent with a selective role of NO-GC in IHCs, NO-GC β 1 mRNA was found in isolated IHCs but not in OHCs. Using transgenic mice expressing the FRET-based cGMP biosensor cGi500, NO-induced elevation of cGMP was detected in real-time in IHCs, but not in OHCs. Pharmacological long-term treatment with a NO-GC stimulator altered auditory nerve responses, but did not affect OHC function and hearing thresholds. Interestingly, NO-GC stimulation exacerbated the loss of auditory nerve response in aged animals, while attenuating the loss in younger animals. This suggests NO-GC as a target for early pharmacological prevention of auditory fiber loss (synaptopathy) and proposes NO-GC to provide selective benefits for hearing function, by maintaining functional integrity of auditory nerve fibers in early life rather than at old age.

This work was supported by the Deutsche Forschungsgemeinschaft (Grants FOR 2060 project FE 438/6-1, FR 1725/3-1, RU 713/3-2); Action on Hearing Loss (RNID Grant 54); and University of Tübingen, Tübingen, Germany (Fortüne 2339-0-0), and the Hahn Stiftung (Index AG).

O-54 The synaptic ribbon is critical for sound encoding at high rates and with temporal precision

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We studied the role of the synaptic ribbon for sound encoding at the synapses between inner hair cells (IHCs) and spiral ganglion neurons (SGNs) in mice lacking RIBEYE, the core-component of the ribbon. Electron and immunofluorescence microscopy revealed a lack of synaptic ribbons and an assembly of several small active zones (AZs) at each synaptic contact. Spontaneous and sound-evoked firing rates of SGNs and their compound action potential were reduced, indicating impaired transmission at ribbonless IHC-SGN synapses. The temporal precision of sound encoding was impaired and the recovery of SGN-firing from adaptation indicated slowed synaptic vesicle (SV) replenishment. Activation of Ca²⁺-channels was shifted to more depolarized potentials and exocytosis was reduced for weak depolarizations. Presynaptic Ca²⁺-signals showed a broader spread, compatible with the altered Ca²⁺-channel clustering observed by super-resolution immunofluorescence microscopy. We postulate that RIBEYE disruption is partially compensated by multi-AZ organization. The remaining synaptic deficit indicates ribbon function in SV-replenishment and Ca²⁺-channel regulation.

O-55 Impaired sound encoding in PSD-95 knockout mice**Gulnara Yamanbaeva^{1,*}, Sangyong Jung², Man Ho Wong³, Nicola Strenzke¹***1 InnerEarLab, University Medical Center Göttingen**2 Neuro modulation and Neuro Circuitry Group Singapore Bioimaging Consortium (SBIC), Biomedical Sciences Institutes (BMSI)**3 European Neuroscience Institute*** Presenting author*

Introduction: Sound is encoded by inner hair cells, each forming 8-20 ribbon synapses with auditory nerve fibers (ANF). The scaffolding protein PSD-95 contributes to clustering of AMPA receptors in the postsynaptic membrane. Using PSD-95 knockout mouse (PSD-95 KO), we investigated the role of PSD-95 in AMPA receptor clustering and action potential generation in the auditory system.

Methods: To study sound encoding in PSD-95 KOs, we performed recordings of auditory brainstem responses (ABRs) and extracellular in vivo single unit recordings from the ANF and cochlear nucleus neurons. Furthermore, using confocal and STED microscopy, we imaged inner hair cell ribbon synapses.

Results: ABRs recorded from adult mice had normal thresholds, but a reduced amplitude of the wave I, suggesting impaired temporal precision and/or rates of synaptic transmission, while the other ABR waves were normal. Single unit recordings revealed lower spontaneous spike rates in ANFs. Single unit thresholds and frequency tuning were normal. Onset and adapted spike rates in response suprathreshold tone burst stimulation were reduced and the time constant of fast adaptation was reduced. The delay and jitter of the first spike in response to stimulus onset was increased. STED data indicated alterations in the arrangement of postsynaptic glutamate receptor clusters of PSD-95 KO ANFs.

Conclusion: PSD-95 scaffolding protein is essential for the glutamate receptor clustering in ANFs. The absence of this protein results in impaired sound encoding in PSD-95 KO mice, presumably due to changes in the number, arrangement or mobility of AMPA receptors.

O-56 The role of IGF1-related pathways in the aging of the ear

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Introduction

IGF-1 plays important role in the developing cochlea. IGF-1 is essential for inner ear morphogenesis and maturation and its absence leads to sensorineural hearing loss. Our previous work showed the efficacy of IGF-1 for protection of cochlear hair cells against aminoglycosides and its therapeutic effect on hearing recovery in patients with sudden deafness. In the present study, we investigated the involvement of IGF-1 in age-related hearing impairment in mice.

Materials and Methods

C57Bl/6J mice at the ages of 6, 20 and 32 weeks were used in this study. The cochlear function was assessed by ABR and DPOAE. Morphological changes in cochleae were estimated in cross sections with HE staining and in surface preparation. The expression levels of genes of interest were assessed by qPCR.

Results

ABR and DPOAE demonstrated significant elevation of thresholds in 32-week-old mice in comparison with 6-week-old ones (control group), while 20-week-old mice showed significant elevation of thresholds only for 40Hz frequency. Significant loss of outer hair cells was found only in 32-week-old mice. HE staining showed degeneration of stria vascularis and spiral ganglion. qPCR showed significant differences in the expression levels of *Igf-1*, *FoxO3* and *Gsk3b* in 32-week-old mice, and for *Mtor* - in both 20- and 32 week-old mice compared with control group.

Conclusion

Our results indicate that changes in molecules associated with the IGF1/PI3K pathway may play role in age-related degeneration in cochleae of C57BL/6J mice.

O-57 Age-related changes in cochlear nuclei microglia and macrophages in the rat**Paola Perin^{1,*}, Roberto Pizzala¹**¹ University of Pavia

* Presenting author

Age-related changes in the auditory system are known to include changes in peripheral structures and central auditory circuits, from cochlear nuclei onwards [Roth 2015]. However, most CNS studies have so far focused onto neuronal responses, whereas it is becoming increasingly evident that brain aging especially impacts glial cells, shifting them towards more proinflammatory phenotypes [Soreq et al. 2017].

We compared the morphology and distribution of cochlear nuclei (CN) microglia and CN-associated macrophages between 2 month-old and 12-14 month-old Wistar rats, by immunofluorescence labeling for Iba-1 (which labels both microglia and macrophages) and P2y12r (which selectively labels microglia [Mildner 2017]). Macrophages were found associated to the pia (in the CN region not abutting the ventricular surface) and to the choroid plexus epithelium, both on the luminal side (epiplexus cells), and in the stroma.

Within the CN parenchyma, in both young and aged rats the density of Iba1+ and P2y12r+ cells was similar, although the spatial distribution was more regular in the young. Microglia from aged rats, moreover, morphologically differed from that of young rats. In particular, activated and dystrophic microglia were found in aged rats but not in young rats. Dystrophic microglia displayed tortuous processes with uneven size and intensity of Iba-1 labeling, giving the cell a very distinctive beaded or spiny appearance, and was often found in loose clusters. Activated microglial cells displaying larger soma and thicker processes were irregularly distributed. Epiplexus cells, which made up the majority of choroid plexus macrophages, also displayed age-related morphological differences in sphericity and surface complexity.

Since microglia and macrophages are main players in both neuroinflammation/inflamming [di Benedetto et al. 2017] and auditory plasticity [Janz and Illing 2014, Kaur et al. 2015], the present results suggest they may be involved in age-related hearing changes.

O-58 Subgroups of meniere's patients with different patho-morphological and clinical traits as identified by temporal bone MR-imaging

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Introduction and aims

Among patients with Meniere's disease (MD) a large variability exists with regard to the manifestation of clinical symptoms. This "phenotypic heterogeneity" is a main reason for the lack of disease-specific diagnostic criteria and it renders MD a diagnostic challenge. The existence of multiple etiopathologies and distinct clinical phenotypes in MD was proposed but, to date, has not been demonstrated. Here, we aimed to establish new clinical imaging-based criteria to distinguish distinct MD patient subgroups based on two MD-specific pathologies – degeneration and hypoplasticity – of the endolymphatic sac (ES).

Materials and methods

Retrospective study: 1) Gadolinium-enhanced magnetic resonance imaging (Gd-MRI, 3T) data of temporal bones from MD patients (n=76) was used to determine the angular trajectory (angle b) of the vestibular aqueduct (VA) in the axial plane as a radiographic distinguishing marker for ES degeneration ($b < 120^\circ$) or hypoplasticity ($b > 140^\circ$), 2) Chart review and collection of audiological/vestibular data from MD patients, 3) Statistical patient subgroup comparisons.

Results

Gd-MRI-based measurements of b identified four MD patient subgroups with uni- or bilateral radiographic signs of either ES-degeneration or -hypoplasticity. Patient subgroup comparisons revealed significant phenotypic group differences.

Conclusion

Four subgroups of MD patients with distinct pathomorphological and clinical traits were identified, using temporal bone Gd-MRI. Subgroup diagnosis in MD will in the future presumably enable a more specific diagnosis and allow to prognosticate crucial features in the course of MD for individual patients.

O-59 The effects of vestibular galvanic stimulation improving balance control of patients with myelopathy**Ludimila Labanca^{1,*}, Tatiana R Silva², Fabrice Giraudet¹, Paul Avan¹, Denise U Gonçalves²***1 University Clermont Auvergne**2 Federal University of Minas Gerais*** Presenting author*

Recent studies have shown galvanic vestibular stimulation (GVS) as a useful tool for postural instability rehabilitation. The GVS can stimulate the central nervous system and create new neuronal links which enable partial or total recovery of a function that has been lost due to neurological disorder. The myelopathy associated with T-lymphotropic virus 1 (HTLV-1) infection is characterized by medullary inflammatory alterations and ensuing postural instability. Once there is no treatment for HTLV-1 infection, this study aimed at evaluating if GVS could bring benefit to balance disorders of patients with HTLV-1 associated myelopathy (HAM). Three patients with HAM were submitted to five series of GVS, once a week, for a period of four weeks. All of them were evaluated before and after the GVS treatment (cervical and ocular vestibular evoked myogenic potentials, soleus responses, Romberg, Berg Balance Scale (BBS) and Visual Analog Scale (VAS)). After GVS sessions all patients presented instability improvement evaluated by Romberg, BBS and VAS and better performance during the walking test. This pilot study indicates a possible new therapeutic tool for HAM patients, but it still requires further investigation.

Abstracts symposium

S-1 Cochlear gene therapy for Usher III

Lawrence Lustig

S-2 Direct cortical effects of deafness genes: an insight into the contrasted results of hearing restoration

Christine Petit

S-3 **The microanatomy and ultrastructure of the human cochlea – functional aspects with special reference to cochlear implantation**

Helge Rask-Andersen and Wei Liu

Department of Surgical Sciences, Head and Neck Surgery, section of Otolaryngology, Uppsala University Hospital, Departments of Otolaryngology, Uppsala University Hospital, SE-751 85, and Uppsala, Sweden.

Today's inner ear surgery imposes better understanding of human inner ear microanatomy, ultrastructure and physiology. Ear surgeons need to be familiar with its complex anatomy and to understand its responsiveness to exposure and manipulation of the fragile structures. This presentation deals with some recent findings of the microstructure of the human cochlea relevant for hearing preservation surgery but may also improve our understanding of its physiology. Super-resolution fluorescence structured illumination microscopy (SIM) was performed at the Uppsala SciLife national facilities in Sweden (<http://www.scilifelab.se/#>). A lateral precision of approximately 80 nm and 250 nm axially were obtained. The study was based on surgically obtained material obtained after ethical consent and patient approval. The protein distribution in various cells in the human lateral wall was analyzed. We studied voltage-dependent Na⁺ and K⁺ channels and Ca⁺⁺ (VGCCs). The architecture of the human ion transport systems essential for the generation of the DC field potential such as Na⁺/K⁺-ATPase and Na⁺/K⁺-2Cl ion transporter systems, K⁺ diffusion potentials, tight junction barrier proteins and gap junction networks in the lateral wall were studied. Its architecture and relevance in hearing preservation CI-surgery is discussed. The human auditory nerve has unforeseen properties to endure despite the deterioration of the sensory epithelium, of great benefit for the CI patient. The slow retrograde degeneration may relate to the configuration and molecular expression envisaged in the human spiral ganglion cell soma. Description of microglia-associated cells presumably involved in an innate immune defensive system in the human cochlea is made.

Acknowledgements

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S-4 Temporary neurotrophic drug delivery to the inner ear for long-term effects

Dyan Ramekers, Sjaak F.L. Klis, Huib Versnel

Department of Otorhinolaryngology and Head & Neck Surgery, Brain Center Rudolf Magnus, University Medical Center Utrecht, the Netherlands

After substantial loss of cochlear hair cells, treatment with neurotrophins prevents degeneration of spiral ganglion cells (SGCs) forming the auditory nerve. For this treatment to be relevant for human cochlear implant users, the neuroprotective effect must be long-lasting, the thus rescued cells must be functional, and the means of delivery must be clinically practicable. We have shown that temporary treatment with brain-derived neurotrophic factor (BDNF), delivered with an osmotic pump, leads to SGC preservation far beyond the treatment period in deafened guinea pigs. As a direct measure of electrical excitability of the rescued SGCs, electrically evoked compound action potentials (eCAPs) indicated that the BDNF treatment led to functional preservation in addition to mere anatomical SGC preservation in these animals. Before translation to the human situation is possible, a more clinically applicable means for temporary drug delivery to the inner ear should be investigated. Preliminary data show that BDNF-soaked gelfoam on the round window membrane has similar preservative effects, both structurally and functionally, on the SGC population as the more invasive conventional delivery methods.

S-5 Translating hearing health discoveries: challenges and opportunities

Anne Schilder

S-6 Use of single cell gene expression data for reconstruction of spatial and temporal aspects of the developing, mature, and regenerating inner ear

Stefan Heller

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Flow cytometry and single cell transcriptomics are two technical advances with potential to revolutionize the molecular biological analysis of the inner ear. These technologies allow us to obtain quantitative information of mRNA and other RNA species of individual inner ear cells. The high complexity of single cell gene expression data in turn creates challenges for data analysis, visualization and communication of findings. Our laboratory has recently started with utilizing flow cytometry, single cell transcriptomics, and data analysis strategies to investigate open questions related to early development, tonotopy, and hair cell regeneration. Our work has revealed challenges and resulted in novel strategies with respect to distinguish transcriptomic features that are spatially driven from changes in cells' transcriptomes that are temporally driven such as in developing otic cell lineages.

In this presentation, I will show examples for spatial and temporal reconstruction of the developing inner ear, as well as the tonotopic map of the organ of Corti, and first results aimed at resolving the order of signaling events that initiate, execute, maintain, and terminate hair cell regeneration in the chicken inner ear. For these analyses, we have developed a novel bio-informatics algorithm called CellTrails that allows us to resolve temporal and spatial trajectories and to identify bifurcations in cell lineages. In addition, we developed a method enabling the comparison of cellular trajectories using a strategy analogous to traditional sequence alignment methods. I will show an example for utilization of our data analysis tools to extract the temporal order of hair bundle genes in the bifurcating developmental lineage of vestibular type I and type II hair cells using multiplex single cell qRT-PCR data as well as single cell RNA-Seq data.

S-7 Developing molecular therapeutics for human inner ear disease

Hinrich Staecker

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The last 30 years have seen an explosion in our understanding of the molecular basis of hearing loss. The pathways underlying the development of the ear as well as the numerous molecular components that make hearing work have been extensively explored. So far this has not translated into the development of new types of therapeutics for what is actually the most common neurodegenerative disease in man.

A significant portion of our focus has been on understanding the pathways that control the genesis of auditory and vestibular hair cells in an effort to apply this in patients. We will review some of the key discoveries in the patterning of the neurosensory epithelium of the inner ear and what factors go into taking that information and developing a hair cell regeneration drug that is testable and human subjects.

S-8 Pendrin and inner ear development

Philine Wangemann

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Mutations of *SLC26A4* /pendrin are a leading cause of hereditary hearing loss that entails a pathological enlargement of the inner ear. *Slc26a4* Δ/Δ mice lack functional pendrin expression, recapitulate the enlargement, and fail to acquire hearing and balance. Growth and development of the embryonic inner ear depends on a dynamic balance between fluid secretion and fluid absorption. Functional experiments and gene expression profiling point to the vestibular labyrinth as site for fluid secretion and the endolymphatic sac as site of fluid absorption.

The importance of the endolymphatic sac for the development of hearing is evident from the finding that restoration of pendrin expression solely in the endolymphatic sac is sufficient to prevent enlargement and to restore hearing and vestibular function in *Slc26a4* Δ/Δ mice.

Supported by NIH-R01-DC012151.

S-9 The use of electroporation therapy to improve the electro-neural interface of the cochlear implant

Jim Patrick

S-10 Hair cell and neural responses to sound from the inner ear of cochlear implant subjects: Multiple uses with potential clinical significance

Douglas Fitzpatrick

Associate Professor

University of North Carolina at Chapel Hill

Until recently, cochlear implant (CI) subjects were usually severe to profoundly deaf, suggesting that responses to sound from the inner ear should be small or absent. However, using intraoperative recordings from the round window, there is almost always some residual response to sound, and some responses are large. The responses are primarily from hair cells rather than the auditory nerve, so they are only loosely related to hearing thresholds. In addition, many current CI subjects have greater residual hearing, where large cochlear responses are expected and are indeed found.

One use of the information about residual physiology is to compare it to speech perception outcomes with electric stimulation. The 'total response (TR),' or summed spectral components in the responses to multiple stimulus frequencies, was compared to results in monosyllabic word tests in adults and children. The TR accounted for ~48% of the outcome variability in most adults and ~38% in children 6 years and older, but only ~16% in younger children. The difference in young children may relate to mostly congenital rather than progressive causes of hearing loss.

A second use is for the responses to sound to serve as a monitor for electrode position and surgical trauma during insertion. Responses can be recorded intracochlearly through the electrode array, or from an extracochlear site. Intracochlear responses sometimes increase during insertion, consistent with the tip electrode approaching responding elements, but often show no increases, and occasionally decrease. Extracochlear responses are typically flat, an expected indication of an atraumatic insertion, but sometimes decrease. With either recording location, responses can drop and then recover, which complicates an interpretation of a response drop as an indication of trauma.

The generators of the responses to sounds consist of outer and inner hair cells and the auditory nerve, which together produce the cochlear microphonic, compound action potential, auditory nerve neurophonic and summing potential. Each potential shows a wide variety of morphologies across CI subjects. Ongoing experiments in gerbils utilizing ototoxins and neurotoxins are being used to unravel these complex responses, to maximize the information about cochlear status that can be obtained.

S-11 Hearing devices bypassing the middle ear, stimulating the cochlea acoustically; a classification for patients with conductive hearing impairment**Ad Snik, Emmanuel Mylanus, Martijn Agterberg***Department of Otorhinolaryngology, Radboud University Medical Centre Nijmegen*

The healthy middle ear works as a highly efficient transformer of acoustic energy from air-borne sound waves to sound waves in the cochlear fluid. Without an effective middle ear system, e.g. in case of a bony aural atresia, hearing is poor. However, owing to the vibrating head in a sound field, the cochlea still can be activated. If the sound level exceeds 60 dB SPL, a normal functioning sensitive cochlea will detect the head vibrations; these head vibrations cause the cochlear fluid to move owing to (primarily) inertial forces. Fluid movements are possible because of the mobile cochlear windows.

To stimulate the cochlea, bypassing a malfunctioning middle ear, a vibrating mini-actuator might be applied, surgically coupled to one of the cochlear windows, or to a stapes prosthesis (if the oval window is immobile as is the case in otosclerosis). The other option is to 'shake' the head with a more massive vibrating actuator. In this case, the externally generated vibrations are well conducted by the skull bone in all directions, referred to as bone conduction.

Both solutions to bypass the middle ear are not optimal. Therefore, the amplifiers driving the actuators should be powerful, especially when using bone conduction. High amplification might lead to problems with the maximum output of the device (amplifier with actuator), feedback and device noise that might be audible when cochlear hearing is (sub) normal. In most cases, it is possible to minimize these drawbacks by using dedicated sound processing algorithms. However, such manipulations affect the gain of the device and eventually sound quality. The connection between an external sound processor and the skull (when applying a bone-conduction device) or an actuator coupled directly to the cochlea, also introduces limitations.

Nevertheless, such hearing devices are indispensable for patients with conductive or mixed hearing loss. A classification of available amplification options will be presented, based upon the maximum output and device noise. This classification includes devices using bone conduction and devices that directly stimulate the cochlea. Next, complexity of these devices, including surgical aspects, will be discussed as well as safety and stability.

Over the last decades, technological achievements in this field are impressive and, fortunately, ongoing.

S-12 Biomechanical characteristics of hearing

Alex Huber

S-13 Restoring hearing with a new auditory midbrain implant**Hubert H. Lim**

Institute for Translational Neuroscience Scholar Departments of Biomedical Engineering and Otolaryngology, University of Minnesota

The cochlear implant is considered one of the most successful neural prostheses to date, which was made possible by visionaries who continued to develop the cochlear implant through multiple technological and clinical challenges. However, patients without a functional auditory nerve or implantable cochlea cannot benefit from a cochlear implant. The focus of the talk is to review the development and translation of a new type of central auditory prosthesis for this group of patients that is known as the auditory midbrain implant (AMI) and is designed for electrical stimulation within the inferior colliculus. The rationale and results for the first AMI clinical study using a multi-site single-shank array will be presented initially. Although the AMI has achieved encouraging results in terms of safety and improvements in lip-reading capabilities and environmental awareness, it has not yet provided sufficient speech perception. Animal and human data will then be presented to show that a two-shank AMI array can potentially improve hearing performance by targeting specific neurons of the inferior colliculus and minimizing suppressive effects induced by temporal stimulation patterns presented on individual electrodes. A new two-shank AMI device has been developed that is expected to improve hearing performance over the previous single-shank device and is currently being investigated in a clinical trial funded by the National Institutes of Health. This clinical study is a joint collaboration among Hannover Medical School, International Neuroscience Institute, Cochlear Limited, Bionics Institute and University of Minnesota. Positive outcomes from this clinical trial will motivate new efforts and developments toward improving central auditory prostheses for those who cannot sufficiently benefit from cochlear implants.

S-14 Optogenetic studies of evoked potentials and behavior using a novel auditory brainstem implant based on light

Daniel Lee

Posters overview

Posters overview

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Poster abstracts

P-1 Neuro-otological treatment for patients with dementia and hearing loss in psychiatric hospital

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Introduction

Japan is a hyperaging society. The number of patients with dementia are increasing and might be 4.62 million people in 2012. We reported the audiometric tests (AT) in patients with dementia and the usefulness of hearing aid (HA).

Subjects

The subjects were 98 patients (28 men, 70 women, age range, 61-95; mean age 79.9 years) with dementia: Alzheimer's in 60 (61.2%), vascular in 9 (9.2%), mixed in 4 (4.1%), Lewy Body (DLB) in 9 (9.2%), frontotemporal (FTD) in 13 (13.3%), others in 4 (4.1%). We used revised Hasegawa's dementia scale (HDS-R) as a cognitive function test. The results were classified as follows: normal:21; mild: 1620; moderate: 1115; severe: 10. AT results were classified as follows: normal: pure tone average (PTA)25 dB; mild: 26-40dB; moderate: 4170dB; severe: 71dB.

Results

In HDS-R, 36 (36.7%) cases gave normal results, 23 (23.5%) showed mild, 22 (22.4%) showed moderate and 17 (17.3%) showed severe. In AT, on the right ear, 5 (5.1%) cases gave normal results, 22 (22.2%) showed mild, 58 (59.2%) showed moderate and 13 (13.3%) showed severe. On the left ear, 7 (7.1%) cases gave normal results, 25 (25.5%) showed mild, 44(44.9%) showed moderate and 22 (22.4%) showed severe. Bilateral AT results showed normal in 3 (3.1%) case, right hearing loss (HL) in 3 (3.1%), left HL in 3 (3.1%) and bilateral HL in 89 (90.8%). In total therefore, 95/98 (96.9%) had HL. We recommended patients with HL to wear HA and 37/95 (38.7%) of them purchased HA and 22/95 (23.1%) of them continued to wear HA.

Discussion

It is important for them to detect their HL and usefulness of HA. We have to be careful for the possibility of pseudo-dementia (cognitive dysfunctions caused by HL or earwax).

P-3 Induction of mitophagy in the HEI-OC1 auditory cell line and activation of the Atg12/LC3 pathway in the Organ of Corti**Cristian Setz^{1,*}, Yves Brand¹, Soledad Levano¹, Daniel Bodmer¹**¹ University of Basel

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Mitophagy is a selective intracellular mechanism by which malfunctioning mitochondria are removed. Since mitochondrial dysfunction has been reported in several neurodegenerative disorders, its possible involvement in acquired hearing loss remains requires investigation. Autophagy has been shown to play a role during early inner ear morphogenesis, the development of the vestibular organ as well as in adult cochlear hair cells exposed to ototoxic insults in mammals. In the past few years, autophagy has been described within auditory cells exposed to ototoxic agents. Mitophagy, a selective autophagic cell mechanism targeting mitochondria, has not yet been studied in the cochlea. In this study, we searched for molecular indicators of mitophagy within HEI-OC1 cells as well as in the organ of Corti (OC). We sought the expression of PINK1/parkin mRNA in 5-day-old C57BL/6 mice's cochleae using RTPCR, which detected all cochlear compartments. The induction of mitophagy in HEI-OC1 was detected by objectivizing the translocation of fluorescence-tagged LC3 to mitochondria using confocal microscopy after a 6-hour incubation with a well-described mitophagy-inducing agent: the mitochondrial uncoupler carbonyl cyanide m-chlorophenyl hydrazone (CCCP). Gentamicin exposure generated no mitochondrial translocation of LC3. Protein levels of COXIV, Atg5/12 and LC3 were evaluated by an immunoblot analysis after a 24-hour CCCP treatment as well as gentamicin. Our data suggests the presence of mitophagy after CCCP exposure in HEI-OC1 cells by showing a downregulation of COXIV in the immunoblot, a significant oxygen consumption rate in cells treated with CCCP as well as significant morphological changes of mitochondria by electron microscopy. We demonstrated changes in the expression of Atg12 and LC3 proteins in both the OC and HEI-OC1 cells after CCCP exposure but not after gentamicin. Our data indicate that gentamicin had no impact in the activation of mitophagy—neither in HEI-OC1 cells nor in the OC, suggesting mitophagic-independent mechanisms underlying aminoglycoside ototoxicity.

Author keywords: Autophagy / Cochlea / HEI-OC1 / Inner ear / Mitophagy / Organ of Corti

P-4 The organ of hearing and human hearing Part 2: Frequent perceptions standards

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Different people have different frequency thresholds for perception. This phenomenon does not have biophysical justification, however, the theory of hearing [1] allows establishing a functional connection between the maximum threshold frequency and the length of the cochlear duct.

For the cochlear duct, the experimental fact of G. von Békésy (1960) on reducing the perception of the frequency of sound in the high-frequency (HF) region can be taken as a statistical phenomenon: Békésy claimed that every six months a person loses 80Hz (per year ~1%) of the HF sound range. For the sounds perceived by man, we establish a statistical law that expresses the dependence of the perceived magnitude of the frequency range on time.

Suppose that during a time Δt in the HF region with the boundary frequency of perception $f=f_{mo}$, part of the perceived frequencies up to f_{max} is lost by the value $\Delta f=f_{mo}-f_{max}$ from the total sound range. It can be assumed that the reduction in the perceived range by $-\Delta f$ over time is proportional to the initial value of the range itself and the duration of the process, that is, $-\Delta f=r \cdot f_{m} \cdot \Delta t$, where r is the constant sound loss of high frequencies by the auditory organ with the unit of measurement $[r]=1/\text{year}$.

For the idea of G. von Békésy, the constant loudness of HF has the meaning of the probability of shifting the boundary of the upper threshold frequency of perceived sound towards its decrease and is an individual characteristic of each person.

Passing in our equation from finite differences to infinitesimal, we obtain the differential equation $df=-r \cdot f \cdot dt$, dividing the variables in which, we have $df/f=-r \cdot dt$.

Integrating it in definite integrals, we obtain a solution in the form of the function $f_{max}(t)=f_{mo} \exp(-rt)$, whence $r=1/t \cdot \ln[f_{mo}/f_{max}(f)]$. This equation is frequency-time law for cochlear duct.

P-5 The organ of hearing and human hearing Part 3: Age frequency standards

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Using frequency-time law for cochlear duct $r=1/t \cdot \ln[f_{\text{mo}}/f_{\text{max}}(f)]$ and the idea of G. von Békésy [1] about the rate of shifting the boundary of the upper threshold frequency of the perceived sound in the direction of its decrease as the initial condition, we calculate the value of the coefficient of soundness of high frequencies. Since, according to G. von Békésy, for a typical process, a person for the first year of life ($\Delta t=1$ year) loses perception of the upper frequency of sound by approximately 1%, then after this time interval its threshold boundary decreases from $f_{\text{mo}}=20\text{kHz}$ to $f_{\text{max}}=0.99 f_{\text{mo}}=19.8\text{kHz}$. Then $r=1/\Delta t \cdot \ln(f_{\text{mo}}/f_{\text{max}})$ quantitatively $r=0.01/\text{year}$.

The solution of the differential equation [2] $f_{\text{max}}(t)=f_{\text{mo}} \cdot \exp(-rt)$ is the time-frequency law of the age evolution of the cochlear duct, showing the rate of contraction of the upper perceived frequency under ideal conditions.

It can be assumed that the lower frequency varies according to a similar law, but with an increase in the limit. Changing the sign in the exponent, we can write $f_{\text{min}}(t)=f_0 \cdot \exp(-rt)$, where $f_0=20\text{Hz}$ is the lower limiting frequency of the perceived sound. The actual hearing will have an individual value of the r -coefficient for each patient.

Thus, we came to the conclusion that the decrease in the upper and the growth of the lower threshold boundary of the perceived frequencies occurs according to an exponential law.

P-6 The antioxidant N-Acetyl-L-Cysteine (NAC) as a pharmacological candidate for age-related hearing loss**Sergio Gonzalez-Gonzalez^{1,*}, Aurore Marie¹, Susanna Malmstrom¹, Sylvie Cosnier-Pucheu¹**¹ CILcare

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Introduction

Age-related hearing loss (ARHL) is the most common sensory disorder in the elderly population. The senescence-accelerated prone strain 8 (SAMP8) mouse model presents accelerated senescence and has been identified as a model of gerontological research. SAMP8 displays sequential degeneration of cochlear hair cells, spiral ganglion neuron and stria vascularis which mimic human ARHL. The molecular mechanisms associated with SAMP8 senescence involve oxidative stress and altered levels of antioxidant enzymes leading to chronic inflammation and apoptosis. Here, we studied the effect of NAC, an antioxidant, on SAMP8 hearing loss to determine the potential interest of this model in the study of new therapies.

Material and methods

To characterize hearing loss in SAMP8, we added NAC in the drinking water at 61 mM and we measured the auditory brainstem response (ABR) and the distortion product otoacoustic emissions (DPOAEs), two auditory parameters, every two weeks during two months.

Results

We observed a strong decrease of ABR thresholds at all frequencies and a significant increase of DPOAE amplitude in the NAC treated group compared to vehicle.

Conclusion

NAC reduces the accelerated senescence process by decreasing ABR thresholds and protecting cochlear hair cells, strongly suggesting that antioxidants could be a pharmacological target for ARHL.

P-7 Deficiency of mitochondrial tRNA modification causes progressive hearing loss.

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Introduction

Mitochondrial dysfunction is considered to be associated with aging and age-related hearing loss. However, the detailed mechanism and pathophysiology of hearing loss remain unknown.

Transfer RNAs (tRNAs) contain a wide variety of posttranscriptional modifications that are important for accurate decoding. Mammalian mitochondrial tRNAs (mt-tRNAs) are modified by nuclear-encoded tRNA-modifying enzymes (Wei, 2013). Cdk5 regulatory subunit-associated protein 1 (cdk5rap1) is responsible for 2-methylthio (ms2) modifications of mt-tRNAs. And deficiency in ms2 modification markedly impaired mitochondrial protein synthesis. This resulted in respiratory defects in cdk5rap1 knockout (KO) mice.

We reported the influence of a mitochondrial dysfunction caused by the ms2 modifications of mt-tRNAs on age-related change utilizing cdk5rap1KO cells last year, however, cdk5rap1 ms2 modification deficiency is still unknown to affect with hearing function in vivo. We herein investigated the influence of mt-tRNA modification of hearing function utilizing cdk5rap1KO mice.

Materials and Methods

Auditory brainstem Response (ABR) was measured in cdk5rap1 KO mice and hetero mice at postnatal 1, 3, 5, 12 months. Each stage cochlea was dissected, cryosectioned and immunostained with anti-Tuj1 antibody to count the number of spiral ganglion cells. Other cochlea was demonstrated surface preparation study.

Results

ABR thresholds in cdk5rap1 KO mice (postnatal 1, 3 months) showed no difference from hetero mice on the same stage. While, ABR thresholds in cdk5rap1 KO mice (postnatal 5, 12 months) showed significantly higher any frequency than those in hetero mice at the same stage. The numbers of spiral ganglion cells in cdk5rap1 KO and hetero mice (postnatal 5, 12 months) decreased compared to those in cdk5rap1 KO and hetero mice (postnatal 1, 3 months). The numbers of spiral ganglion cells in cdk5rap1 KO mice (postnatal 5, 12 months) decreased compared to those in hetero mice at the same stage.

Conclusions

Our previous study and these results suggest that ms2 modifications of mt-tRNAs may induce an apoptotic program in the spiral ganglion cells and accelerated their aging, thereby causing aging-hearing loss. However, other factors must be investigated.

Author keywords: Progressive hearing loss / Mitochondrial dysfunction / Mitochondrial tRNA modifier / cdk5rap1

P-8 The organ of hearing and human hearing Part 1: Biophysical substantiation of age standards

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According to the basic equation of the morphofunctional acousto-wave theory of hearing [1], the maximum possible frequency f_m of the perceived sound is associated with the length of real cochlear duct $L_r = L_o \cdot 2^{\log(f_m / f_{mo})}$ with standard length of the cochlear duct $L_o = 32\text{mm}$, perceiving the maximum frequency $f_{mo} = 20\text{kHz}$.

Theoretical justification suggests that this correspondence is caused by destruction of the cochlear duct with time and a reduction in its length [2]. Let's justify this: let the sound waves act on the apical bundle of cochlear duct membranes from the moment of human birth for t years. During this time its length will decrease from initial (standard) to $L < L_o$.

This ratio assumes that the natural course of the process leads to the fact that amount of destruction ($-\Delta L$) is directly proportional to the length of the cochlear duct and the duration of the process $\Delta L = k \cdot L \cdot \Delta t$, where the positive coefficient k (with unit of measurement $[k] = 1/\text{year}$) determines the relative decrease in length of cochlear duct during its age evolution per unit time $k = -(\Delta L / L) / \Delta t$. This coefficient k can also be defined as the rate of linear destruction of the cochlear duct during its natural development.

Passing in the last formula to differentials, we obtain the equation $k = -(dL/L)/dt$, integrating, we obtain the law of age evolution of the cochlear duct $L(t) = L_o \cdot \exp(-kt)$.

The value of the coefficient k is determined by equality of the basic equation of the acoustic theory to the last established relation, whence $k = 2 \cdot \ln 2 \cdot \log(f_{mo} / f_m)$.

It's found that for every six months a person loses 80Hz in the region of high-frequency sounds. Calculations in a result (for a standard cochlear duct) $k = 0.0060/\text{year}$.

The obtained ratio is of great practical importance: it allows using experimental data to predict not only standard but also real auditory processes, and also to study reconstruction of auditory effects.

P-9 The organ of hearing and human hearing Part 4: Age morphological standards

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According to the acoustic theory of hearing [1], there is a functional relationship between the maximum perceived frequency of sound and the linear biological parameters of the inner ear, which are the length of the cochlear duct L_d and the width of the ligament of its L_a membranes in the apical zone. Using this relationship, we obtain the linear-temporal law of the age evolution of the cochlear duct of the human inner ear $L_d(t) = L_o \cdot \exp(-kt)$ and for the apical membrane ligament the analogous equation $L_a(t) = L_{ao} \cdot \exp(kt)$. In these ratios, k is the speed of contraction of the cochlear duct and the rapidity of growth of the width of the apical ligament with the unit of measurement $[k] = 1/\text{year}$, $L_o = 32\text{mm}$ is the length of the standard cochlear duct that receives the maximum frequency $f_{mo} = 20\text{ kHz}$, $L_{ao} = 0.5\text{mm}$ - width of the apical ligament of the standard cochlear duct.

Calculations show that under standard conditions the coefficient $k = 0.006/\text{year}$. This approach can lead us to calculate the working length of the cochlear duct $L_e = L_d - L_a$ - that part of it that converts sound energy into the energy of auditory sensation.

For a real ear, the value of the coefficient k can be different due to other external conditions and possible pathological conditions, more often, towards an increase: the real coefficient $kr = k + \Delta k$, so $L_d(t) = L_o \cdot \exp(-kr \cdot t)$ and $L_a(t) = L_{ao} \cdot \exp(kr \cdot t)$. In this case, the length of the actual cochlear duct $L_e = L_o \cdot \exp(-kr \cdot t) - L_{ao} \cdot \exp(kr \cdot t)$. The degree of destruction of biological structures and the degree of change in linear parameters can be characterized by $\epsilon L = \Delta L_e / L_e = (L_e - L_e r) / L_e$, where $L_e r$ is the working length of the actual cochlear duct.

It can be shown that the rate of shortening of the cochlear duct k and the frequency coefficient of sound loss r are related to each other by the relation $k = 2 \cdot \ln 2 \cdot r$.

P-10 Central Auditory Processing on HTLV-I: a case report**Aline Castro^{1,*}, Ludimila Labanca¹, Luciana Resende¹, Denise Gonçalves¹***1 Universidade Federal de Minas Gerais*** Presenting author*

Brazil is one of the highest endemic area of Human T-cell Lymphotropic Virus type 1 infection worldwide. HTLV-I associated myelopathy/tropical spastic paraparesis (HAM/TSP) is a progressive disease whose inflammatory nature can affect the entire neuraxis. Auditory-induced electrophysiological measures such as P300 have shown association between cognitive decline and HTLV-1. However, its influence on Central Auditory Processing (CAP) has not been investigated. This study aims to report major findings at CAP assessment of a 51 years old HTLV-I infected man. He was submitted to the following tests to assess the peripheral and central pathways: pure tone and speech audiometry, P300, Staggered Spondaic Words (SSW), Synthetic Sentences Identification (SSI), Masking Level Difference (MLD), Pitch Pattern Sequence (PPS), Gaps in Noise (GIN) and Speech in Noise (SNT). Mean pure tone audiometry threshold was 20 dBHL with 100% of speech recognition. P300 had increased latency (401,97ms). SSW showed a large number of right-ear errors (55%) and Type A pattern. Ipsilateral SSI was altered on the left ear (50%). The functional deficits observed may be related to inefficient nerve conduction along the central auditory pathway or the inability of primary cortex to integrate information between the hemispheres or with associative areas. Only tests with higher cognitive demand and linguistic load showed abnormality, suggesting top-down influence upon auditory perception. Considering patient's normal hearing sensitivity, the HTLV-I may be the cause of individual's cognitive deficit that negatively affects the auditory functional response, which is something unprecedented since HAM/TSP is an eminently medullary disease.

P-11 Auditory training to remediate Auditory Processing Disorder: effectiveness and the role of adherence

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Objective

To investigate the effectiveness of Auditory Training (AT) in Auditory Processing Disorder (APD) and the role of adherence to AT.

Design

Quasi-experimental study.

Sample

Fifty participants with APD, aged from eight- to 16-year-old, were equally divided into two groups.

Method

All participants underwent initial and final Auditory Brainstem Response (ABR), Middle Latency Response (MLR), P300, Staggered Spondaic Words (SSW), Pitch Pattern Sequence (PPS), Masking Level Difference (MLD) and Speech in Noise (SiN) tests. Trained group (G1) underwent eight 40-minutes AT sessions and received guidance and material to practice unsupervised AT at home. G0 (control) was assessed at equal time intervals, but was not enrolled in AT. Patient adherence was considered at least 15 minutes of unsupervised AT three times a week. ANOVA and T-tests were performed using SPSS 19.0 and considering $p < 0.05$.

Results

G1 showed greater improvements in final assessment of electrophysiological and behavioral tests. MLR amplitude ($p < 0.001$; CI: -0,616/- 0,274), P300 latency ($p = 0.004$; CI: 6,917/35,563), P300 amplitude ($p = 0.001$; CI: -2,366/-0,628) and PPS score ($p = 0.005$; CI: -24,101/-4,571) were the most sensitive measures of AT effects. AT adherence (72%) was related to significant improvement in MLR amplitude ($p < 0.001$), P300 latency ($p = 0.007$) and amplitude ($p = 0.006$), and a decreased number of auditory impaired skills. Caregiver's perception of improvement was greater in participants who showed better electrophysiological and behavioral responses on final assessment.

Conclusion

AT improved auditory skills and neural synchrony in APD children and adolescents. Behavioral and electrophysiological measures and caregiver's perception of improvement were better amongst adherent patients. Thus, patient adherence is key to AT effectiveness.

P-12 The myelin protein zero-deficient mouse as a new animal model for auditory neuropathy

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Introduction

Auditory Neuropathy (AN) is characterized by the dysfunction of the hair follicle synapses or the cochlear nerve, while the hair cells have a physiological function. The affected patients have a hearing impairment with a very limited language comprehension. Patients with hereditary polyneuropathies can also suffer from an AN and have a limited hearing ability in addition to the motor and sensitive disorders. The P0-deficient mouse is an established model of Charcot Marie Tooth Disease (CMT), a severe form of polyneuropathy. These animals exhibit pronounced hypomyelination and an axon loss of peripheral nerves.

Material and methods

In order to evaluate whether there is also a hearing impairment in P0-deficient animals, P0^{-/-} mice and wild-type control animals at the age of 3, 6, 9 and 12 months were studied by hearing physiology and histology. The frequency-specific brain stem audiometry (BERA) and the investigation of the otoacoustic emissions (DPOAE) were performed. The cochlea and auditory nerves were examined immunohistologically and electron microscopically.

Results

At the age of 9 months, P0^{-/-} animals in the BERA had a significantly increased hearing threshold compared to the control animals, while the DPOAE showed a regular function of the cochlear amplifier and thus the outer hair cells. Histologically, there was no significant difference in the number of outer hair cells and spiral ganglion cells to the control animals. The peripheral portions of the auditory nerves showed signs of dysmyelination and reduced axonal diameters.

Conclusions

The P0-deficient mouse represents a suitable animal model for the AN, as morphological and physiological similarities of this disease are present. Therefore, this mouse model could lead to a better understanding of the progressive development of this disease and the study of possible new therapeutic approaches.

P-14 Glucocorticoid receptor mediated stress worsens sound responsiveness and central neural gain after acoustic trauma**Philipp Eckert^{1,*}, Marie Manthey¹, Kamyar Kasini¹, Mirko Jaumann¹, Wibke Singer¹, Ulrike Zimmermann¹, Marlies Knipper¹, Lukas Rüttiger¹***¹ University of Tübingen - Tübingen Hearing Research Center*** Presenting author*

Recent findings suggested that stress induced through a social stress paradigm exhibits an impact on IHC synapse vulnerability (Singer et al. 2013). As Glucocorticoid Receptor GR and Mineralocorticoid Receptor MR are expressed in mature hair cells and spiral ganglion neurons (Terakado et al. 2011; Yao and Rarey 1996), a direct effect of GC on sound processing in addition to its anti-inflammation actions (O'Byrne and Pedersen 1998) has to be considered. To investigate the influence of GC on hearing in more detail, we analyzed to what extent endogenous glucocorticoid levels in individual rat specimens with and without acoustic trauma might modify auditory processing. We tested (i) the number of CtBP2/RIBEYE-positive ribbon synapses of the IHC as a measure for the number of auditory nerve fiber contacts and thereby elevated the degree of altered cochlear input (Kujawa and Liberman 2009) (ii) the DPOAEs as measure of OHCs electromechanical responses (iii) the auditory brainstem response (ABR) thresholds and the amplitudes of the first ABR wave, which reflects the summed activity of the auditory nerve (Johnson and Kiang 1976) as well as the amplitudes of the ABR wave IV, which is generated in the lateral lemniscus and inferior colliculus (IC) (Melcher and Kiang 1996) as an indicator for the disproportionally elevation is a correlate of central neural gain (Heeringa and van Dijk 2016; Rüttiger et al. 2013; Singer et al. 2013). (iv) We measured the urinary corticosterone level (the prevailing stress hormone in rodents) and (v) the influence of mifepristone as GR antagonist and spironolactone as MR antagonist. We observed a striking GR rather than MR mediated contribution to acoustic trauma induced auditory processing deficits. The results will be discussed in the context of stress contribution to hidden hearing loss.

P-15 The Ca²⁺ channel $\alpha 2\delta 3$ subunit expressed in spiral ganglion neurons is a candidate for an auditory processing disorder**Simone Kurt^{1,*}, Jutta Engel¹**¹ CIPMM

* Presenting author

Although many forms of hearing impairment and deafness in men result from deficits in the auditory periphery, particularly the cochlea, hearing disorders exist that reside in the central auditory system, which is responsible for transferring, processing and finally perceiving the auditory information. Mice lacking the auxiliary $\alpha 2\delta 3$ calcium channel subunit of voltage-gated Ca²⁺ channels have practically normal hearing thresholds but distorted ABR waveforms pointing to an auditory processing disorder. $\alpha 2\delta 3$ is expressed in spiral ganglion neurons and is essential for a normal morphology and structure of auditory nerve fibre terminals, the endbulbs of Held (Pirone*, Kurt* et al., J Neurosci 2014).

Using electrophysiological recordings from mouse auditory midbrain we examined the impact of endbulb of Held synapse malfunction for temporal coding. Temporal coding deficits in turn lead to auditory discrimination learning deficits as we have analyzed in behavioral experiments. Taken together, our results demonstrate a causal link between the function of specific (endbulb of Held) synapses at the junction between the peripheral and central auditory pathway and impaired central processing and perception, which might be responsible for auditory processing disorders.

P-16 In vivo single unit recordings from the inferior colliculus of otoferlin Ile515Thr mutant mice**Maïke Pelgrim^{1,*}, Gulnara Yamanbaeva¹, Ellen Reisinger¹, Nicola Strenzke¹**¹ University Medical Center Göttingen

* Presenting author

Otoferlin is an inner hair cell specific protein, which is essential for hearing. In its absence neurotransmitter exocytosis is abolished. In human patients the Ile515Thr point mutation causes only a mild increase in hearing thresholds, but a severe speech perception deficit, impaired adaptation to continuous sounds and auditory fatigue. Mice with this mutation reflect this auditory synaptopathy. In the Otof^{I515T/I515T} inner hair cells the amount of otoferlin at the membrane of the active zones is reduced and synaptic vesicles are enlarged. This indicates that otoferlin is critical for the reformation of properly sized and fusion-competent synaptic vesicles.

We now analyze how this peripheral deficit affects sound encoding in higher brain areas of the auditory pathway, particularly the inferior colliculus.

Single units from auditory nerve fibers of Otof^{I515T/I515T} mice show normal spontaneous spiking, frequency tuning and thresholds, but reduced spike rates with a striking dependence on repetition rate and stimulus duration. In the inferior colliculus similar changes could be observed. Its neurons show normal spontaneous spiking, frequency tuning and thresholds, but a decreased spike rate when challenged with long stimuli. The phase locking to amplitude modulated tones is impaired and a longer silent interval between paired tones was required to detect the second tone.

Single unit recordings from Otof^{I515T/I515T} mice indicate an unusual sound encoding deficit with a usedependent reduction of spike rates. We believe that this reflects an impaired vesicle reformation at the inner hair cell ribbon synapse due to reduced levels of functional otoferlin. The peripheral deficit in the encoding of amplitude modulated and paired tones cannot be fully compensated up to the level of the inferior colliculus, although the defect seems not to be as severe as in the auditory nerve. The resulting gap detection deficit likely contributes to the communication problems of human patients with otoferlin mutations.

P-17 Changes in longitudinal eCAP measures following ototoxic treatment indicate progressive auditory nerve degeneration

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The electrically evoked compound action potential (eCAP) is thought to contain a wealth of subject-specific information that could be used to the benefit of individual cochlear implant (CI) users. Especially within-subject variability (either across ears or over time) may prove to be a reliable indicator of cochlear health, as it eliminates substantial cross-subject variability. Another way to evaluate variability is to assess the effect size when manipulating the shape of the current pulse. In the present study we recorded eCAPs longitudinally in guinea pigs with varying phase duration and inter-phase gap (IPG).

Normal-hearing guinea pigs were implanted with an intracochlear electrode array. Four weeks after implantation the animals were deafened by co-administration of kanamycin and furosemide. Using a MED-EL PULSAR CI, awake eCAP recordings were performed at least weekly during the entire period of implantation (up to 11 weeks). In each session eCAPs were recorded in response to single biphasic current pulses of which the current level, phase duration and inter-phase gap were systematically varied as previously described (Ramekers et al. [2014] *J Assoc Res Otolaryngol* 15: 187-202).

Gradual changes in eCAPs over time after implantation involved increases in maximum amplitude and slope, and a decrease in threshold. After deafening the amplitude and slope progressively decreased; remarkably, a transient decrease in threshold was observed, which was restored to normal levels typically within a week. The eCAP latency decreased immediately after deafening, and fluctuated over the course of subsequent weeks. The introduction of an IPG resulted in changes in these eCAP measures that corresponded to values previously observed in between-subject comparisons (e.g., Ramekers et al. 2014). Most noticeably, before deafening the eCAP latency was independent of IPG, but gradually the eCAP latency increased, matching the IPG increase several weeks after deafening.

We conclude that performing eCAP recordings in awake guinea pigs is feasible, even with current levels needed to evoke saturation-level eCAPs. The stability of eCAP recordings over time allows for a close examination of changes in neural responsiveness to CI stimulation. Whereas absolute eCAP measures varied greatly across animals but also within-subject over time, the subtle IPG effects are much more suitable for the longitudinal assessment of cochlear health, as well as across-subject comparison.

P-18 Developmental localisation of lipoma HMGIC fusion partner-like 5 (LHFPL5) and transmembrane channel-like protein 1 (TMC1) in mouse hair cell bundles

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LHFPL5 (aka TMHS; Xiong et al, 2012) and TMC1 (Pan et al, 2013) are thought to be part of the mechano-electrical transducer channel complex at stereociliary tips. TMC1 and transduction are absent in LHFPL5 knockouts, whilst LHFPL5 binds to protocadherin 15 and thus may be targeting TMC1 for activation by the tip link (Beurg et al., 2015). We have examined the developmental distribution of LHFPL5 and TMC1 using immunofluorescence and immunogold labelling. Cochleae from CD/1 mice aged between P0 and P21 were fixed in 4% paraformaldehyde and incubated in anti-LHFPL5 antibody or anti-TMC1 antibody and then either fluorescent secondary antibody for confocal microscopy or 10 nm gold-conjugated secondary antibody for transmission electron microscopy (TEM). Fluorescence intensity for LHFPL5 is high in P0 and P3 hair bundles, being reduced from P6 onwards; quantitative TEM shows a similar change. By TEM at P0, LHFPL5 is present along the lengths of stereocilia including the tips, by P3 on the tips, lateral and ankle links of all stereocilia, and by P12 and P21 predominantly on the tips of shorter stereocilia in the ranked rows. Faint fluorescent labelling for TMC1 was observed occasionally at P9, but more strongly and consistently at P12 and P21. Preliminary immunogold labelling at P9 shows similar localisation to LHFPL5, i.e. primarily at the tips. These data suggest that although LHFPL5 is expressed from P0 onwards it is refined to the shorter stereociliary tips in maturing hair bundles. Since TMC1 also seems to be expressed on the tips, this confirms that these two proteins may interact to form part of the MET complex. The presence of LHFPL5 from P0 may imply that it is available to stabilise and target TMC1 as soon as the latter is expressed by the hair cell.

P-19 Computed tomography and magnetic resonance imaging evaluation in pediatric unilateral sensorineural hearing loss**Bo Gyung Kim^{1,*}, Se A Lee¹, Sang Woo Sun¹, Jae Hyun Jung¹, Jong Dae Lee¹***¹ Soonchunhyang University*** Presenting author***Objectives**

Children with unilateral sensorineural hearing loss (USNHL) are not actively evaluated by physicians. The diagnostic tool for evaluation of USNHL is also controversial, and no strategy for diagnosing USNHL through imaging studies has been established. We examined the results of temporal bone computed tomography (TBCT) imaging and magnetic resonance imaging (MRI) studies on children with USNHL.

Methods

Eighty-nine patients with USNHL were reviewed. Of these patients, 21 underwent both TBCT and MRI, 51 underwent temporal MRI only, and 17 underwent TBCT only.

Results

The etiology of USNHL could be determined through imaging studies in 20 patients. The most common abnormal finding (65%) was a narrow internal auditory canal (IAC), compared to that of a normal ear, identified on TBCT, or cochlear nerve aplasia on temporal MRI. Incomplete partition (20%), common cavity (10%), and labyrinthitis ossificans (5%) were seen in imaging studies. The hearing threshold was lower in USNHL patients with normal findings (76.1 ± 28.7 dB) than in USNHL patients with abnormal findings on TBCT or temporal MRI (100.1 ± 22.3 dB).

Conclusion

Cochlear and cochlear nerve abnormalities can be detected through imaging studies in approximately 25% of patients with USNHL. Therefore, we suggest that children should undergo TBCT after USNHL is confirmed through audiologic evaluation.

P-20 The human “cochlear battery” – Claudin-11 barrier and ion transport proteins in the lateral wall of the cochlea**Wei Liu^{1,*}, Annelies Schrott-Fischer², Rudolf Glueckert², Hao Li¹, Charlotta Kämpfe¹, Helge Rask-Andersen¹***1 Uppsala University Hospital**2 Medical University of Innsbruck*** Presenting author***Background**

The cochlea produces an electric field potential essential for hair cell transduction and hearing. This biological “battery” is situated in the lateral wall of the cochlea and contains molecular machinery that secretes and recycles K⁺ ions. Its functioning depends on junctional proteins that restrict the para-cellular escape of ions. The tight junction protein Claudin-11 has been found to be one of the major constituents of this barrier that maintains ion gradients (Gow et al. 2004, Kitajiri et al. 2004). We are the first to elucidate the human Claudin-11 framework and the associated ion transport machinery using super-resolution fluorescence illumination microscopy (SR-SIM).

Methods

Archival cochleae obtained during meningioma surgery were used for SR-SIM together with transmission electron microscopy (TEM) after ethical consent.

Results

Claudin-11-expressing cells formed parallel tight junction (TJ) lamellae that insulated the epithelial syncytium of the stria vascularis and extended to the suprastrial region. Intercellular gap junctions were found between the barrier cells and fibrocytes

Conclusions

TEM, confocal microscopy and SR-SIM revealed exclusive cell specialization in the various subdomains of the lateral wall of the human cochlea. The Claudin-11-expressing cells exhibited both conductor and isolator characteristics, and these micro-porous separators may selectively mediate the movement of charged units to the intrastrial space in a manner that is analogous to a conventional electrochemical “battery.” The function and relevance of this battery for the development of inner ear disease are discussed.

P-21 The study of the stria vascularis changes of the inner ear on the model of aminoglycoside antibiotic ototoxicity**Natalia Petrova^{1,*}, Vera Perevozchikova¹, Ekaterina Petrova¹, Evgeniya Ilinskaya¹***1 North-Western State Medical University named after I.I. Mechnikov*** Presenting author***Introduction**

The problems of the etiopathogenesis of sensorineural hearing loss are actively studied by scientists all over the world. The leading role in the development of hearing loss is given to the stria vascularis changes.

Objective

To study the stria vascularis ultrastructural changes of the animals inner ear in with experimental sensorineural hearing loss.

Materials and methods

The study includes 16 healthy guinea pigs, which were divided into 2 groups (8 animals in each): experimental - received gentamicin in ototoxic dose; intact group did not receive any drugs. After modeling sensorineural hearing loss by means of gentamicin-sulfate in ototoxic dose, by the method of in vivo isolation snails was extracted the vascular streak of an inner ear, after which the standard preparation and staining with hematoxylin and eosin was subjected to morphological study using light microscopy.

Results

The ultrastructure of the stria vascularis in the intact group was characterized by the absence of expansion of intercellular spaces, preserved cytoplasm, moderately expressed secretory activity, normal mitochondria. Distinctive features of the stria vascularis structure in animals group receiving gentamycin were stasis and fullness of capillaries, adherent erythrocytes in the lumen of the vessel, expansion of intercellular spaces, reduction and deformation of cells, coarsely dispersed condensed chromatin, rarefaction of the cytoplasm, destruction of cristae and rarefaction of the mitochondrial matrix, secretory activity, cell destruction.

Conclusion

Thus, deep changes in both blood vessels and epithelial cells of the vascular stripe have been revealed, with experimental hearing loss caused by aminoglycoside antibiotics, which is probably the morphological substrate of developing hearing loss.

P-22 Monitoring of the inner ear function during and after cochlear implant insertion using Cochlear Microphonics

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Introduction

To preserve residual hearing during cochlear implant (CI) surgery it is desirable to use intraoperative monitoring during the electrode insertion. A promising method is the recording of cochlear microphonics (CM). The aim of the monitoring is to identify critical steps as well as to modify the ongoing insertion procedure immediately if necessary.

Method

During the insertion of hearing preservation electrodes, different modes of intraoperative CM recordings were performed. In one mode the potentials were recorded extracochlear using a cotton wick electrode at the promontory wall before, during and after insertion. In a second mode the potentials were recorded intracochlear during, or directly after, insertion and postoperative during the follow up appointments. Here the CI electrode was used for recordings. The stimulation was done acoustically using tone bursts. The extracochlear recordings were done up to now with 50 patients, the intracochlear recordings with 30 patients (MedEl and Advanced Bionics).

Results

Extracochlear recorded CMs showed peaks of maximal 0.5 μV in the according spectra for most patients. Intracochlear peaks of up to 30 μV were detected. In the first data, the amplitude of long term CMs seem to be in line with the audiometrical pure tone thresholds.

Conclusion

The recording of CMs is very good possible with all methods. The amplitudes of intracochlear recorded CMs were detected to be much larger than the extracochlear recorded CMs.

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P-23 Cochlear optogenetics drives avoidance behavior in normal hearing and deaf gerbils**Alexander Dieter^{1,*}, Christian Wrobel², Daniel Keppeler¹, Gerhard Hoch¹, Marcus Jeschke³, Tobias Moser⁴***1 Institute for Auditory Neuroscience and InnerEarLab, University Medical Center Göttingen**2 Institute for Auditory Neuroscience, Collaborative Research Center 889, University of Göttingen; Department of Otolaryngology, Ruhr University Bochum**3 Institute for Auditory Neuroscience, University Medical Center Göttingen; Auditory Neuroscience and Optogenetics, German Primate Center, Göttingen**4 Institute for Auditory Neuroscience, Collaborative Research Center 889, Auditory Neuroscience and Optogenetics, German Primate Center; Göttingen*** Presenting author*

Electrical stimulation of spiral ganglion neurons (SGNs) via cochlear implants (CI) represents the state of the art means of hearing restoration in profoundly hearing impaired, enabling speech recognition in most users. However, the electric current spreads in the saline environment of the cochlea, which in turn leads to activation of large subsets of SGNs, limiting the number of independently usable stimulation channels. This inherent limitation of CIs could be overcome by using optogenetic stimulation. Here, SGNs are genetically modified to express channelrhodopsin_b and subsequently stimulated with light. Focusing light to activate small subsets of SGNs would then allow to increase frequency resolution of artificial sound encoding.

Here, channelrhodopsin-2 variant CatCh was injected into the spiral ganglion of adult gerbils, leading to light sensitivity of SGNs and an optical fiber was implanted into the cochlea. Using a shuttlebox paradigm animals were trained on a detection task in which they learned to avoid mild electrodermal stimuli to the feet upon perception of a stimulus (blue laser pulse delivered through the optical fiber) via locomotion. A different set of animals was trained acoustically and deafened afterwards in which we restored avoidance behavior cued by optogenetic stimulation but not acoustic stimulation.

We demonstrate that optogenetic stimulation of SGNs drives avoidance behavior with thresholds as low as 2.5 mW (for 1 ms pulses) and 0.1 ms (at 25 mW) in normal hearing animals. Furthermore, optogenetic stimulation could restore avoidance behavior in acoustically pre-trained animals in a gerbil model of ototoxic deafness. Finally, avoidance behavior that was trained using optogenetic stimulation was transferred to acoustic stimulation and vice versa, suggesting similarity of percepts.

In conclusion, this study demonstrates that stimulation of channelrhodopsin-expressing SGNs with blue light creates a percept strong enough to cue behavior in both normal hearing and deaf animals at least for several weeks.

P-24 Hearing bionic rehabilitation in a child with bilateral cochlear and facial nerve dysplasia**Marchioni D., Carner M., Bianconi L.** *(ENT Department, University of Verona)***Genovese E., Palma S.** *(ENT Department, University of Modena)*

A male newborn presented a right facial nerve paralysis and was referred to our clinic by the Hearing Screening Program with bilateral FAIL at otoacoustic emission test. We performed auditory brainstem response (ABR) and could not identify the wave V at 90 db bilaterally. The patient was studied with a brain MRI. On the right side only a tiny vestibular nerve was detected, the facial nerve was spotted outside the IAC, anteriorly. On the left side the facial nerve was very thin, the vestibular nerve was not detectable. The patient was wearing bilateral hearing aids, since four months of age, with no acoustic beneficial. When parents accepted surgery, he underwent ABR and electrocochleography (ECoG) under general anaesthesia: on the right side the wave V was identified at 120 dB HL, on the left side there wasn't any brainstem response even at the highest stimulation. During ECoG testing only on the right side it was possible to identify the action potential (AP) at 90 dB HL, latency 2.30 ms. We decided to perform an auditory brainstem implant (ABI) on the left side and a cochlear implant (CI) on the right side to give the patient the best chances of rehabilitation. On the left side the anatomy of the cerebellopontine angle (CPA) was abnormal, the trigeminal nerve (V) had an anomalous partition and the facial nerve was identified very anteriorly, while no cocleovestibular nerve was evidenced. On the right side the CI was positioned through a promontorial cochleostomy. Ossicular chain and lateral semicircular canal anomalies were evidenced. One month after surgery the ABI was activated and two months later the child presented a score of 2 at the Category of Auditory Performance (CAP) test. The CI, activated three months after surgery, consolidated the hearing performance of the child with the ABI and allowed the detection of all sounds at the Ling Six Sound test.

P-25 The effect of platinum black plated electrodes in EABR measurement**Hideaki Ogita^{1,*}, Koji Nishimura¹, Juichi Ito¹***¹ Shiga Medical Center Research Institute*** Presenting author*

Platinum black has the porous structure, which enlarges the surface area and lowers the impedance. However, platinum black electrodes have not been used in cochlear implants because platinum black electrodes can lose part of their platinum during mechanical insertion, and deteriorate in biological fluids (G. M. Clark et al., 1977). In recent years, some processing methods have been reported which can fabricate mechanically stable platinum black (S.A. Desai et al., 2010, R. Kim et al. 2013).

We are developing a new cochlear implant (CI). Decreasing the power consumption is one of the tasks for designing the new CI, because the output of our new CI is limited. Decreasing the impedance of the electrode-electrolyte interface is one of the solutions to decrease the power consumption. In the present study, we evaluate the effect of platinum black electrodes for decreasing the resistance by using electrically evoked auditory brainstem responses (EABRs).

We used two kinds of ball electrodes. One of the electrodes was plated with platinum black. The other was pure platinum. The diameter of the ball was about 0.6mm. We used Hartley guinea pigs as experimental animals. We inserted those electrodes into the scala tympani of the cochleae, and we measured EABRs. Stimuli were produced by a constant-current stimulator. They had 100 μ s of biphasic rectangle waves. The intensity was increased by 10 μ A step. During the EABR measurements, we measured the voltage for stimulation simultaneously. We compared the threshold and the voltage between platinum black electrodes and pure platinum electrodes.

P-26 Polydopamine coating of cochlear implant electrode arrays enables stable colonization with adipose-derived stem cells**Philipp Schendzielorz^{1,*}, Andreas Radeloff², Kristen Rak¹, Sebastian Schraven¹, Johannes Völker¹, Rudolf Hagen¹***1 University Hospital Würzburg**2 University Hospital Oldenburg*** Presenting author***Introduction**

Adipose-derived stem cells (ASC) produce growth factors and neurotrophic factors that enhance survival of spiral ganglion neurons and neurite outgrowth. Colonization of cochlear implant arrays with ASCs might improve the bioelectrical interface between electrodes and auditory neurons. The hydrophobic silicone surface of the electrode carrier hinders a stable cell growth on the cochlear implant (CI). This study investigates whether functionalization with polydopamine (PD) of silicone surface of the electrode carrier enhances surface hydrophilicity and enables growth of ASCs.

Methods

Silicone samples and electrode arrays were coated with PD or PD and collagen. Surface hydrophilicity and morphology of the PD-coated silicone samples were determined. Coated and uncoated silicone samples and CIs were cultivated with ASCs. The proliferation, viability and production of neurotrophic factors were analyzed. Uncoated CIs, Coated CIs with and without ASCs were inserted in an inner ear model to measure insertion forces.

Results

A complete and thin PD coating of the silicone surface was achieved. Hydrophilicity, cell proliferation and cell viability were significantly enhanced by PD surface modification. Growth factors and neurotrophic factors were detected in ELISA. A stable colonization with ACS of CIs was observed before and after insertion. PD-coating with or without collagen did not increase insertion forces in an inner ear model.

Conclusion

PD coating of the silicone surface of CIs enables a stable and sufficient colonization with ASCs. Simultaneous application of ASCs as a continuous source of neurotrophic factors might improve outcomes of cochlear implantation.

P-27 The precision of eCAP thresholds in human subjects depends on the number of waveform averages

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Introduction

An essential component of cochlear implant fitting is the determination of stimulation thresholds. These thresholds can be obtained objectively by recording the electrically evoked compound action potential (eCAP). A commonly applied approach is based on linear extrapolation of the eCAP amplitude growth function (AGF) to zero amplitude. The present study determines the precision of the eCAP threshold as a function of the number of waveform averages.

Materials and methods

AGFs were obtained in 7 Advanced Bionics cochlear implant users from 3 electrode contacts in the base, middle and apex. 256 eCAP waveforms were recorded per stimulus level. Best-estimate reference thresholds were calculated from AGFs using the average of all 256 waveforms. Precision of the eCAP threshold was determined by generating probability distributions using a Monte Carlo approach. AGFs were constructed using averaged eCAPs from 8 to 128 randomly sampled waveforms. From the eCAP threshold probability distributions, the 95% confidence intervals (95% CI) were determined and expressed as fractions of the reference.

Results

At the default number of 32 averages in the Advanced Bionics system, the 95% CIs were 0.97 - 1.02 for the apical and medial electrodes and 0.96 - 1.01 basally. However, individual 95% CIs could deviate more than 25% from the reference. Doubling of the number of averages improved the median lower bound by approximately 1 to 2% and the upper bound by 0.4 to 0.7%. Lower bounds of the 95% CI were typically larger than the upper bounds.

Conclusion

In this study we have found that at 32 averages, the median bounds of the 95% CI of the eCAP threshold are within a range of 5% of the best-estimate value obtained at 256 averages. However, at 32 averages individual 95% CIs may still deviate substantially from the best-estimate value.

This study was supported by Advanced Bionics

P-28 The conditional deletion of bHLH transcription factors by Islet1-Cre in inner ear development**Iva Macova^{1,*}, Martina Dvorakova¹, Romana Bohuslavova², Tetyana Chumak³, Josef Syka³, Bernd Fritzscht⁴, Gabriela Pavlinkova²***1 Charles University, Institute of Biotechnology CAS**2 Institute of Biotechnology CAS**3 Institute of Experimental Medicine CAS**4 University of Iowa*** Presenting author*

Inner ear development is governed by cascades of transcription factors. Our research is focused on LIM-homeodomain protein Islet1 and its possible cooperation with basic helix-loop-helix family of transcription factors, Atoh1 and Neurod1. We used mouse model Islet1-Cre to delete Atoh1 or/and Neurod1 genes. Atoh1 is early hair cell marker whereas Neurod1 is important for the differentiation and survival of cochleovestibular neurons.

After conditional deletion of Atoh1, neither hair cells nor supporting cells are found in the inner ear and as a consequence, nerve fibers are missing at postnatal day 0. The loss of Neurod1 results in cochlear shortening and disorganization of hair cells in apex. The number of spiral ganglion neurons of Neurod1 deletion mutant is reduced to 17 % compared to control, although radial innervation in the cochlea seems unaffected. Additionally, the cochlear nucleus is smaller. These morphological changes are associated with significant hearing impairment of adult Neurod1 mutant mice. Double deletion of Atoh1 and Neurod1 genes by Islet1-Cre causes similar changes in sensory epithelium observed in mice with Atoh1 single conditional excision. However, inner ear innervation of double Atoh1/Neurod1 mutant forms a unique and yet undescribed pattern. Despite minimum of spiral ganglion cells, nerve fibers are condensed in the place of the missing sensory epithelium with expanded loops beyond the lost organ of Corti.

In conclusion, transcription factor Islet1 plays a key role in the specification of neurosensory cells in the inner ear. Our results confirm necessity of Atoh1 for hair cell differentiation and Neurod1 for cochleovestibular ganglion formation. An ongoing challenge is to define the mechanistic contributions of the double deletion of Atoh1 and Neurod1 in neuronal fiber guidance.

P-29 Enhancement of neurite outgrowth in spiral ganglion neurons on graphene substrates by electrical stimulation through cochlear**Rongrong Guo¹, Mingliang Tang^{1,*}***1 Southeast University*** Presenting author*

Cochlear implantation is now the best therapeutic method for severe sensorineural hearing impairment. However, the key to successful gain of hearing relies on the remaining sufficient functional spiral ganglion neurons. Our previous studies suggest that graphene is suitable for cell growth (neurons, microglia, stem cells, etc.) and could enhance neurite sprouting and outgrowth in the primary hippocampal neurons. Furthermore, electrical stimulation is reported to be capable of guiding neuronal growth. In this study, we aim to promote neurite outgrowth in spiral ganglion neurons by electrical stimulation. Spiral ganglion neurons were cultured on graphene substrates, which were fabricated by chemical vapor deposition method. Electrical stimulation was delivered through cochlear receiving certain music into the graphene substrates. Our preliminary results indicate that neurite growth of spiral ganglion neurons could be improved by this small device, and the optimized pattern of stimulation was further investigated. Our study will lay the foundation for improving the efficiency of cochlear implantation when cell transplantation is incorporated.

P-30 Insm1a is required for zebrafish posterior lateral line development**Yingzi He^{1,*}***1 Affiliated Eye and ENT Hospital, State Key Laboratory of Medical Neurobiology, Fudan University*** Presenting author*

Insulinoma-associated 1 (Insm1), a zinc-finger transcription factor, is widely expressed in the developing nervous system and plays important roles in cell cycle progression and cell fate specification. However, the functions of Insm1 in the embryonic development of the sensory system and its underlying molecular mechanisms remain largely unexplored. Here, through whole-mount in situ hybridization, we found that the zebrafish *insm1a* gene was expressed in the posterior lateral line (pLL) system, including both the migrating pLL primordium and the deposited neuromast cells. In order to decipher the specific roles of *insm1a* in zebrafish pLL development, we inhibited *insm1a* expression by using antisense morpholino knockdown strategy. The *insm1a* morphants exhibited primordium migration defects that resulted in reduced numbers of neuromasts. The knockdown of *insm1a* caused a deficiency of differentiation of hair cells, and this defect could be a secondary consequence of disrupting rosette formation in the pLL primordium. Additionally, we showed that disrupted *insm1a* decreased the proliferation of pLL primordium cells, which likely contributed to these pLL defects. Furthermore, we showed that loss of *insm1a* resulted in elevated Wnt/ β -catenin signaling in the primordium accompanied by Fgf target gene downregulation, which inhibited the expression of chemokine signaling genes. Taken together, this study reveals a pivotal role for *insm1a* in regulating pLL formation during zebrafish embryogenesis.

P-31 Genome-wide profiling reveals sequential translational responses of hair cells to cisplatin intoxication

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New protein production can rapidly and locally control cellular responses to stress. mRNA species that occupy translating ribosomes at consecutive periods after neuronal trauma provide sequential snapshots of translational responses to injury. We will report a profile of translating ribosomes from undisturbed and cisplatin intoxicated hair cells. We will describe the extent of post-transcriptional control for each hair-cell expressed mRNA and the role of metabolic enzymes and cytoskeletal proteins that are under strong post-transcriptional control in response to cisplatin toxicity. Our results should enable the discovery of target protein with otoprotective activity for clinical applications.

P-32 Anomaly of the human vestibular aqueduct: A case report**Charlotta Kämpfe Nordström¹, Hao Li¹, Weil Liu^{1,*}, Helge Rask-Andersen¹**¹ Uppsala University

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Introduction: The vestibular aqueduct (VA) is a canal which passes through the temporal bone from the vestibule to the posterior surface of the petrous pyramid, housing the endolymphatic duct (ED) and sac (ES). The VA normally runs medially to the semicircular canals and common crus almost parallel to the posterior semicircular canal (PSSC). Enlargement of the VA is associated with hearing loss and blockage or narrowing of the canal has been linked to Meniere's disease but to our knowledge no other kind of malformation or aberrant course of the canal is known.

Case report: One case of aberrant course of the VA was observed while examining a collection of 324 inner ear casts belonging to the human temporal bone collection of Uppsala University. In this case, the VA first runs cranially parallel to the common crus (CC) and then makes a bend, passing through the sub-arcuate fossa of the superior semicircular canal to continue its course towards the posterior fossa.

Conclusion: This aberrant course of the VA challenges the time-course and what is known about the embryological development of the semicircular canals and ED and ES. This case of aberrant course of the VA represents a malformation of the inner ear that is not known before. Its functional consequences is unknown.

P-33 Wnt/ β -catenin interacts with the FGF signaling to control neuromast development in zebrafish lateral line**Huawei Li^{1,*}, Dongmei Tang¹***1 EENT Hospital, Fudan University*** Presenting author*

Control of cell proliferation is a critical process during the sensory organs development. While the canonical Wnt/ β -catenin signaling pathway plays a critical role in regulating cell proliferation and differentiation; the exact molecular mechanisms induced by Wnt/ β -catenin activation that mediate these changes in the development of sensory organs remains poorly defined. In this study, we use the zebrafish mechanosensory organ-lateral line neuromast to understand how cell proliferation is regulated during development. Our results showed that overactivation of the Wnt pathway promoted the neuromast proliferation, resulting in an increased expression of the specific FGF target genes and ultimately leading to significantly more proliferating cells and differentiated cells, whereas suppression of the Wnt signaling inhibited the cell proliferation and differentiation in neuromast and rendered a significant reduction of FGF markers expression. Meanwhile, blockade of FGF signaling using pharmacological inhibitor or transgenic line disrupted the cell proliferation and differentiation in the neuromast. Moreover, the proliferation induced by Wnt activation was totally inhibited after FGF inhibition. Conversely, overactivation of the FGF pathway by basic fibroblast growth factor (bFGF) treatment resulted in enhanced proliferation and increased hair cell formation during developmental stage similarly to Wnt activation, whereas no significant change in Wnt target gene expression were detected after over-activating FGF. Furthermore, bFGF treatment led to a partial rescue of neuromast defects in the absence of Wnt activity. In summary, these data suggest that FGF acts downstream of Wnt signaling during the periods of proliferation and hair cell differentiation stage and that the balance of the activation of Wnt and FGF signaling pathways is essential for proper neuromast development.

P-34 Development of a setup for the in-vitro modelling of the auditory pathway by the application of differentiated neuronal stem cells.**Kristen Rak^{1,*}, Johannes Völker¹, Christine Völker¹, Philipp Schendzielorz¹, Andreas Radeloff², Rudolf Hagen¹***1University of Wuerzburg**2University of Oldenburg*** Presenting author*

During the development of hearing impairment, pathological lesions arise not only in the cochlea, but also in the neural structures of the auditory pathway. Specifically, these changes are characterised by a loss of neurons and degeneration of the auditory nuclei. In recent years, neural stem cells have been described in the spiral ganglion, the cochlear nucleus and the inferior colliculus. These cells have been hypothesized as providing a substrate to counteract degeneration of the auditory pathway. However, a basic scientific understanding of fundamental questions regarding the application of these cells is still lacking, particularly with regard to a possible interaction of these stem cells. Research outcomes from these studies will serve as a precondition for translational application in regenerative medicine.

To investigate this interaction a cell culture setup was developed which allowed cells to be propagated separately as stem cells in neuronal stem cell medium and then cultivated as differentiated individual cells in a differentiation medium together. After a defined time in culture, the cells were fixed and stained with specific antibodies against neuronal (β -III Tubulin), non-neuronal (GFAP and MOG) and synaptic (Synaptophysin) proteins. In addition calcium imaging experiments were performed.

An interaction of all three neuronal stem cell types with a time-dependent aspect was found in culture. The cells differentiated in the network and formed all three cell types of the neuronal cell line. A synapse formation was observed between neuronally differentiated cells. Preliminary functional studies revealed neuronal activity.

In summary, neuronal stem cells from different nuclei of the auditory pathway were able to be cocultivated with the new setup. The differentiated stem cells showed a neuronal-like interaction. In future projects, these cells will also be investigated at the molecular biological and functional level in this in-vitro modelling of the auditory pathway.

P-35 Nanoporous platinum coatings for surface modification of cochlear electrode contacts**Kim Dana Kreisköther^{1,*}, Nina Ehlert¹, Peter Behrens¹, Jennifer Schulze², Athanasia Warnecke²**¹ Leibniz Universität Hannover² Klinik für Hals-Nasen-Ohren-Heilkunde

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By enhancing the long-term biointegration and the contact between electrode and nerve fibers, the function of the cochlear electrode can be improved. This can be realized by chemical modification of the electrode surface or by integrating a drug delivery system [1] in e.g. modified nanoporous platinum (NPt) coatings on electrode contacts. For loading pores with active agents of various size, the pore diameter is adjusted by use of templates like Pluronic®F127 and polystyrene latex beads (PLBs). To control the release behaviour of active agents like rolipram or BDNF, which induced enhanced SGN neuronal survival [2], the NPt coating is modified with several functionalized thiols.

NPt is deposited electrochemically on dense platinum. Within the first method, Pluronic®F127 is dissolved in Pt(IV)solution. During the platinum deposition, Pluronic®F127 is incorporated into the forming platinum coating and removed subsequently. These NPt surfaces are modified with selfassembled monolayers of different thiols. Within the second method, dense platinum is coated with PLBs. After template formation, platinum is deposited in the voids of the PLB layer and PLBs are removed subsequently. The NPt coatings were characterized by SEM, sorption measurements, impedance spectroscopy, and cell culture investigations with NIH3T3 fibroblasts, spiral ganglion cells (SGNs) and bone-derived mesenchymal stem cells (BDMSCs). The thiol modification was determined via contact angle measurements and XPS.

Both methods lead to NPt. By using Pluronic®F127 pores with a size of about 10 nm are obtained, by using PLBs the pore diameter corresponds to the bead size in the range of 50-500 nm. Impedance measurements of the NPt coatings show improved impedance behaviour in the lower frequency range. Cell culture investigations indicate a good cell compatibility and SGN neurite growth. Rolipram release experiments show an adjustable rolipram amount depending on the modification.

This work was supported by the DFG Cluster of Excellence EXC 1077/1 „Hearing4all“.

P-38 Alginate-beads containing BDNF-producing MSCs support the survival of auditory neurons in vitro**Jana Schwieger^{1,*}, Annika Hamm², Andrea Hoffmann², Thomas Lenarz¹, Verena Scheper¹***1 Department of Otolaryngology, Hannover Medical School**2 Department of Orthopaedic Surgery, Hannover Medical School*** Presenting author*

The nerve-electrode-interface is one factor influencing the functionality of the cochlear implant. Increasing the number of surviving spiral ganglion neurons (SGN) and regeneration of their neurites e.g. by addition of neurotrophic factors (NTF) like BDNF (brain-derived neurotrophic factor) could improve this interface. For a longer lasting neuroprotective effect on the SGN, a chronic application of NTF is a prerequisite. One possible way for chronic drug delivery is the implantation of genetically modified cells into the cochlear. The encapsulation of these cells in a bioinert alginate-matrix could prevent patients against uncontrolled migration of the cells. Furthermore the alginate-matrix may shield the cells from immune cells, preventing immune reactions.

Mesenchymal stem cells (MSCs) were isolated by density gradient centrifugation from human bone marrow of one selected donor, expanded and lentivirally modified to produce human BDNF. 2500 MSCs were encapsulated in ultra-high viscosity-alginate and beads were formed. Dissociated SGN of neonatal rats were co-cultivated with these alginate-MSC-beads for 48 hours in 200 µl medium. MSC expansion medium was chosen as negative control, medium spiked with 50 ng/ml BDNF was included as positive control. After neuron-specific staining the number of surviving SGN was counted and the survival rate calculated. The amount of produced BDNF was analyzed in the harvested supernatant by ELISA. The bead stability was macroscopically evaluated.

The alginate-MSC-beads were stable during trial period. The ELISA-detected BDNF concentration within the supernatant varied from 50 to 600 pg/ml. A significant neuroprotection could be observed compared to negative control. Moreover, the alginate-MSC-beads were as potent as the positive control in protecting SGN.

Alginate-encapsulated, BDNF-producing MSCs are a promising candidate for long-term delivery of NTF to the inner ear for neuroprotection. Further investigations have to show how long the MSCs can survive and produce BDNF.

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P-39 Effect of biphasic charge balanced pulses on growth factor releasing MSCs encapsulated in alginate**Verena Scheper¹, Andrea Hoffmann², Tanja Schubert¹, Thomas Lenarz¹, Jana Schwieger¹***1 Department of Otorhinolaryngology, Hannover Medical School**2 Department of Orthopedics, Hannover Medical School*

The survival of spiral ganglion neurons (SGN) is critical for the efficacy of cochlear implants (CI). Application of growth factors has been shown to reduce SGN degeneration after deafness. To avoid time-delayed neuronal degeneration growth factors should be applied chronically. Lentivirally modified human mesenchymal stem cells (MSCs) producing BDNF (brain-derived neurotrophic factor) may serve as chronic BDNF-source. Those MSCs are encapsulated in alginate to shield them from the immune system. Since the purpose is to implant the alginate-MSCs in CI-patients being electrically stimulated (ES) via the CI we investigated the effect of ES on the MSC survival and BDNF-production.

MSCs were embedded into alginate and placed in a custom made stimulation chamber. To investigate if the encapsulated MSCs survive ES a clinically irrelevant high electric charge density was used. Alginate-embedded MSCs were stimulated at 1 kHz with 2000, 1000 or 400 μ A amplitude using biphasic 400 μ s pulses with 120 μ s interphase gap. Cells without ES served as control. After 24 hours surviving MSCs were counted and the supernatant was analyzed for BDNF concentration by ELISA.

Using 2 or 1 mA electric current a significantly decreased cell number was observed between stimulated and unstimulated MSCs. In some cases of maximal stimulation the alginate directly located at the electrode was charred. ES at 400 μ A did not change the cell number significantly compared to unstimulated controls. Additionally, the BDNF production and alginate stability was unaffected by ES.

The survival and BDNF release of genetically modified MSCs and the alginate stability are not influenced by a moderate ES paradigm exceeding the clinically applied current densities. Therefore the used MSCs in combination with the UHV-alginate are a promising method for chronic inner ear growth factor therapy with simultaneous ES as applied in CI patients.

Supported by the German Research Foundation grant SCHE 1636/2-1.

P-40 Kanamycin versus kanamycin-furosemide protocols to study ototoxicity in the guinea pig**Monika Kwiatkowska^{1,*}, Melek Demirbas¹, Michael Bös¹, Marcus Müller², Hubert Löwenheim²***1 Acousia Therapeutics GmbH, Tübingen, Germany**2 Tübingen Hearing Research Centre (THRC), Germany*** Presenting author*

Aminoglycoside antibiotics are commonly used, even though they are associated with irreversible sensorineural hearing loss. To study aminoglycoside ototoxicity and to identify novel otoprotective compounds, it is necessary to establish standardized in vivo animal models of cochlear damage. Simultaneous systemic administration of kanamycin and the loop diuretic furosemide has become routine in experimental protocols. The combination of aminoglycoside and loop diuretic is known to show synergistic effects on ototoxic damage and cause bilateral loss of sensory hair cells and a degeneration of auditory neurons. In this study we used a local application to avoid systemic side effects of the ototoxic drug. We applied (a) a combination of kanamycin sulfate (kana.su) and furosemide, (b) kana.su alone, and (c) kanamycin sulfate diacetate (kana.suda) to the middle ear of guinea pigs. Various concentrations and exposure times were tested. Hearing thresholds were analyzed by recording acoustically evoked compound action potentials of the auditory nerve at one and two weeks after application. Simultaneous local application of kana.su (200 mg/ml) and furosemide (50 mg/ml) resulted in a severe to profound hearing loss across all frequencies. One-hour exposure to kana.su solution alone did not affect hearing threshold. However, aqueous solubility of kana.su is limited to 100 mg/ml. To improve solubility kana.suda was used. A solution of 400 mg/ml kana.suda was tested at 1 h exposure and resulted in severe to profound hearing loss. A 15 min exposure caused a moderate threshold loss. In conclusion, local drug application of kana.su or kana.suda avoids systemic toxicity. The contralateral ear remains preserved and serves as an intra-animal control. The use of a monotherapy reflects the clinical setting. Kana.suda circumvents the combination with a second ototoxic drug which may confound the mechanisms of ototoxic hair cell death. This in vivo model will be used to develop novel otoprotective compounds.

P-41 Nanoporous silica nanoparticles as delivery systems for surface modification of cochlear electrodes**Nadeschda Schmidt^{1,*}, Peter Behrens¹, Jennifer Schulze², Athanasia Warnecke²**¹ Leibniz Universität Hannover² Hannover Medical School

* Presenting author

Nanoporous silica nanoparticles (NPSNPs) have already offered their great potential as delivery platforms in previous studies due to their advantageous properties. The properties include a high surface area, a high porosity (up to 50%) and the amenability for surface modification, because the silanol groups at the internal and external surface can be used to adjust the surface properties. Moreover, NPSNPs have shown a good biocompatibility and are biodegradable.[1,2] Our approach is the application of such delivery systems on the surface of the cochlear implants, e.g. on the silicone surface. The established delivery systems for neuroprotective factors can promote different cell processes like differentiation or proliferation. Previous studies have demonstrated that the brain-derived neurotrophic factor (BDNF) is a promising agent due to its ability to improve the survival and growth of spiral ganglion neurons (SGNs).[3] For that reason we investigated the delivery of BDNF by NPSNPs.

NPSNPs were prepared via sol-gel process from alkaline aqueous solution. Cetyltrimethylammonium bromide (CTAB) acted as structure directing agent to build up the porous system. The particle surface was modified with different trialkoxysilanes to equip the surface with various functional groups.[4] We investigated the immobilization of BDNF on the surface of the NPSNPs and its release. The immobilized and released amounts of BDNF were determined by using an ELISA. The cytocompatibility of the NPSNPs was investigated with fibroblasts and the neuroprotective effect of immobilized and released BDNF was studied with SGNs.

The synthesized NPSNPs were approximately 40 nm in size and had a high specific surface area (500-1000 m² g⁻¹). The modified NPSNPs were successfully used as delivery system for BDNF. They exhibit a good cytocompatibility with fibroblasts and the delivered amounts of BDNF increased the survival rate of SGNs.

This work was supported by the Cluster of Excellence Hearing4all.

P-42 Synthesis of fluorescent, polysaccharide-modified nanoporous silica nanoparticles for localized drug release and bio imaging**Tim-Joshua Pinkvos^{1,*}, Inga Wille¹, Cornelia Blume², Peter Behrens¹***1 Leibniz Universität Hannover, Institut für Anorganische Chemie**2 Leibniz Universität Hannover, Institut für Technische Chemie***Presenting author*

Nanoporous silica materials offer a good biocompatibility, large and permanent pore volume and versatility with regard to adjustable surface properties and are therefore often used within the field of drug delivery systems.[1,2]

Polysaccharides possess a large variety of different properties, concerning their cellular uptake and adhesion to cell surfaces. Attaching polysaccharides to nanoporous silica nanoparticles (NPSNPs) can affect their location and distribution in tissues or living organisms. Moreover, polysaccharides can be used to cap the pores of the NPSNPs to cage a drug, leading to a zero release. If a suitable enzyme is present, the polysaccharide is degraded and the drug is released.[2] Such enzymes can be introduced into certain places to obtain a localized drug delivery. In order to track such NPSNPs during their uptake and to know where the release happens, they can be equipped with fluorescent properties.

In order to obtain such particles, we synthesized NPSNPs and modified them with fluorescein isothiocyanate (FITC) according to a synthesis by Lin et al.[3] Afterwards the polysaccharides were attached to the surface. As polysaccharides, we employed starch derivatives, and polysialic acid. The latter is involved in the development of neuronal tissue. The attachment happened according to a synthesis route, that has been worked out within our group.[1] To attach the starch derivatives we used a procedure developed by Bernardos et al.[2] We were able to show the presence of the starch derivatives using the Tollens' test, while the Purpald[®] test showed the presence of the polysialic acid. Furthermore we were able to record a change concerning the pH-dependent zeta-potential due to the surface modification. The fluorescence was observed under irradiation with UV light for the nanoparticles with attached FITC.

This work was supported by the DFG Cluster of Excellence EXC 1077/1 „Hearing4all“.

P-43 Autophagy protects auditory hair cells against neomycin-induced damage**Zuhong He, Lingna Guo, Qiaojun Fang, Xia Gao, Renjie Chai**

Aminoglycosides are toxic to sensory hair cells (HCs) by inducing the production of reactive oxygen species (ROS) that in turn activate apoptotic pathways. Autophagy is an essential and highly conserved self-digestion pathway that plays important roles in the maintenance of cellular function and viability under stress. However, the role of autophagy in aminoglycoside-induced HC injury is unknown. In this study, we systematically investigated the role of autophagy in aminoglycoside-induced HC damage. First we found that autophagy activity was significantly increased, including enhanced autophagosome-lysosome fusion, in both cochlear HCs and HEI-OC-1 cells after neomycin/gentamicin injury. This suggested that autophagy might be correlated with aminoglycoside-induced cell death. We then used rapamycin, an autophagy activator, to increase the autophagy activity and found that the ROS levels, apoptosis, and cell death were significantly decreased after neomycin/gentamicin injury. In contrast, treatment with the autophagy inhibitor 3-methyladenine (3-MA) or knockdown of autophagy-related proteins (ATGs), including ATG5, BECN1 (Beclin1), and ATG7, resulted in reduced autophagy activity and significantly increased ROS levels, apoptosis, and cell death after neomycin/gentamicin injury. Lastly, after neomycin injury, the antioxidant N-acetylcysteine (NAC) could successfully prevent the increased apoptosis and HC loss induced by 3-MA treatment or ATG knockdown, suggesting that autophagy protects against neomycin-induced HC damage by inhibiting oxidative stress. We also found that the dysfunctional mitochondria were not eliminated by autophagy (mitophagy) in HEI-OC-1 cells after neomycin treatment, suggesting that autophagy might not directly target the damaged mitochondria for degradation. This study demonstrates that moderate ROS levels can promote autophagy in order to recycle damaged cellular constituents and maintain cellular homeostasis, while the induction of autophagy can inhibit apoptosis and protect the HCs by suppressing ROS accumulation after neomycin/gentamicin injury. Our results suggest that autophagy might be a new therapeutic target for the prevention of aminoglycoside-induced HC death.

Author keywords: aminoglycosides / apoptosis / autophagic flux / autophagosome / hair cell protection / lysosome / oxidative stress

P-44 Speech recognition and cochlear processing; the mediating role of genetic hearing impairment**Ronald Pennings^{1,*}, Joop Leijendeckers¹, Arjan Bosman¹, Ad Snik¹***¹ Radboudumc*** Presenting author*

The relation between speech recognition and hearing loss is not straightforward; impaired cochlear processing of sounds might play a role as induced by deficient genes. To study that, data obtained in nine groups of patients with genetic hearing loss were used; speech recognition was related to cochlear processing as assessed by psychophysical measurements. The study showed that differences in speech perception between patient groups could be explained by psychophysical variables. Different types of genetics-related cochlear impairment were distinguished. In summary, speech was primarily associated with genetics-related malfunctioning of the cochlea and not to the degree of hearing loss.

P-45 Molecular networks in neonatal mice cochlear and vestibular epithelial non-sensory cells**Jose Antonio Lopez-Escamez^{1,*}, Alvaro Gallego-Martinez¹, Teresa Requena¹**¹ Otolology & Neurotology Group CTS495, Centre for Genomics and Oncology Genyo, ibs.Granada

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Introduction

Cochlear and vestibular epithelial non-sensory cells are essential to maintain the cellular architecture in the Organ of Corti and the vestibular epithelium integrity in the vestibular sensory organs. Intercellular junctions and extracellular matrix interactions are essential to prevent an abnormal ion redistribution resulting in loss of endocochlear potential. The aim of this study is to generate a molecular networks map in cochlear and vestibular non-sensory cells to define clusters of proteins for hearing or vestibular loss of function.

Methods

We retrieved RNA-seq datasets from P0 mouse epithelial sensory and non-sensory epithelial cells from gEAR portal (<http://umgear.org/index.html>) and obtained gene expression fold-change between non-sensory cells and hair cells (HC) for each gene. Differentially expressed genes (DEG with a foldchange >3) between non-sensory and HC were selected to search for protein-protein interactions using STRING database (<https://string-db.org/>) to design molecular networks for non-sensory and HC in the cochlea and the vestibular organs.

Results

Five hundred one and 323 genes were differentially expressed in the cochlear and vestibular nonsensory cells in the early neonatal mouse. The top five DEG were respectively *Cldn11*, *Epyc*, *Ogn*, *Scg2* and *Rspo2* for the cochlear non-sensory cells and *Lect1*, *Ntn1*, *Aldh1a1*, *Cldn4* and *Sfrp5* for the vestibular cells. Molecular networks had a significant enrichment of GO biological functions, cellular components and functional processes and specific protein clusters were identified in both cochlear and vestibular non-sensory cells.

Conclusions

We have predicted a molecular network map with several protein clusters for non-sensory epithelial cells of the Organ of Corti and vestibular organs. This map will facilitate the functional studies of novel candidate genes for hearing or vestibular loss in non-sensory epithelial cells.

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P-46 FDXR mutations cause sensorial neuropathies, a new mitochondrial Fe-S disease

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** Presenting author*

Hearing loss and retinis pigmentosa have mostly genetic origins, some of them being related to sensorial neuronal defects. Here, we report eight subjects from four independent families presenting with auditory neuropathy and optic atrophy. Whole-exome sequencing revealed biallelic mutations in FDXR in affected subjects of each family. FDXR encodes the mitochondrial ferredoxin reductase, the sole human ferredoxin reductase which is implicated in the biosynthesis of iron-sulfur clusters (ISC) and in the heme formation. ISC proteins are involved in enzymatic catalysis and gene expression, DNA replication and repair. We observed deregulated iron homeostasis in FDXR mutant fibroblasts and indirect evidence of mitochondrial iron overload. Functional complementation in a yeast strain deleted for ARH1, the human FDXR counterpart, established the pathogenicity of these mutations. These data emphasize the wide clinical heterogeneity of mitochondrial disorders related to ISC synthesis.

P-47 A missense variant in NLRP12 gene in a family with meniere's disease and a topic dermatitis suggests NLR-mediated inflammation**Teresa Requena^{1,*}, Dheeraj R. Bobbili², Rudi Balling², Patrick May², Jose A. Lopez-Escamez³***1 GENYO - Centre for Genomics and Oncological Research – Pfizer/University of Granada/ Junta de Andalucía**2 Luxembourg Centre for Systems Biomedicine, University of Luxembourg**3 Instituto de Investigación Biosanitaria ibs. Granada Complejo Hospital Universitario***Presenting author***Introduction**

Meniere's disease (MD) is a spectrum of rare disorders characterized by recurrent vertigo attacks associated with low frequency sensorineural hearing loss and tinnitus, with a strong familial aggregation (familial-prevalence 8-10%). Evidences from epidemiology suggest a genetic susceptibility involving multiple genes and an autoimmune background. Variants in immune response genes can modify hearing loss progression and proinflammatory cytokines are elevated in a subset of patients. We have performed whole-genome sequencing (WGS) in a family with MD and atopic dermatitis.

Methods

WGS data were processed with a script developed by the Luxembourg Centre for Systems Biomedicine based in Annovar that annotate with 87 databases. We filtered the Single Nucleotide Variants (SNVs) by high-quality, minor allele frequency (MAF < 0.001) from gnomAD. 790 unrelated Spanish individuals were used to exclude local variations. Then we prioritized the SNV according to different pathogenic scores including CADD, Revel and FATHMM. All variants were validated by Sanger sequencing. qPCR were performed to confirm the expression in inner ear human tissue and to define the effect of the variant.

Results

After filtering and prioritizing, we have identified two novel heterozygous variants in the VHL and NLRP12 genes. Linkage analysis and clinical data discarded VHL as causal gene in this family. NLRP12 variant affects most of the isoforms described in Ensembl and Uniprot. Our results showed that NLRP12 is expressed in the human inner ear tissue. Also differential expression levels between carriers and controls were observed.

Conclusions

Our results support that mutations in NLRP12 may cause autoinflammatory inner ear disease in patients with MD and autoimmune background.

Funding

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P-48 NOX3-deficient mice study emphasizes NOX3 as new drug target for noise- and age-induced hearing loss**Francis Rousset^{1,*}, Marta Coelho¹, Vivianne Kokje², Antoine Marteyn¹, Karl-Heinz Krause¹, Pascal Senn²***1 University of Geneva**2 University Hospital of Geneva*** Presenting author*

Research over the last decades has identified a major role for reactive oxygen species (ROS) in hearing loss. NADPH oxidases (NOX) are a family of seven isoenzymes expressed in mammals dedicated to the production of ROS. While the physiology of NOX enzyme is at least partially understood (it includes bacteria killing, otocilia formation or thyroid hormone synthesis), there is increasing evidence that dysregulation of NOX activity contributes to pathological states. High levels and specific expression in the inner ear identifies NOX3 as a source of oxidative stress possibly contributing to hearing loss. Using mice harbouring loss of function mutations of the NOX3 complex (NOX3- or p22phox-deficient), this study aims to address the role of NOX3-derived ROS in hearing pathologies.

We used two models of hearing loss: (i) natural age related hearing loss in A/J mice and (ii) noise overexposure in the C57Bl/6 mouse background. A robust quantifiable hearing loss was observed in both models. In A/J mice, age-related hearing loss was markedly observed at high frequencies (16-45 kHz) and progressed over time. A marked decrease in the number of outer hair cells of the organ of Corti was associated with hearing loss. In addition, the number of synaptic connections between the spiral ganglion neurons and the inner hair cells was strongly reduced in aged mice (6 months old). In noise-induced hearing loss (C57Bl6 mice), no significant differences could be observed in the architecture of the sensory epithelium after noise exposure. However the number of ribbons was significantly reduced in noise exposed mice. Interestingly, in both models, NOX3-deficient animals exhibited a remarkably preserved architecture of the sensory epithelium. Accordingly, hearing thresholds of NOX3-deficient animals were significantly lower compared to the WT littermates. Based on loss-of-function mutant mice, this study sheds light on the potential role of NOX3-generated ROS in hearing loss.

P-49 Highly efficient diagnostic testing using panel-based next generation sequencing and CNV detection in patients with hereditary hearing loss**Sarah Fehr^{1,*}, Antje Rinckleb¹, Anke Tropitzsch², Hubert Löwenheim², Andrea Sprecher³, Konstanze Hörtnagel¹, Saskia Biskup¹***1 Praxis für Humangentik Tübingen**2 University ENT Clinic of Tübingen, Tübingen, Germany**3 CeGaT GmbH*** Presenting author*

Genetic heterogeneity complicates the molecular diagnosis of hereditary hearing loss (HHL). Particularly, the contribution of copy number variations (CNVs) to hearing loss is most likely underestimated and screening by standard high-throughput sequencing methods does not allow for CNV detection.

We aimed to incorporate a method of direct detection of CNVs as part of our standard analysis pipeline (target enrichment, NGS library preparation and sequencing on the Illumina platform, bioinformatic analysis, and medical evaluation). Combined with bioinformatic analysis, single base substitutions, small deletions and insertions, as well as copy number variations (CNVs) in genes associated with non-syndromic and syndromic HHL can reliably be detected.

In a group of 300 patients clinically diagnosed with profound hearing loss we identified causative mutations in >40% of cases (~6% GJB2). In addition to previously-reported CNVs in the gene STRC, we also detected causative CNVs in known deafness genes, which were confirmed by a second method.

In conclusion, we have implemented CNV detection in a panel-based NGS pipeline which is a highly sensitive, fast, and cost efficient tool for comprehensive genetic diagnosis of HHL. These results have positive consequences for counseling of patients and families, and may provide information regarding novel gene- or even mutation-specific treatment options in HHL.

P-50 Mutation in COL11A2 causes dominant hearing loss with cochlear malformation in a Chinese family

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Objective

Autosomal dominant non-syndromic hearing loss (DFNA) is a genetically heterogeneous disorder. So far, 36 pathogenic genes have been identified. In this study, we aim to characterize the clinical feature and the genetic cause of a Chinese DFNA family.

Methods

Whole exome sequencing was performed on the proband. Co-segregation between the hearing loss phenotype and the potential causative mutations was verified in all family members by Sanger sequencing. Pure tone audiometry and temporal bone computed tomography (CT) were performed to determine clinical features.

Results

Audiologic profiles of the affected family members revealed a profound hearing loss affecting all frequencies. Computed tomography (CT) examination of the proband and her mother revealed malformation of the cochleae. A novel COL11A2 mutation, NM_080679: c.C1600T (p.P534S), was identified in this family segregating with the congenital hearing loss. The mutation caused substitution of a high conserved amino acid in the triple helical region of collagen 2(XI). The mutation was not detected in public population databases (1000 genome, ExAC, ESP6500 and gnomAD).

Conclusions

We identified a novel COL11A2 mutation in a Chinese DFNA family. To the best of our knowledge, this is the first report of a COL11A2 mutation causing nonsyndromic hearing loss with cochlear malformation. Our findings added new insights to the genotype-phenotype correlation spectrum of COL11A2 associated hearing loss.

P-51 Inner ear-enriched transcript Epiphycan is necessary for normal auditory function**Yukiko Hanada^{1,*}, Yukiko Nakamura¹, Yoshiyuki Ozono¹, Yasumitsu Takimoto¹, Shoichi Shimada¹, Hidenori Inohara¹***¹ Graduate School of Medicine, Osaka University*** Presenting author***Introduction**

Recently research of inner ear specific transcripts has revealed novel information about hereditary hearing loss and a mechanism of normal hearing. In this study we have focused on Epiphycan(Epyc), a member of small leucine-rich repeat proteins(SLRPs) family, as a enriched transcript in the inner ear.

Methods and results

C57BL/6J male mice at 7 weeks were used for this study. We performed realtime quantitative PCR using various tissue and confirmed Epyc is abundantly and specifically expressed in the cochlea. In situ hybridization was performed to investigate Epyc mRNA localization in the cochlea and showed Epyc mRNA was localized in supporting cells of the organ of Corti. We generated Epyc KO mice using CRISPR/Cas9 technology. We measured hearing threshold of WT and Epyc KO mice, and thresholds of Epyc KO mice were elevated at high frequencies compared to WT mice.

Conclusion

Epyc is an inner ear-enriched transcript and localized in supporting cells in the cochlea. Epyc is necessary for normal hearing function. Further study is needed to elucidate its role in maintainance and development of hearing ability.

P-52 Mitochondrial variants in spanish patients with Meniere disease**Alvaro Gallego-Martinez^{1,*}, Margarita Ferriz¹, Teresa Requena¹, Jose Antonio Lopez-Escamez²**

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Introduction

Meniere's disease is a chronic disease affecting inner ear characterized by vertigo attacks, low-to-mid frequency sensorineural hearing loss, tinnitus and aural fullness. Familial aggregation studies supports a potential mitochondrial inheritance in MD. In this study, we sequenced 29 mitochondrial genes in patients with MD to explore this hypothesis.

Methods

We performed a custom targeted-enrichment sequencing (Haloplex, Agilent) in 890 patients with MD and 40 controls. Samples were pooled (10 samples per pool) to reduce sequencing cost. Variant calling was performed using GATK along with Samtools+Picard tools. The variants were filtered through population frequency, pathogenicity score, in-house controls and quality filters and the remaining candidate variants were validated by Sanger sequencing. Functional implications were modelled through 3D-protein modelling in candidate genes.

Results

Nine variants were considered potential candidate for sporadic cases of MD in our dataset. These variants were found in ATPase6, ND1, ND3, COII, tRNA(Lys/Ter), tRNA(Ala) genes. Sanger sequencing validation was performed in all pools to estimate the allelic frequency in patients with MD population.

Conclusions

Mitochondrial inheritance may explain some sporadic cases in MD. A number of candidate variants have been validated in sporadic cases of MD.

Funding

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P-53 Discovery of CDH23 as a significant contributor to age-related sensorineural hearing loss in Koreans**Hye-Rim Park^{1,*}, Min Young Kim¹, Jin-Hee Han¹, Seung-Min Lee¹, Doo-Yi Oh¹, Bong-Jik Kim², Byung Yoon Choi¹***1 Seoul National University Bundang Hospital, Seoul National University College of Medicine, Seongnam, Korea**2 Wide River Institute of Immunology, Seoul National University College of Medicine, Seoul Korea***Presenting author*

CDH23 mutations have mostly been associated with prelingual severe-to-profound sensorineural hearing loss (SNHL) in either syndromic or nonsyndromic SNHL (DFNB12). Herein, we show the contribution of CDH23 variants to age-related nonsyndromic SNHL (NS-SNHL).

We screened 32 Korean adult probands with postlingual NS-SNHL sporadically or in autosomal recessive fashion using targeted panel or whole exome sequencing.

We detected four (12.5%, 4/32) potential postlingual DFNB12 families that carried the recessive CDH23 variants, qualifying for our criteria.

Three of the four families carried one definite pathogenic CDH23 variant previously known as the prelingual DFNB12 variant in trans with rare CDH23 variants.

To determine the contribution of rare CDH23 variants to the postlingual NS-SNHL, we checked the minor allele frequency (MAF) of CDH23 variants detected from our ethnicity-matched Korean postlingual NS-SNHL cohort and ethnicity-matched prelingual Korean NS-SNHL cohort.

Among the 2040 normal control chromosomes, the allele frequency of these CDH23 variants in our postlingual cohort was 12.5%, which was significantly higher than that of the 2040 control chromosomes (5.53%), confirming the contribution of these rare CDH23 variants to age-related postlingual NS-SNHL.

Furthermore, minor allele frequencies of these rare CDH23 variants from the age-related postlingual NS-SNHL group was significantly higher than that from the prelingual NS-SNHL group.

Our study clearly demonstrates an important contribution of CDH23 mutations to age-related postlingual NS-SNHL in Korea and shows that the phenotypic spectrum of DFNB12 can be broadened even into the presbycusis, depending on the pathogenic potential of variants.

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P-54 Using next generation sequencing to analyse expression patterns in the ageing mouse inner ear**Nick Schubert^{1,*}, Marcel van Tuinen¹, Kristof Joosten¹, Sonja Pyott¹***¹ University of Groningen, University Medical Center Groningen, The Netherlands*** Presenting author*

Hearing loss is the most common sensory disorder. Difficulty hearing commonly occurs with ageing and has enormous economic, societal and personal consequences. Identifying molecular pathways that are involved in age-related hearing loss (ARHL) is necessary for the development of treatments for this disease. Next-generation sequencing (NGS) has revolutionised genomic research during the last decades and made it possible to investigate transcriptional changes and molecular pathways in an easy and cost-effective way.

We micro-dissected the organ of Corti (OC) and stria vascularis (SV) of C57BL/6 mice aged six weeks and two years. The inner ear is fully developed at six weeks of age, whereas C57BL/6 mice at two years of age have lost the ability to hear. The transcriptome of these different tissues was acquired using NGS. Differential expression (DE) and gene ontology (GO) enrichment analysis were performed using R/Bioconductor software and online tools.

1950 genes were differentially expressed (adjusted FDR < 0.1) in the aged inner ear. A subset of this list, containing 1057 genes, were only expressed in the SV, whereas 666 genes were only expressed in the OC. GO enrichment analysis revealed that different biological processes (BP) are up-regulated in the OC (22 BP) and SV (23 BP). The OC of aged mice showed to up-regulate neuronal development, vesicle transportation and exocytosis. The aged SV showed an up-regulation of immune processes and apoptosis.

This study shows different biological processes are involved in ageing of the inner ear. The OC and SV show unique expression patterns and up-regulation of different biological processes during ageing. This study provides a genetic basis for future research in investigating the ageing inner ear to find potential new treatments for ARHL.

P-55 Intra-tympanic administration of pioglitazone, a peroxisome proliferator-activated receptor gamma agonist, protects from noise-induced hearing loss

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Introduction

Pioglitazone is an oral antidiabetic agent used to control glucose in patients with type 2 diabetes. Pioglitazone and other peroxisome proliferator-activated receptor (PPAR- γ) agonists have pleiotropic effects on pathways involved in inflammation, oxidative stress, and metabolic dysfunction. We developed a formulation of pioglitazone (1.2% w/w) for intra-tympanic injection, and evaluated its effectiveness to reduce noise-induced hearing loss.

Methods

Acute acoustic trauma was induced in rats by exposure to pure tone noise of 10 kHz for 60 min at 120 dB SPL. Animals were treated with single i.t. injections of pioglitazone or placebo in separate groups at 1, 24, and 48 hrs after noise. Hearing was evaluated by auditory brainstem response (ABR) and immunohistochemical assessments.

Results

Our results demonstrated that pioglitazone reduces noise-induced hearing loss. The greatest efficacy was observed in rats receiving pioglitazone immediately (1 hr) after noise exposure. Significant, although lesser protection, was observed when pioglitazone was administered 24h or 48 h after noise exposure. Interestingly, the early administration of pioglitazone acts against oxidative stress imbalance induced by noise, whereas, when administer at late time points from acoustic trauma the protective effect of the drug is related to its ability to counteract cochlear inflammation.

Conclusions

These results suggest that intra-tympanic Pioglitazone administration may be an effective treatment for hearing loss. data obtained for different schedules for drug delivery provide evidences that the early drug administration is more effective in improving hearing loss and preventing hair cell loss reducing the early onset of oxidative stress cascade activation. These results suggest that a single dose of drug can be more effective if injected in the peri-traumatic period before that the cell death pathways are well established. Taken together these data are encouraging for clinical translation.

P-56 Study of molecular and pathological mechanism of protective effect on noise-induced hearing loss (NIHL) by hydrogen-saturated saline**Liwei Chen¹, Daishi Chen¹, Shiming Yang¹, Xuejun Sun², Wei Sun³, Ning Yu¹, Suoqiang Zhai¹***1 Chinese PLA General Hospital**2 Second Military Medical University**3 University at Buffalo The State University of New York*

To evaluate the effect of hydrogen-saturated saline protecting intensive narrow band noise induced hearing loss in guinea pigs. Guinea pigs were divided into 3 groups: hydrogen-saturated saline group, normal saline group and control group. For saline administration, the guinea pigs were given daily abdominal injections (1ml/100g) three days before and 1 hour before narrow band noise exposure (2.5-3.5 kHz 130 dB SPL, 1 h). The guinea pigs in control group received no treatment. After noise exposure, auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE) were tested to examine cochlear physiology. Scanning electron microscopy (SEM) and succinat dehydrogenase (SDH) staining were applied to examine hair cell morphological damages. The changes of free radicals and inflammatory cytokines in the cochlear were also examined before noise exposure, immediately after and 7 days after noise exposure. Results showed that the guinea pigs pretreated with hydrogen-saturated saline had less noise-induced hair cell damage and hearing loss. By detecting levels of free radicals and inflammatory cytokines in the cochlea, we found that the malondialdehyde (MDA), lipid peroxidation (LPO) and hydroxyl ($\cdot\text{OH}$) levels were significantly lower in the hydrogensaturated saline group after noise trauma. After noise exposure, the concentrations of proinflammatory cytokines (IL-1, IL-6, TNF- α) and ICAM-1 in the cochlea of guinea pigs in the hydrogen-saturated saline group were dramatically reduced compared to those in the normal saline group. We speculated that hydrogen-saturated saline can decrease the amount of those harmful free radicals and inflammatory cytokines caused by noise trauma. Our findings suggest that the hydrogensaturated saline can be effective in preventing intensive narrow band noise induced hearing loss in guinea pigs, and its mechanism may be achieved through both the anti-oxidative and antiinflammatory effects.

P-57 Otolin-1 in biological fluids: a possible biomarker for inner ear diseases**Emilio Avallone^{1,*}, Heike Schmitt^{1,*}, Thomas Lenarz¹, Giorgio Lilli^{1,*}, Anke Lesinski-Schiedat¹***1 Hannover Medical School – Germany***Presenting author***Introduction**

The expression of Otolin-1 mRNA is highly restricted to the inner ear; in particular it is identified in support cells of the vestibular maculae and semi-circular canals cristae, it is also a component of the tectorial membrane. The aim of the study is to analyze the presence of the protein Otolin-1 in serum, urine and saliva samples of human subjects. We hypothesize that through the rupture of the membranous labyrinth that occurs in the Ménière's Disease different levels of the protein are present in these fluids compared to healthy subjects.

Materials and methods

The study involved patients suffering from Ménière's Disease hospitalized in our clinic. The blood, urine and saliva sampling was performed in the morning after patients have observed about 12 hours of fasting. The blood was collected, centrifuged at 1500g for 20 minutes and the supernatants were collected. The samples were stored at -20 °C until the analysis by the use of the ELISA-kit for human OTOLIN-1.

Results

The preliminary results show for the first time the presence of the protein Otolin-1 in urine and saliva samples of patients with the Ménière's Disease. Otolin-1 was detected in pg/ml range as well in urine, saliva and serum samples.

Conclusion

Our results indicate that Otolin-1 is also present in urine and saliva; this would allow to reduce the invasiveness of sample collection and increase the patients compliance. Referring to literature high levels can also be found in patients with other pathologies such as BPPV. The hypothesis is that OTOLIN-1 would get rid in excess amounts, following a breakdown of the membranous labyrinth that occurs in Ménière's Disease. As well as with other biomarkers such as myoglobin, CK and troponin in cardiology Otolin-1 can be suitable as possible biomarker of this disease, according to its possible detection in urine and serum.

P-58 In vitro corrosion of platinum electrode contacts of cochlear implants induce adverse biological effects on cells**Kirsten Wissel^{1,*}, Gudrun Brandes¹, Nils Pütz², Gian Luigi Angrisani³, Jan Thieleke⁴, Thomas Lenarz¹, Martin Durisin¹***1 Medical School Hannover**2 TU Dresden**3 Leibniz University Hannover**4 Leibniz University Hannover***Presenting author*

Increase of electrical impedance of inserted cochlear implants can be due to electrode cracks or cellular interactions with the electrode. To analyze the effects of eroded platinum surfaces of electrode contacts on cells, an in vitro cell culture model was established. Human CI electrode was electrically stimulated in a 0.5 % aqueous NaCl solution for four weeks and the concentration of the platinum dissolute (Pt-Diss) was determined by mass spectrometry. The murine fibroblast (NIH 3T3) and the human neuroblastoma (SH-SY5Y) cell line were exposed to Pt-Diss and – for comparison – to platinum nanoparticles (Pt-NP, 3 nm) with varying concentrations. The biological activity of the cell lines were demonstrated by the WST-1 assay as well by transmission electron microscopy. It could be shown that their cell death was inducible after exposition to less than 8 µg/ml of the corrosion products (Pt-Diss) in a concentration dependent manner. In contrast, Pt-NP concentrations more than 50 µg/ml revealed mitochondrial swelling in NIH3T3 and SH-SY5Y cells. Therefore, the present findings indicate that only Pt-ions provoke cell death by interacting with the respiratory chain.

P-59 Age-related structural changes in synaptic ribbons of mouse cochlear inner hair cells lacking otoferlin**Séverin Belleudy^{1,*}, Margot Tertrais¹, Yohan Bouleau¹, Didier Dulon¹**¹ Université de Bordeaux

* Presenting author

Hearing relies on faithful synaptic transmission at the afferent ribbon synapses contacting the inner hair cells (IHCs). At these glutamatergic synapses, IHCs have a unique presynaptic organelle, the synaptic ribbon, which is essential for aggregating synaptic vesicles and organizing Ca^{2+} channels at the active zone. The synaptic ribbons are generally classified into two main functional subgroups according to spontaneous rate (SR) and threshold sensitivity. For still unknown reasons, low-threshold high SR synapses have small ribbons while high-threshold low SR use larger ribbons. The morphological and functional changes occurring in the organization of the synaptic ribbons during development and aging are not fully characterized. To test if the development of a morphological spatial gradient of ribbons requires synaptic activity, we here quantified the size and spatial distribution of the synaptic ribbons in IHCs from mouse lacking otoferlin (the putative calcium sensor controlling vesicle exocytosis). Furthermore, we compared the aging of the synaptic ribbons in normal wild-type mice with deaf mice lacking otoferlin. We found a progressive decrease in the number of ribbons per IHCs with aging in wild-type mice: from an average of 16 ribbons at age P15 to 9 ribbons at age P365. Remarkably, during that period, the mean size of the ribbons was increasing by 3.8 fold with a topographic distribution more concentrated at the base of the IHCs. In IHCs lacking otoferlin, the loss of ribbons with aging was much accelerated and the remaining ribbons were also found of large size. In conclusion, we propose that, during aging, the preferential loss of low-threshold high SR synapses (small ribbons) is not related to synaptic activity since a similar pattern, although accelerated, was observed in mice lacking otoferlin.

P-60 Can neuroprotective substances reduce the cisplatin ototoxicity in vitro?

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Cisplatin is widely used for tumor therapy. Unfortunately, one of the common side effects is hearing loss. In this study, PDE-4 inhibitor, BDNF and GDNF, all known for protecting the spiral ganglion cells in the inner ear, were tested for their otoprotective potential against cisplatin toxicity in the organ of Corti (OC) *in vitro*.

The membranous cochleae were prepared from 3-5 day old Wistar rats cultured over 48 h. The neuroprotective substances were immediately added to the medium: PDE type4 inhibitor (Rolipram: 0.1 nM; 1 nM; 10 nM), BDNF (brain-derived neurotrophic factor 10 nM; 50 nM; 100 nM), GDNF (glial cell-derived neurotrophic factor 50 nM; 100 nM; 200 nM). After a cultivation period of 24 h, cisplatin was added (20 µM) and the OCs cultured again for 24 h. The OCs were fixed and the neurites in the peripheral process and the inner (IHC) and outer (OHC) hair cells were stained and analyzed.

None of the investigated substances showed a negative effect on the number of hair cells as well as on the neurites. The treatment with cisplatin reduced the number of both hair cell types without the neurotrophic factors significantly ($p < 0.001$) without affecting the number of neurites.

Rolipram, PDE type 4 inhibitor, reduced cisplatin ototoxicity of the IHC significantly in all regions of the OC a dose depend manner, the OHC were protected only with 10 nM Rolipram. The application of 100 nM GDNF prevented the OHC against cisplatin damage in all regions of the OC significantly. A slightly protection also shown for the IHC cultured in 100 nM and 200 nM GDNF. In contrast the application of BDNF did not effected cisplatin damage on the hair cells compared to the cisplatin treated OC cultured without BDNF.

Our experiments demonstrate the otoprotective potential of several neurotrophic substances against cisplatin.

P-61 Confocal microscopy 3-D reconstruction of synaptic ribbon alterations in vivo models of noise-induced hearing loss and ototoxicity**Rolando Rolesi^{1,*}, Fabiola Paciello², Antonella Di Pino¹, Diana Troiani³, Anna Rita Fetoni², Gaetano Paludetti¹***1 Institute of Otolaryngology Università Cattolica, School of Medicine, Rome, Italy**2 Institute of Otolaryngology Università Cattolica, School of Medicine, Rome, Italy; Institute Cell Biology and Neurobiology, CNR, Monterotondo (Italy),**3 Institute of Human Physiology, Università Cattolica, School of Medicine, Rome, Italy***Presenting author***Introduction**

Synaptic ribbon quantification is a powerful estimate of synaptic integrity at inner hair cell (IHC)-auditory nerve synapse. Acoustic overexposure in a variety of animals results in swelling of the auditory nerve fibers and their synaptic contacts with the inner hair cells (Kujawa and Liberman, 2015). The loss of IHC/afferent fiber synapses reduces the robustness of stimulus coding in low signal to noise conditions, such as speech in noise (Kujawa and Liberman, 2009). In order to evaluate the effect of different exogenous factors (noise and ototoxic drugs) on synaptic function and the role of glutamate excitotoxicity in cochlear injury, we performed a 3-D reconstruction of synaptic ribbons in Wistar rats in different models of hearing loss.

Methods

Adult Wistar rats exposed to chronic noise (100 dB, 10kHz, 60 min/10 days), acute noise (120 dB, 10 kHz, 60 min) and ototoxic drug (styrene, 400 mg/kg, 3 weeks/5 days/week) were used. Auditory brainstem responses (ABR) were used to assess the magnitude of hearing loss. Latency/amplitude/ intensity curves were derived from ABR registrations. Surface preparations of the organ of Corti were immunolabelled with antibodies to CtBP2 (component of the presynaptic ribbon) and GluA2 subunit (postsynaptic glutamate receptor). Confocal microscopy technology was used to obtain 3-D reconstruction of the organ of Corti.

Results

Noise exposure affects the number of ribbon synapses and quantitative differences were observed between noise and ototoxicity-induced cochlear damage. Functional results of latency and amplitude of ABR waveform analysis were consistent with morphological observations.

Conclusion

This study provide evidences on the role of auditory nerve synapse loss in different models of cochlear injury, suggesting that noise or ototoxic drugs targets different cochlear cell compartments. Taken together, to investigate the effects of noise and ototoxicity on cochlear synaptic transmission could be useful to understand mechanisms of cochlear damage induced by different exogenous factors.

P-62 Effects of insulin on glucose transport mechanisms in the mouse organ of Corti in vitro**Nathan Huerzeler^{1,*}, Vesna Petkovic¹, Marijana Sekulic-Jablanovic¹, Matthew B. Wright², Daniel Bodmer¹***1 University Hospital Basel, Basel, Switzerland**2 Strekin AG, Basel, Switzerland*** Presenting author*

Several but not all studies in humans have demonstrated a potential association between diabetes and inner ear dysfunction leading to both morphological alterations and sensorineural hearing loss. However, little is known about the role of insulin signaling in the cochlea. We performed immunohistochemistry and gene expression analysis to characterize the expression pattern of the insulin receptor (IR) and various glucose transporters in the mouse cochlea. We also performed glucose uptake assays in the presence of insulin and the insulin sensitizer pioglitazone in cultured mouse organ of Corti (OC).

Western blots of protein extracts from OCs showed a high expression of IR and Glucose transporter 3 (GLUT3). Immunohistochemistry in cochlear sections demonstrated that the IR is specifically expressed in supporting cells. GLUT3 was found to be expressed in outer as well as in inner HC, in the basilar membrane (BM), the Stria vascularis (SV), Reissner's membrane (RM) and the spiral ganglion (SGN). Glucose transporter 1 (GLUT1) was detected with low signal in the BM, SV and RM and a high signal in the SGN. Glucose transporter 4 (GLUT4) was expressed on a low level in the BM and the SV.

Fluorescence glucose uptake assays revealed that HC take up glucose and that addition of insulin (10nM or 1µM) approximately doubled the rate of uptake. Pioglitazone (10µM), added with insulin, conferred a small but nonsignificant further increase in glucose uptake at the higher insulin concentration. Gene expression analysis in OC extracts confirmed high expression of IR, GLUT1 and GLUT3, which was unchanged in OCs treated with pioglitazone +/- insulin.

Together, these data show that functional units for active glucose transport are present in the OC. Hair cells appear to respond to insulin, however by a pathway not involving the canonical insulinresponsive glucose transporter GLUT4.

P-63 The effects of the somatostatin analogue pasireotide, and the role of NFAT, in the protection of auditory hair cells from aminoglycoside toxicity**Krystsina Kucharava¹, Marijana Sekulic Jablanovic¹, Matthew B. Wright², Daniel Bodmer¹, Vesna Petkovic¹***1 Department of Biomedicine and the Clinic for Otorhinolaryngology, Basel, Switzerland**2 Strekin AG, Basel, Switzerland.*

The chemotherapy and certain antibiotics such as gentamicin and other aminoglycoside antibiotics damage the hair cells and neurons of the cochlea.

Aminoglycoside-induced hair cell stress initiates an influx of calcium ions (Ca^{2+}) and rapid rise in intracellular calcium concentration. Once the inner ear receives these stressful insults, common pathophysiological mechanisms for the cochlea are activated.

We previously demonstrated, *in vitro*, that the Ca^{2+} -sensitive neuropeptide, somatostatin (SST), and its analogue, octreotide, protect HC from gentamicin-induced apoptosis. Mechanistically, SST binding to G-protein-coupled receptors (SSTR1-SSTR5). Mechanistically, SST binding to G-protein-coupled receptors (SSTR1-SSTR5) inhibits Ca^{2+} channel activity and the cytotoxic calcium ion influx, and associated downstream events, that provokes gentamicin-induced cell-death. In the present study, we report that a next generation SST analogue, pasireotide, prevents gentamicin-induced HC cell death in explants derived from the mouse organ of Corti (OC). We previously demonstrated that the SSTRs are expressed in the mammalian inner ear and the SST and its analogue octreotide can protect HC from gentamicin-induced hair cell death *in vitro*.

In the present study, we report that a next generation SST analogue, pasireotide, prevents gentamicin-induced HC cell death in explants derived from the mouse organ of Corti (OC).

These data suggest that SST analogues antagonize aminoglycoside-induced alterations in cellular Ca^{2+} flux, leading to preservation of HC survival pathways (PI3K, NMDA). SST analogues and NFAT inhibitors therefore represent new therapeutic opportunities for the treatment of hearing loss.

P-64 Animal models for studying auditory damage related to Type 1 Diabetes Mellitus**Ludimila Labanca^{1,*}, Paul Avan¹, Denise U Gonçalves², Sirley A da S Carvalho², Luciana M de Resende², Marion Souchal¹, Cristina A Delgado¹, Fabrice Giraudet¹***1 Université Clermont Auvergne**2 Federal University of Minas Gerais*** Presenting author*

One of the metabolic systemic diseases that can result in sensorineural hearing loss is the Type 1 Diabetic Mellitus (T1DM). By losing beta cells, the production of endogenous insulin is severely reduced, leading to hyperglycemia and hypoinsulinemia. In this prospective study we aimed to investigate the pathophysiology of T1DM associated with hearing impairment by using two mice models: C57BL and CBA mice. T1DM was induced in both models through single intraperitoneal injection of streptozotocin (STZ) at 150 mg/Kg. Body weight, blood glucose, and auditory tests (Auditory Brainstem Response – ABR; Distortion Product Otoacoustic Emission – DPOAE) were evaluated at the baseline and every 2 weeks during a 6-week period. In T1DM groups, the body weight had a significant decline while in the control group it increased. Blood glucose levels were significantly increased in C57BL mice, one week after STZ injection and after two weeks in CBA mice. In the control group there were no changes. During the whole evaluating period, ABR and DPOAE tests did not show any significant difference in thresholds, neither in CBA mice nor in controls. However, we found a significant increase in diabetic C57BL from the second week, after induction of STZ. Mean DPOAE amplitude was found to be significantly reduced in diabetic C57BL six weeks after STZ, but not in CBA mice. Our data suggest that C57BL diabetic mice are more susceptible to cochleae damage than CBA diabetic mice and that these animals models could be a good way for studying cochlear damage (C57BL model) and central auditory pathways (CBA model) related to T1DM.

P-65 Expression of BK channel γ subunits LRRC26 and LRRC52 in inner hair cells**Isabelle Lang^{1,*}, Barbara Niemeyer¹, Jutta Engel¹***1 Saarland University, Center for Integrative Physiology and Molecular Medicine*** Presenting author*

Voltage and Ca^{2+} -activated K^+ (BK) channels of mature IHCs are responsible for fast repolarization of the receptor potential and for the small IHC time constant. BK channels in IHC are independent of Ca^{2+} influx and activate around -60 mV, whereas in heterologous expressions systems, they do not activate below $+100$ mV in the absence of Ca^{2+} . So far, the mechanisms of the very negative activation of BK channels in IHCs are not understood.

Members of the leucin-rich-repeat-containing (LRRC) protein family can function as a regulatory subunit of BK channels (Yan & Aldrich, 2010) transforming them into a purely voltage-gated K^+ channel. In arterial myocytes, LRRC26 is an auxiliary (" γ ") subunit of BK channels and is colocalized with the BK- α channel pore (Evanson, 2014).

We performed transcript analysis for LRRC proteins that can substantially shift the activation of BK channels to more negative potentials: LRRC26 (-140 mV) and LRRC52 (-100 mV). Nested RT-PCR with cDNA reverse-transcribed from reference tissue, organ of Corti and selectively harvested hair cells showed transcripts for LRRC26 and LRRC52 in the organ of Corti and in most inner and outer hair cells.

Mallotoxin (MTX) specifically modulates the conductance-voltage relationship of BK channels expressed in HEK cells (Zakharov, 2005). However, this effect is lost and replaced by a 2-fold faster BK channel activation if LRRC26 is present (Almassy & Begegnisich, 2012). Patch-clamp recordings on the effect of MTX on gating of BK channels in IHCs are in progress.

Taken together, evidence is accumulating that LRRC26 and LRRC52 are expressed in IHCs, which may explain the very negative voltage range of BK channel activation.

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P-66 Improved auditory fidelity by cochlea BDNF- a precondition for adaptive homeostatic plasticity?**Marie Manthey^{1,*}, Dario Campanelli¹, Wibke Singer¹, Lukas Rüttiger¹, Marlies Knipper¹***1 Tübingen Hearing Research Centre (THRC), Molecular Physiology of Hearing, Elfriede-Aulhorn-Str. 5, 72076 Tübingen, Germany*** Presenting author*

Previously, we showed that peripheral BDNF in the cochlea or lower brainstem regions (studied in the BDNF Pax2 Cre mouse), but not central BDNF from the higher cortical brain regions drives auditory fidelity with sensory experience [1]. This improved auditory fidelity includes improved sensitivity of auditory fibers, lowering of hearing thresholds, and enlarged dynamic range, shortening of latency and changed inhibitory strength. The alteration of markers for inhibitory connections spread along the entire auditory pathway as well as hippocampal circuits. We asked, how BDNF Pax2 Cre KO mice with hampered development of auditory fidelity cope with different injury situations induced by noise exposure [2,3]. To answer this question we exposed BDNF Pax2 Cre KO and WT mice to enriching (80dB SPL), mildly traumatic (100dB SPL), and traumatic (120dB SPL) sound and characterised functional (ABR) and molecular markers (plasticity genes, markers for inhibition and excitation) along the auditory pathway. The findings are presented and discussed in the context of sensory experience-induced maturation of auditory fidelity as potential prerequisite for adaptive homeostatic plasticity.

P-67 Defective Gpsm2/Gai3 signaling disrupts stereocilia development in Chudley McCullough syndrome

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The Chudley McCullough syndrome (CMCS) is a rare autosomal recessive neurological disorder characterized by early and severe onset of deafness and brain anomalies (Chudley et al., 1997). Recently, mutations in the G protein signaling modulator 2 (GPSM2) gene were found to be causative of the pathology (Walsh et al., 2010; Doherty et al., 2012). It has been suggested that CMCS is due to a defect in asymmetric division of hair cells progenitors, as Pins is known to control asymmetric cell division, but the exact molecular and cellular bases of the pathology are unclear.

Here, we show that Gpsm2 protein defines a nanodomain at the tips of the tallest stereocilia and that this localization requires the presence of Gai3, myosin15 and whirlin, the last two well-known regulators of stereocilia elongation. In absence of Gpsm2 the stereocilia elongation process is stopped, a shortening of the hair bundle representing the most probable cause for early and severe deafness in the Gpsm2 mutants. We further report that lack of Gnai3 elevates hearing threshold, correlating with hair bundle elongation defects in high-frequency cochlear regions.

Mechanistically, we identify an interaction between Gpsm2 and whirlin, and show that this interaction plays stabilizes whirlin. This interaction is maintained with some of the CMCS variants analyzed, affecting the ability of the myosin15/whirlin complex to generate filopodia in a heterologous system. These CMCS mutations also reduce F-actin levels while overexpression of the Gpsm2/Gai3 complex increases F-actin levels. Our data support the idea that Gpsm2/Gai3 are at the interface between the actin and the membrane to regulate the actin polymerization at the tip of stereocilia.

We believe that the novel function we identified for the Gpsm2/Gai3 complex on actin polymerization during stereocilia elongation is at the root of the stereocilia elongation defects and deafness in CMCS patients.

P-68 Loud sound changes mitochondrial morphology in outer hair cells**Alfred L Nuttall^{1,*}, Sarah Foster¹, Teresa Wilson¹***1 Oregon Health & Science University*** Presenting author***Background**

Cellular stress can affect mitochondrial dynamics resulting in increased mitochondrial fragmentation, production of excessive oxidative free radicals, and cell death. Whether mitochondrial dynamics are altered in response to increased energy needs of outer hair cells (OHCs) with loud sound exposure has not been well-characterized.

Objective

To determine whether loud sound affects mitochondrial dynamics in auditory hair cells.

Methods and results

For visualization of OHC mitochondria, PhAMfloxed mice (C57Bl/6 background) were crossed with prestin-CreERT2 mice to generate mice that express a mitochondrial-specific version of the Dendra2 fluorescent protein. These mice were exposed to loud sound (98 dB SPL, 8-16 kHz, 2 hours) and the cochlea harvested at various times following noise exposure. Airyscan imaging revealed primarily punctate mitochondria in the OHCs of control animals. Further, distinct populations of mitochondria were observed: perinuclear, lateral, and apical. In basal OHCs, the perinuclear mitochondria were significantly smaller than those located in the apical region. Additionally, the apical and lateral mitochondria decreased in size progressing from the base to the apex of the cochlea. Following noise exposure, the apical mitochondria in basal OHCs acquired a more punctate morphology and an altered spatial localization.

Conclusions

Regulation of mitochondrial fusion and fission processes, and therefore the morphological state of mitochondria, is believed to modulate mitochondrial function and health. The presence of fragmented mitochondria in normal OHCs suggests that the balance of fission and fusion processes are altered, potentially due to a higher oxidant environment being present in these cells. The existence of distinct populations of mitochondria in OHCs, particularly OHCs in the high frequency region of the cochlea, likely reflects the regional metabolic needs within these cells, and may provide a clue as to the basis for the increased sensitivity of these OHCs to damage from loud sound exposure.

Funding

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P-69 POLD1 variants leading to reduced polymerase activity can cause hearing loss without syndromic features**Doo-Yi Oh^{1,*}, Min Young Kim¹, Jin Hee Han¹, Hye-Rim Park¹, Seungmin Lee¹, Byung Yoon Choi¹**¹ Seoul National University Bundang Hospital

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Purpose

The largest catalytic subunit of the DNA polymerase delta (Pol δ) complex, encoded by *POLD1* in humans, has domains with polymerase (pol) and exonuclease (exo) activities. Single heterozygous *POLD1* mutations in these domains have been recently reported to cause syndromic deafness as a part of multisystem metabolic disorder or predisposition to cancer. However, the phenotypic outcomes of diverse combinations of *POLD1* genotypes have not been elucidated in humans.

Methods

Whole exome sequencing of five members of a multiplex family segregating autosomal recessive sensorineural hearing loss without any syndromic feature (NS-SNHL) have revealed novel compound heterozygous variants (p.S197HfsX54 and p.G1100R) of *POLD1*. Deducing that p.S197HfsX54 would result in a null allele, the pol and exo activities from recombinant p.G1100R Pol δ variant were measured. Further, we measured replicative Pol δ activities also from extracts of patient-derived Pol δ -mutant cells.

Results

The recombinant p.G1100R Pol δ showed a reduced pol activity by 30-40%, but exhibited normal exo activity. The pol activity in cell extracts from the affected subject carrying the two *POLD1* mutant alleles was about 33% of normal controls.

Conclusion

Significantly decreased pol activity of Pol δ , but not a complete absence, with normal exo activity could lead to NS-SNHL.

P-70 Polyphenols in noise induced hearing loss (NIHL)**Antonella Di Pino^{1,*}, Rolando Rolesi¹, Fabiola Paciello², Diana Troiani³, Anna Rita Fetoni¹, Gaetano Paludetti¹***1 Institute of Otolaryngology Università Cattolica, School of Medicine, Rome, Italy**2 Institute of Otolaryngology Università Cattolica, School of Medicine, Rome, Italy; Institute Cell Biology and Neurobiology, CNR, Monterotondo (Italy)**3 Institute of Human Physiology, Università Cattolica, School of Medicine, Rome, Italy***Presenting author***Introduction**

Several experimental models demonstrated that hair cells (HCs) damage after Noise exposure is highly related to cochlear redox unbalance which leads to an overproduction of reactive oxygen species (ROS) with subsequent lipoperoxidative cell damage. Many antioxidants such as Ferulic Acid (FA), Rosmarinic Acid (RA) and Caffeic Acid (CA) have been proved to counteract redox unbalance and to prevent oxidative stress induced hair cells death. These phenolic compounds have been described as an anti-inflammatory, anti-viral, anti-bacterial, anti-neoplastic and antioxidant compound. In this study, we evaluated the effectiveness of these three different polyphenols in order to establish the best modality for antioxidant treatment against NIHL.

Methods

Wistar rats (200-250 g) were used in this study. In Noise groups, animals were exposed for 60 minutes to a pure tone of 120 dB SPL at 10 kHz. Polyphenols was administrated 1h pre trauma and for 3 consecutive days as follows: FA (i.p at 75-150-600 mg/kg), RA (i.p. at 2,5-5-10-20 mg/kg) and CA (i.p. at 15- 30 and 50 mg/Kg) in all experimental groups. Auditory functional analyse was evaluated by Auditory Brainstem Responses (ABR), morphological analysis was performed using Rhodamine-Phalloidin staining (Rh-Ph) and immunofluorescence studies was spending in order to quantified cochlear superoxide amount and lipid peroxidation production.

Results

Our results demonstrate that FA, RA and CA can: (a) attenuate hearing loss by reducing ABR threshold shift at 1, 3 and 7 days after the acoustic trauma; (b) polyphenols decrease hair cell loss as shown by Rh-Ph staining as highlight in cochleogram and (c) decrease cell damage in the cochlea by reducing superoxide amount and lipid peroxidation.

Conclusion

Our results demonstrate that FA, RA and FA reduce the oxidative cochlear damage caused by noise trauma. Consequently, polyphenols can provide a promising approach against the oxidative stress induced by NIHL.

P-71 Spontaneous calcium transients in mouse interdental cells during the critical period of cochlear development**Thore Schade-Mann¹, Pauline Schepsky^{1,*}, Jutta Engel¹, Tobias Eckrich¹***1 Saarland University, Center for Integrative Physiology and Molecular Medicine*** Presenting author*

The tectorial membrane (TM) is essential for normal hearing. It is attached to the spiral limbus and the stereocilia of the outer hair cells and consists of collagens and glycoproteins, which account for about 50 % of TM material and are exclusive to the inner ear. Mutations of these proteins not only lead to aberrant TM formation but also to severe hearing loss. During the first postnatal week interdental cells (IDC) massively secrete TM proteins into the extracellular space. So far, little is known about the physiology of IDCs and about how they build up the TM. We performed Ca^{2+} imaging in acutely dissected inner ear explants from postnatal day 1 (P1) to P18 using the Ca^{2+} indicator Fluo 8-AM and a confocal laser scanning microscope (Zeiss LSM 710). 5 – 20 % IDCs generated spontaneous Ca^{2+} transients, which did not propagate to neighbouring cells, at a low rate (≥ 1 event/10 min). Two principal types of spontaneous IDC Ca^{2+} transients were observed: a) single peaks with fast upstroke and decay and (b) longer, complex transients with double or multiple peaks. Interestingly, both rate of activity and signal types changed as a function of age. Applying 1 – 10 μM ATP or UTP evoked oscillatory Ca^{2+} transients in nearly all IDCs at P1 – P5. Responses to ATP and UTP differed significantly after the onset of hearing, indicating a change in purinergic receptor expression during maturation. The mechanisms behind the Ca^{2+} transients / oscillations remain to be elucidated.

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P-72 The expression and localization of DGK ζ in normal and noise exposed guinea pig cochlea**Chikako Shinkawa^{1,*}, Tsukasa Ito¹, Makoto Chiba¹, Yasukazu Hozumi², Kaoru Goto¹, Seiji Kakehata¹***1 Yamagata University Faculty of Medicine, Japan**2 Akita University Faculty of Medicine, Japan*** Presenting author*

Cochlear hair cells are the mechanoreceptors of hearing in mammals and these hair cells collect information, then transmit this information along the auditory pathway to the auditory cortex where it processed as sound. Damage to these hair cells can result in sensorineural hearing loss. If the cause of hair cell loss can be determined then it may become possible to prevent such loss. Until now nobody has investigated the expression and the role of Diacylglycerol kinase (DGK) in the cochlea which catalyzes the phosphorylation of diacylglycerol (DG) to phosphatidic acid (PA) leads to play an important role in the intercellular lipid signal transduction. Recent studies reveal the relationships between DGK isozymes and diseases. In this study we focused on the relation between hair cell damage and DGKs. First we performed RT-PCR to elucidate the expression of DGK ζ which has been reported to have the functional implication on cellular pathophysiology under the stress conditions. Then we used immunohistochemical approach for DGK ζ and confirmed that DGK ζ localizes to the nucleus of inner hair cells (IHC), outer hair cells (OHC), supporting cells (SC) and spiral ganglion cells (SG) but not glia cells in spiral ganglion. Moreover DGK ζ translocated to the cytoplasm after noise exposure on OHCs, which are damaged by noise exposure. Recent studies have reported that DGK ζ shuttles between the nucleus and the cytoplasm in neurons under the stress conditions and this shuttle of the DGK ζ affects on the apoptosis of neurons. These results suggest that DGK ζ is involved in the OHC death after noise exposure.

P-73 Oxidative stress caused by cisplatin on the inner ear cell line OC-k3**Edi Simoni¹, Erica Gentilin¹, Milvia Chicca², Alessandro Martini¹, Laura Astolfi^{1,*}**¹ Bioacoustics Research Laboratory, Department of Neurosciences, University of Padua, Italy² Department of Life Sciences and Biotechnology, University of Ferrara, Italy

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The production of reactive oxygen species (ROS) plays a key role in cell death and signalling in auditory tissues and is involved in several types of hearing loss, including sensorineural, age-related, hereditary, ototoxic and noise-induced. Cisplatin is a common antineoplastic agent employed in clinical treatments of solid tumours, but its application is known to cause severe side effects, such as neurotoxicity, nephrotoxicity and ototoxicity. This drug is also known to interfere with the production of endogenous antioxidants able to protect the inner ear against ROS toxicity. In order to test *in vitro* the relationship between cisplatin and oxidative stress in the inner ear, we examined the effects of administration of different doses of cisplatin on an immortalized mouse cell line derived from the organ of Corti (OC-k3), analysing cell viability, cell morphology, metabolism and expression of proteins involved in oxidative stress and apoptosis. The results show that cisplatin causes in OC-k3 cells a dose-dependent increase of oxidative stress, activating cell death pathways and inducing apoptosis after 24 h of exposure. The OC-k3 cell line appears a reliable model for studies of cell death pathways related to oxidative stress in auditory pathologies. Based on these studies, *in vitro* protection protocols by antioxidants against ROS produced by exposure to ototoxic drugs could be developed and translated to *in vivo* models and clinical trials, integrating advanced hearing loss therapies.

P-74 Specifying the calcium channels of primary neurons of the rat cochlear nucleus by analyzing the calcium activity

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In 2011 neuronal stem cells were first described in the cochlear nucleus (Rak et al. 2011). In order to get more information on the function of these cells their calcium channels which develop during differentiation are of interest, since calcium currents indirectly reflect the activity and differentiation of neurons.

The cochlear nucleus from p6 rats was microscopically dissected. The tissue was dissociated and single cells plated in stem cell medium. After 3 days medium was changed to differentiation medium. For calcium imaging experiments cells were loaded with the calcium sensitive fluorophore Oregon Green on day 4 of cell differentiation. Experiments were performed with addition of the calcium channel inhibitors nifedipine, omega-conotoxin MVIIC, kurtoxin and SNX-482 or control agents. After the measurements cells were fixed with paraformaldehyde and analyzed by immunocytochemistry.

It could be shown that the calcium channel inhibitors in different manifestation increase the intracellular calcium concentration. This can be explained by the blockade of the calcium channels on the somata and dendrites of the neurons. Furthermore, neurons of the cochlear nucleus expressed calcium channels of the L-, N-, P/Q- an R-type in different distribution in the outer cell membrane of the somata and dendrites during the cell differentiation.

These results show, that different calcium channels are involved in the formation of the electrical activity of the cochlear nucleus neurons. This knowledge expands understanding of the development and function of the auditory system.

P-75 P/Q-type Ca²⁺ currents of spiral ganglion neurons and endbulbs of Held specifically require the auxiliary subunit $\alpha 2\delta 3$ **Friederike Stephani^{1,*}, Stefan Münkner¹, Jutta Engel¹***1 Saarland University, Center for Integrative Physiology and Molecular Medicine*** Presenting author*

Spiral ganglion (SG) neurons connect hair cells with central auditory neurons and are indispensable for auditory signal transmission. Proper function and morphology of auditory nerve fiber synapses require the auxiliary Ca²⁺ channel subunit $\alpha 2\delta 3$ (Pirone et al., J Neurosci 2014). To determine the role of $\alpha 2\delta 3$ for SG neurons, we analyzed Ca²⁺ currents in cultured SGNs (Lv et al., J Neurosci 2010) from $\alpha 2\delta 3^{+/+}$ and $\alpha 2\delta 3^{-/-}$ mice at postnatal day (P) 5 and 20. SG neurons express a variety of Ca²⁺ currents at both ages. L-type Ca²⁺ currents, which were isolated by blocking with 10 μ M nimodipine, contributed ~30 % to total I_{Ca} in $\alpha 2\delta 3^{+/+}$, which was unaltered in $\alpha 2\delta 3^{-/-}$ mice (P20). P/Q-type Ca²⁺ currents, which were isolated using 1 μ M ω -agatoxin IVA, contributed to ~60 % of total I_{Ca} in $\alpha 2\delta 3^{+/+}$. In contrast, P/Q-type Ca²⁺ currents were reduced to only 20% of total I_{Ca} in $\alpha 2\delta 3^{-/-}$ SGNs indicating that P/Q-type channels of SG neurons had a clear preference for $\alpha 2\delta 3$. Our data show that normally sized P/Q-type Ca²⁺ currents in P20 SGNs require $\alpha 2\delta 3$. The total I_{Ca} was unchanged between genotypes indicating partial compensation by other Ca²⁺ currents which is currently being investigated.

We further analyzed endbulb of Held synapses at bushy cells using VGLUT-1 labeling. At P7, the number of boutons rather than the size was significantly reduced in $\alpha 2\delta 3^{-/-}$ mice. At P20, however, the number of boutons was similar but the VGLUT1-labeled area was significantly smaller in $\alpha 2\delta 3^{-/-}$ compared with $\alpha 2\delta 3^{+/+}$ mice. We conclude that $\alpha 2\delta 3$ plays a decisive role for the development of endbulbs of Held even before the onset of hearing. Furthermore, it is essential for proper expression of presynaptic P/Q type Ca²⁺ currents.

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P-76 Viral gene transfer of short otoferlins partially restores the fast component of synaptic exocytosis in auditory hair cell from OTOF knock-out mice

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Transmitter release at auditory inner hair cells (IHCs) ribbon synapses involves sustained exocytosis of glutamatergic vesicles during voltage-dependent activation of L-type (Cav1.3) calcium channels (Glowatzki and Fuchs, 2002; Brandt et al., 2003). Remarkably, IHCs do not use the conventional two-C2 domains synaptotagmins (Syt1 and Syt2) as calcium (Ca^{2+}) sensors to trigger synaptic vesicle fusion (Beurg et al., 2010). Otoferlin, a large six-C2 domains protein (C2A-F), has been proposed to function as a high affinity Ca^{2+} sensor that controls the fast and indefatigable release of synaptic vesicles at the IHCs ribbon synapses (Roux et al., 2006; Beurg et al., 2010; Vincent et al., 2014). However, the precise molecular events by which individual otoferlin C2-domains contributes and/or regulates the synaptic vesicle cycle is still incompletely understood. In the present study we have characterized the role of the Cterminal C2-domain of otoferlin. For that purpose, we used an in vivo cochlear viral gene transfer to newborn mutant mice. The Adeno-Associated Virus (AAV) mediated efficient transfer of several otoferlin short forms (otoferlin C2-EF, C2-DEF, C2-ACEF or C2-ACDF) into mouse IHCs lacking otoferlin. The expression of these various otoferlin short forms failed to restore hearing. Surprisingly, IHC patch-clamp recordings showed that the expression of these short otoferlin forms resulted in a partial restoration of the fast component of synaptic exocytosis but not of the sustained component. These results confirm that otoferlin is involved in both the fast vesicle fusion and vesicle replenishment (Pangrsic et al. 2010), and suggest that a cooperativity between the six-C2 domains structure is required for an efficient priming-mobilization of synaptic vesicles in IHCs. Interestingly, the partial exocytotic restoration of the fast component was associated with a recovery to near normal amplitude of the fast inactivating Ca^{2+} currents (Vincent et al., 2017), suggesting that Cav1.3 channel short isoforms interacts with otoferlin.

P-77 Investigating the role of Radixin in modulation of outer hair cell stereocilia stiffness**Sonal Prasad^{1,*}, Anders Fridberger¹**¹ Neurobiology

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Mammalian hearing depends on deflection of stereocilia on the sensory outer hair cells (OHCs) of the inner ear. Previous data from our group indicate that the stiffness of outer hair cell stereocilia is actively regulated (1). The molecular mechanism that regulate the deflection of stereocilia has not been determined.

The main aim of this study is to investigate the mechanistic pathway that underlie the stiffness modulation of stereocilia. Our hypothesis is that the membrane-cytoskeleton linker protein radixin (Rdx), which is present at a high concentration in stereocilia in mammals, could contribute to stiffness regulation. Radixin is found at the base of stereocilia in guinea pigs and in bullfrog saccular hair cells (2). Absence of radixin leads to deafness because of progressive degeneration of hair cell bundles. Rdx activation is known to be regulated by the membrane lipid phosphatidylinositol 4,5-bisphosphate (PIP2), which is also required for adaptation in hair cells. To test this hypothesis, we use the radixin blocker DX-52-1 which binds strongly and specifically to radixin (3). Time-resolved confocal imaging was used to visualize the sound-evoked motion of stereocilia in an in vitro preparation of the guinea pig temporal bone.

We found that the DX-52-1 inhibitor leads to an irreversible decline in stereocilia movements and in the amplitude of the cochlear microphonic potential. However, DX-52-1 caused a paradoxical increase in electromotility. It is however unclear how a protein present in stereocilia can have such a effect on the motility of the cell body.

In this study we examine the mechanism of action and effects of Rdx inhibition on the sound-evoked motion of stereocilia. These data suggests the importance of proteins especially the role of radixin molecule to be functionally important for outer hair cell bundle mechanics in the inner ear hearing loss.

P-78 Putative role of the distal portion of the endolymphatic sac in sensing and regulating endolymphatic calcium concentration in the inner ear

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Introduction and aims

Exceptionally low calcium (Ca^{2+}) concentrations in the endolymphatic fluid ($[\text{Ca}^{2+}]_e$, 24 – 260 μmol) are crucial for proper auditory and vestibular function in the inner ear. The endolymphatic sac (ES) has previously been proposed to be critically involved in maintaining $[\text{Ca}^{2+}]_e$. However, a conclusive model on how the ES can contribute to endolymphatic Ca^{2+} homeostasis at the molecular level is still missing. This study aimed (i) to identify Ca^{2+} transport proteins in the ES epithelium by immunohistochemical methods and (ii) to develop a model of $[\text{Ca}^{2+}]_e$ homeostasis by the ES.

Materials and methods

1) Immunolabeling of paraffin-embedded murine ES tissue sections (male C57BL/6, 6 – 8 weeks). 2) Quantifying of DAB-immunolabeled epithelial cells along the proximal-todistal axis of the ES. 3) Immunofluorescence double labeling of ES cell-type specific Ca^{2+} transport-related proteins.

Results

Calcium-sensing receptor CaSR, transient receptor potential cation channels subtypes TRPV5 and TRPV6, sarco/endoplasmic reticulum Ca^{2+} -ATPases SERCA1 and SERCA2, $\text{Na}^+/\text{Ca}^{2+}$ exchanger NCX2, and plasma membrane calcium ATPases PMCA1 and PMCA4 were localized in ES epithelial cells, demonstrating (i) a distinct subcellular localization pattern in the apical or basolateral membrane domains, or the cytoplasm, (ii) a spatial (proximal-to-distal) labelling gradient with strongest labelling in the most distal ES portion, and (iii) a distinct cell-type specific localization pattern in designated mitochondria-rich (MRCs) or ribosome-rich cells (RRCs).

Conclusion

In the distal portion of the murine ES epithelium, distinct subpopulations of cells were identified with supposedly different functions in endolymphatic Ca^{2+} homeostasis, i.e. CaSR-expressing MRCs that constitute potential “sensors” of $[\text{Ca}^{2+}]_e$, and RRCs that express different Ca^{2+} transport proteins and therefore constitute potential “mediators” of transepithelial Ca^{2+} fluxes. Loss of the distal ES's Ca^{2+} transport system is of potential pathophysiological significance in diseases associated with ES pathologies, such as large vestibular aqueduct syndrome or Meniere's disease.

P-79 Generation of functional Cx26-gap junction plaque forming cells that exhibit spontaneous Ca²⁺ transients from induced pluripotent stem cells**Ichiro Fukunaga^{1,*}, Kaori Hatakeyama¹, Ayumi Fujimoto¹, Osamu Minowa², Katsuhisa Ikeda¹, Kazusaku Kamiya¹**¹ Juntendo University Faculty of Medicine² RIKEN

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Introduction

Mutation of the Gap Junction Beta 2 gene (*GJB2*) encoding connexin 26 (Cx26) is the most frequent cause of hereditary deafness worldwide. Recently, we reported that disruption of the Cx26-dependent gap junction plaque (GJP) is associated with the pathogenesis of *GJB2*-related deafness (Kamiya *et al.*, *J Clin Invest.* 2014) and the cochlear gene transfer of *GJB2* using an adenoassociated virus significantly improved GJP formation and the auditory function (Iizuka *et al.*, *Hum Mol Genet.* 2015). Embryonic stem (ES)/induced pluripotent stem (iPS) cells are an important tool for studying the molecular mechanisms underlying inner-ear pathology as well as for generating cells for replacement therapies. However, previous studies have targeted the generation of inner-ear hair cell-like cells from ESCs/iPSCs. It has not been reported that ES/iPS cells differentiate into Cx26-GJP forming cells such as cochlear supporting cells. In this study, we developed a new strategy for differentiation of mouse iPS cells into Cx26-GJP forming cells.

Method

We examined the strategy to induce Cx26-GJP forming cells from mouse iPS cells using modified methods of previous studies (Koehler *et al.*, *Nature.* 2013).

Results

After the aggregate formation, Cx26-expressing GJP-forming cells (iCx26GJC) were observed in a part of the aggregate. Aggregates were subcultured in adherent culture, and proliferation of iCx26GJCs was observed. To investigate whether the iCx26GJC were functional, we performed scrape-loading assay and Ca²⁺ imaging. In the iCx26GJC culture, we observed that Lucifer yellow diffused beyond the wounded parental cells. Furthermore, iCx26GJC exhibited spontaneous Ca²⁺ transients and their propagation as in the developing cochlea.

Conclusion

In this study, we demonstrate the differentiation of mouse iPS cells into functional Cx26-GJP forming cells, as in the cochlea (Fukunaga *et al.*, *Stem Cell Reports.* 2016). By using these cells, it is expected to establish the inner ear cell therapy for hearing recovery in *GJB2*-related hereditary deafness.

P-80 Generation and differentiation of iPS cells from Connexin26 conditional knockout mice**Kaori Hatakeyama^{1,*}, Ichiro Fukunaga¹, Ayumi Fujimoto¹, Osamu Minowa², Katsuhisa Ikeda¹, Kazusaku Kamiya¹**¹ Juntendo University Faculty of Medicine² RIKEN

* Presenting author

Objectives

Hereditary deafness affects approximately one in 1600 children. Gjb2 gene encoding connexin26 (Cx26) plays important role in intercellular communication of cochlea Gap junction between the inner ear cells (Kamiya, J Clin Invest, 2014). We previously developed Cx26 knockout mice (CX26^{fl/fl} P0- Cre) by using Cre-loxP system. In this study, we generated induced pluripotent stem (iPS) cells from CX26^{fl/fl} P0-Cre mice to recapitulate the pathogenesis caused by Cx26 deficiency. Our final goal is to produce the inner ear cell-like cells from the patients with GJB2 related hearing loss for drug screening and cell therapy.

Methods

We dissected cochlear fibrocytes of CX26^{fl/fl} P0-Cre mice and C57BL/6 mice because Cx26 gene is specifically deleted in inner ear of this mouse. These cells were reprogramed to generate iPS cells by using Sendai-virus vector with reprogramming factors, Klf4, Oct3/4, Sox2 and c-Myc, and characterized the pluripotency. Some iPS clones with GJB2 deletion were selected and induced to differentiate into cochlear gap junction forming cells.

Results

The iPS cells generated from CX26^{fl/fl} P0-Cre (KO-iPS) and C57BL/6 inner ear cells were differentiated into cochlear gap junction forming cells. KO-iPS recapitulated the initial pathology, "Gap junction disruption" as in cochlea of CX26^{fl/fl} P0-Cre (Fukunaga, Stem Cell Reports, 2016, 7(6), 1023-1036).

Conclusions

In this study, we generated iPS cells from the cochlear fibrocytes of CX26^{fl/fl} P0-Cre and C57BL/6 mice by SeV vector with reprogramming genes. The inner ear cell-like cells from these iPS cells recapitulated the molecular pathology of cochlea, and this suggest that these cells can be used as in vitro model of CX26 related hearing loss. Disease specific iPS cells derived from mutant animal models will be powerful tool for drug screening and cell therapy targeting GJB2 related hereditary deafness.

P-81 Hearing impairment induced by shock wave exposure**Kunio Mizutari^{1,*}, Eiko Harada¹, Yasushi Satoh¹, Akihiro Shiotani¹***1 Department of Otolaryngology, Head and Neck Surgery, National Defense Medical College, Japan**4 Department of Pharmacology, National Defense Medical College, Japan***Background**

Blast injuries to head and neck area have increased because of terrorism and conflicts. The ear is one of high-risk organs for blast injury as well as intestinal tract and lung. Sensorineural hearing loss caused by a shock wave is the most critical etiology relating to blast-induced hearing loss. We have established mice models of blast-induced traumatic brain injury and lung injury that are induced by blast-tube. We, herein, present a novel hearing loss model of blast-induced hearing loss.

Methods

Six-week-old CBA/J mice were used. Shock waves were generated by compressed nitrogen in the SUS-tubing. In this experiments, the peak pressure of shock wave was set at 25 kPa. Shock wave irradiation was applied in front of mice head. The thresholds of auditory brainstem responses (ABRs) were measured continuously up to 4 weeks, and histological analysis was performed at 4 weeks after treatment. Hair cell and synaptic ribbon counts were conducted using whole-mount preparation of the organs of Corti.

Results

All treated animals were survived after shock wave irradiation without any side injuries such as perforation of eardrum or cerebral hemorrhage. Therefore, hearing impairment in this study was considered as sensorineural hearing loss. The ABR thresholds were elevated 1 day after treatment. The threshold was recovered gradient, but threshold shifts at high frequencies were remained up to 4 weeks after exposure at all frequencies. The wave I amplitude changes was also seen as the same trend as the threshold changes. Histological analysis revealed that slight outer hair cell loss was observed. And the numbers of synaptic ribbon at the high frequencies area were significantly decreased compared to the control.

Conclusion

Our mouse model revealed that shock wave irradiation generated by shock tube could generate sensorineural hearing loss. This model would replicate important characteristics of blast-induced hearing loss.

P-82 A novel method for cochlear in toto visualization of pre-clinical models of auditory neuropathies**Jennifer Schulze^{1,*}, Lena Nolte², Nadine Tinne², Marc A. Willaredt³, Hans Gerd Nothwang³, Dag Heinemann², Thomas Lenarz¹, Athanasia Warnecke¹, Tammo Ripken²***1 Department of Otorhinolaryngology, Head and Neck Surgery, Hannover Medical School, Hannover, Germany**2 Industrial and Biomedical Optics Department, Laser Zentrum Hannover e.V., Hannover, Germany**3 Neurogenetics, Carl von Ossietzky University Oldenburg, Oldenburg, Germany*** Presenting author*

Many millions of people worldwide are affected by hearing loss. Age-related hearing loss can be caused by loss of hair cells, degeneration of spiral ganglion neurons (SGNs) or metabolic disorders. However, several studies have shown that neuronal damage at the level of the ribbon synapses can occur without any changes in the audiometry of these patients. Patients with this phenotype have difficulties in speech comprehension, especially in a noisy background. Therefore, novel pharmacological and biological therapies should be developed and the molecular and structural changes should be identified. Scanning laser optical tomography (SLOT) is a valuable imaging technique to visualize the murine cochlea in toto without physical slicing [1]. Cochleae of different mouse genotypes were used. The isolated murine cochleae were decalcified with 10% ethylenediaminetetraacetic acid (EDTA) for four days, followed by immunocytochemistry allowing the staining of fine structures within the whole cochlea (hair cells or SGNs). Then, the cochleae were dehydrated by using ethanol and optically cleared with MSBB (Spalteholz fluid) [1]. One main advantage of SLOT is that the whole cochlea can remain intact allowing a visualization of the whole cochlea without any damages or artefacts due to cutting of the tissue. Within the field of hearing research, a variety of different genetic knockout mouse models with several molecular phenotype changes exist, e.g., the Cav1.3^{-/-} knockout mouse. By using SLOT, we could show that at P20 the dendrites of the SGN were visible in wild type and Cav1.3^{-/-} mice whereas at P27 the dendrites of the SGNs were only visible in wild type mice but not in Cav1.3^{-/-} mice. Due to the postnatal onset and progressive loss of cochlear structures, the Cav1.3^{-/-} mice seem to be a promising pre-clinical animal model for otologic gene therapy and pharmacological interventions.

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P-83 Nrf2 is a key target in counteracting the oxidative stress induced cochlear damage**Anna Rita Fetoni^{1,*}, Rolando Rolesi², Fabiola Paciello¹, Antonella Di Pino², Diana Troiani³, Gaetano Paludetti²***1 Institute of Otolaryngology Università Cattolica, School of Medicine, Rome, Italy, Institute Cell Biology and Neurobiology, CNR, Monterotondo (Italy),**2 Institute of Otolaryngology Università Cattolica, School of Medicine, Rome, Italy**3 Institute of Human Physiology Università Cattolica, School of Medicine, Rome, Italy*** Presenting author***Introduction**

Reactive oxygen species and lipoperoxidative damage in conjunction with the imbalance of antioxidant defenses is a common target for sensorineural hearing loss (SNHL) induced by exogenous factors including noise and ototoxicity. The redox-sensitive transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) plays a critical role in the regulation of cellular defenses against oxidative stress, including heme-oxygenase-1 (HO-1) activation. We describe a link between cochlear oxidative stress damage and the activation of the Nrf2/HO-1 pathway in the experimental models of SNHL.

Methods

Animals underwent to cochlear injuries by noise exposure and cisplatin ototoxicity were treated with antioxidant –polyphenols molecules: Rosmarinic acid, curcumin and Ferulic acid. In control noise induced hearing loss and ototoxicity and treated animals, the auditory function was measured and the expression of superoxide production and lipid peroxidation marker 4-hydroxynonenals (4-HNE) and the activation of Nrf2/HO-1 pathway have been studied by using western blotting and immunohistochemistry.

Results

To face the oxidative stress, the endogenous defense system is activated and Nrf2 appears to promote the maintenance of cellular homeostasis under stress conditions. However, in our models the endogenous antioxidant system fails to counteract stress-induced cell damage and its activation is not effective enough in preventing cochlear damage. The polyphenols such as Rosmarinic acid, curcumin, Ferulic acid attenuates SNHL reducing threshold shift, and promotes hair cell survival potentiating the Nrf2/HO-1 signaling pathway as showed by western blotting and immunohistochemical observations.

Conclusions

We demonstrated that polyphenol -mediated HO-1 activation occurs in the organ of Corti via induction of the Nrf2 transcription factor. The heat shock protein HO-1 is candidate target for the rational design of co-therapies aimed at preventing/repairing SNHL induced by exogenous factor.

P-84 Acute but not genetic glucose 6-phosphate dehydrogenase deficiency enhances hair cell death**Thomas Schrepfer¹, Peng Jiang¹, Linghui Luo¹, Jochen Schacht^{1,*}**¹ Kresge Hearing Research Institute

* Presenting author

We have previously reported a crucial role of glucose 6-phosphate dehydrogenase (G6PDH) activity for hair cell integrity. When incubating organ of Corti explants from CBA/J mice (postnatal day 2–3) with the G6PDH inhibitor 6-aminonicotinamide (6-AN), reactive oxygen species and hair cell loss increased. The presence of 6-AN also aggravated aminoglycoside-induced cell death. These results were in agreement with the sensitivity of hair cells to oxidative stress because G6PDH is the primary source of NADPH for cellular redox balance. Furthermore, cells were able to maintain redox defenses in the presence of 6-AN when NADPH was generated by a secondary pathway via *NADP⁺-linked malate enzyme*.

We tested hair cell integrity and responses to aminoglycosides in G6PDH-deficient mice both *in vitro* and *in vivo*. These mice carry a mutation in the X-linked G6PDH gene reducing enzyme expression and activity. Cochlear anatomy and auditory brain stem responses of homozygotes were normal, suggesting that compensatory mechanisms had been induced to maintain a redox balance. Antioxidant defenses were also sufficient to limit aminoglycoside toxicity as gentamicin-induced hair cell death in cochlear explants did not differ between wildtype and homozygotes. However, the addition of 6-AN to incubations with gentamicin elicited differential responses. Inhibition of G6PDH raised hair cell loss by gentamicin (3 μ M for 72 h) from 4% to 46% in wildtype but only to 16% in homozygotes, indicating that G6PDH was not the major source of NADPH in mutants. *In vivo* studies confirmed this notion: wildtype and homozygotes showed the same sensitivity to kanamycin.

The results support the crucial role of NADPH as endogenous protectant. However, a chronic (genetic) G6PDH deficiency does not compromise the ability to maintain a redox balance under normal physiological conditions and under aminoglycoside-induced stress.

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P-85 Influence of electrical stimulation on survival of spiral ganglion neurons *in vitro***Marvin N. Peter^{1,*}, Uta Reich², Athanasia Warnecke¹, Heidi Olze², Agnieszka Szczepek², Thomas Lenarz¹, Gerrit Paasche¹***1 Department of Otorhinolaryngology, Head and Neck Surgery, Hannover Medical School, Hannover, Germany**2 Department of Otorhinolaryngology, Head and Neck Surgery, Charité, Berlin, Germany*** Presenting author*

Patients scheduled for cochlear implantation often have residual hearing. Some of them experience late hearing loss after implantation. The reason for the post-implantation loss of residual hearing is not well understood. It is not expected that surgical trauma is still responsible. Evidence suggests that electrototoxicity could possibly be responsible for this adverse effect. Therefore, the aim of this study is to investigate *in vitro* the survival of spiral ganglion neurons (SGN) under electrical stimulation.

For this purpose, a stimulation setup was developed to provide defined electrical fields at given points of the chamber. Dissociated SGN from rats (p3-4) were cultured for 24 h before they were exposed to biphasic, pulsed electrical stimulation (pulse width 400 μ s, interpulse delay 120 μ s, repetition rate 1 kHz) for another 24 h. The current was varied in the range of 0 to 2 mA. After stimulation, the neurofilament of the SGN was visualized by immunocytochemistry. Neurite growth and cell survival were evaluated with respect to the electrical field at the position of the cells. Cells without electrical stimulation served as control.

Up to a current amplitude of 0.88 mA, SGN survival and neurite outgrowth were close to that of control cells. Total SGN loss was induced by a current of 2 mA. At 1 mA the survival of SGN depended on the location in the chamber thus, on the electric field. Survival and neurite length increased with decreasing field strength.

These first results provide the basis for validation of other stimulation parameters such as pulse width (pulse width of 400 μ s used in the *in vitro* experiments exceeds that in a cochlear implant). We conclude that the developed chamber appears to be suitable for the *in vitro* investigation of effects of cochlear implant on inner ear tissues.

Supported by German Research Foundation grant (WA2806/5-1)

P-86 Preventive medical treatment for assembly-line workers and its impact on the development of professional sensorineural hearing loss**Natalia Petrova¹, Viia Panshina^{1,*}, Julia Maslova¹***1 North-Western State Medical University named after I.I. Mechnikov, St.Petersburg, Russia*** Presenting author*

Sensorineural hearing loss (SHL) is included in top list of occupational diseases (2-4 places), among diseases of the respiratory and cardiovascular systems and diseases of locomotor apparatus. The detection level of SHL depends on the industry and kind of harmful professional factors in it. Most often SHL is formed from the action of industrial noise.

We assessed auditory function of workers in Assembly-line productions, in which the levels of industrial noise was 81-85 dBA. That is higher than the maximum permissible level of 80dBA. We studied dynamics of the development of SHL in different age groups among workers with first degree of hearing loss (average spoken frequency 500, 1000 and 2000 Hz: 20-39 dB, 4000 Hz: ≥ 40 dB). Workers of factory No. 1 were treated by preventive drug therapy in tablets form, and also trained in the selection and change of personal protective equipment (PPE). Workers of factory No. 2 received preventive treatment in the form of injections of medication drugs and physiotherapy. Re-assessment of auditory function was performed after 2 courses of treatment during 1 year.

Conclusions

1. Changing of personal protective equipment is unable to affect the course of the sensorineural hearing loss, what might be indicated of their good selection.
2. We noted the positive dynamics and stabilization processes in symptomatic and audiometric indicators from workers of factory No. 2 in comparison with factory No. 1
3. In the age group from 30 to 49 years we revealed the greatest effect of prevention.

P-87 Characterization of the transcriptomes of the striolar and extrastriolar supporting cells in the mouse utricle**Wenyan Li^{1,*}, Dan You¹, Xiaoling Lu¹, Shan Sun¹, Renjie Chai², Huawei Li¹***1 The EENT Hospital of Fudan University**2 Institute of Life Sciences, Southeast University*** Presenting author*

It has been reported that the supporting cells(SCs) in striolar region could response to the hair cell(HC) loss and activate the Wnt target gene *Lgr5*, serve as the resource of HC regeneration in neonatal mouse utricle. While, the HC regeneration potential of the utricle decreases according to the aging, only limited new HC could be identified after HC loss in adult mouse utricle, which is insufficient for the functional recovery. Thus, it is important to understand the difference between the supporting cells in striolar region and extrastriolar region, which may provide clues for the HC regeneration purpose. Here, we used *Lgr5-EGFP-CreERT2* and *Plp1-tdTomato+* mice to isolate the striolar and extrastriolar SCs after HC damage by flow cytometry. As expected, we found *Lgr5+* striolar SCs had higher proliferation and HC regeneration ability compared with *Plp1+* extrastriolar SCs after the hair cell damage, Wnt signaling activation and Notch signaling inhibition. Next, we performed RNA-Seq to determine the transcriptome expression profiles of these two types of SCs. We conducted analysis of the enriched and differentially expressed genes; and focused on the cell cycle genes, transcription factors, and Wnt and Notch signaling pathway genes. We found 13 cell cycle genes, 102 transcription factors, and 19 cell signaling pathway genes that were differentially expressed in *Lgr5+* striolar SCs or *Plp1+* extrastriolar SCs. Lastly, we made a protein-protein interaction network to further analyze the role of these differentially expressed genes. In conclusion, we illustrated the gene expression differences between the SCs in striolar and extrastriolar region of neonatal mouse utricle; and present a set of genes that might regulate the proliferation and HC regeneration ability of SCs, which might serve as potential new therapeutic targets for HC regeneration.

P-88 Modulating mitotic regeneration via activation of the Wnt/Beta-Catenin pathway**Patrick Atkinson^{1,*}, Yaodong Dong¹, Shuping Gu¹, Elvis Huarcaya Najarro¹, Wenwen Liu¹, Alan Cheng¹***¹ Stanford University*** Presenting author*

Canonical Wnt signaling plays a diverse role in the developing inner ear including otic induction, proliferation, prosensory cell specification, and hair cell differentiation. During mammalian cochlear development, activation of Wnt signaling induces proliferation of prosensory cells and ectopic hair cell formation. In the damaged zebrafish lateral line neuromast and neonatal mouse utricle, Wnt activation promotes mitotic hair cell regeneration. However, it remains to be determined the effect of Wnt activation in the damaged neonatal and adult mammalian cochlea. Here, we stabilized b-catenin, the central mediator of canonical Wnt signaling, after hair cell ablation in the immature and mature cochlea.

The transgenic mice lines *Fgfr3-iCre*, *Pou4f3-DTR* and, *Ctnnb1-flox(exon3)* were used. Hair cells were ablated with via diphtheria toxin injection at postnatal day 1 (P1) or P21 in *Pou4f3-DTR* mice. Tamoxifen and EdU were administered after damage. Neonatal animals were sacrificed at P5 and P7 cochleae were collected for histology; adult animals were sacrificed at P28 or P42 after functional hearing assessment.

In the neonatal *Fgfr3-iCre; Ctnnb1-flox(exon3)* mice, b-catenin stabilization failed to induce proliferation of ectopic hair cell formation. After hair cell ablation in *Pou4f3-DTR* mice, mitotic hair cell regeneration was detectable in the apical turn only. When b-catenin was stabilized after hair cell ablation, the number of EdU-labeled supporting cells and myosin7a-positive hair cells significantly increased, suggesting that Wnt enhances proliferation in the neonatal cochlea. In comparison to P5, cochleae examined at P7 had more EdU-labeled hair cells and supporting cells implying survival and expansion of proliferative cells at least in the short term. In the mature cochlea, no proliferation was observed after b-catenin stabilization alone, or when stabilized after damage. Damage as a result of hair cell loss acts as a permissive signal for spontaneous and Wnt-induced mitotic hair cell formation in the neonatal but not adult cochlea.

P-89 Isolation and characterization of progenitor cells in the human postmortem adult inner ear**Hasan Avci^{1,*}, Aurélie Dos Santos¹, Megan Ealy², Mohamed Bassiouni¹, Marcus Müller¹, Andreas Wagner³, Bernhard Hirt³, Stefan Heller², Hubert Löwenheim¹**¹ Tübingen Hearing Research Centre, Eberhard Karls University Tübingen² Stanford School of Medicine³ Institute of Clinical Anatomy and Cell Analysis, Eberhard Karls University Tübingen

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The mammalian auditory sensory epithelium is considered to be a quiescent organ that undergoes terminal mitosis during embryonic development. It is generally accepted that the adult organ of Corti lacks any intrinsic regenerative capacity because lost sensory hair cells are not replaced. Hair cell loss results in permanent hearing loss, the most frequent human sensory deficit. It has been shown that the adult utricular sensory epithelium of mice contains self-renewing and sphere forming cells which can give rise to hair cell-like cells *in vitro* and *in vivo* (Li et al., 2003). This indicates the presence of native stem cells residing in the adult mouse utricle. In humans, the isolation and characterization of native stem cells from the adult inner ear remains impeded by the very limited access to native human inner ear tissue. In this study, we isolated cells from vestibular and auditory sensory organs obtained from human body donors. We show that dissociated cells of the postmortem human inner ear possess the ability to proliferate and to form spheres. The newly generated cells express stem cell and otic progenitor markers like Nestin, Sox2, Pax2 and Pax8. Furthermore, gene expression analyses reveal that the spheres are capable of differentiating towards cells that express supporting cell and early hair cell markers *in vitro*. These findings indicate the existence of a niche of stem / progenitor cells in the human adult organ of Corti, which could open new opportunities for hair cell regeneration therapies in humans.

The research leading to these results has received funding from the European Research Council under the European Union's Seventh Framework Programme (FP7 grant agreement No. 603029 - Project OTOSTEM).

P-90 Co-activation of Hedgehog and Wnt/ β -catenin signaling enhanced cochlear hair cell regeneration after neomycin damage**Yan Chen¹, Wenli Ni¹, Wenyan Li¹, Renjie Chai², Huawei Li¹***1 EENT hospital of Fudan University**2 Southeast University*

Sensorineural hearing loss is one of the universal disabilities that seriously affect the quality of life. Unlike lower vertebrates, mammalian inner ear have limited hair cell regeneration capacity after damage, as a result, hair cell loss is the major cause of permanent sensorineural hearing loss. In situ cochlear hair cell regeneration should be explored as one of the main approaches to restore hearing that is lost due to hair cell loss. Hedgehog and Wnt/ β -catenin signaling play important roles in the proliferation, differentiation, and cell fate decision of embryonic inner ear progenitor cells. In this study, we aim to explore whether co-regulation of Hedgehog and Wnt signaling could promote cochlear hair cell regeneration by using transgenic mice. Up-regulation of Wnt/ β -catenin signal in mouse neonatal cochlear support cells could promote precursor cells to proliferate, but a very low proportion of the proliferating cells differentiated into hair cells, and while up-regulation of Shh signaling induced few proliferating cells, but the higher percentage of proliferating cells differentiated into hair cells. When the Hedgehog and Wnt signaling were activated simultaneously, hair cell regeneration was enhanced significantly demonstrated by the number of EdU-Myosin7a double positive cells. Results showed that Hedgehog signaling promoted hair cell differentiation of Wnt-activated proliferating supporting cells. This study has important scientific value and application prospect for the treatment of sensorineural hearing loss.

Key words: hearing loss; hair cell regeneration; Hedgehog signaling; Wnt/ β -catenin signaling

P-91 Towards longitudinal monitoring of stem cells after transplantation in the cochlea**Margriet A. Huisman^{1,*}, Timo Schomann¹, John C.M.J. de Groot¹, Laura Mezzanotte², Marcelo N. Rivolta³, Dyan Ramekers⁴, Huib Versnel⁴, Sjaak F.L. Klis⁴, Johan H.M. Frijns¹**¹ Leiden University Medical Center² Erasmus Medical Center³ University of Sheffield⁴ University Medical Center Utrecht

* Presenting author

During the last decades cochlear implant (CI) technology has progressed considerably, resulting in advanced electrode and speech processor strategies associated with high performance levels. However, high between-subject variability in performance, especially under noisy conditions, has not been overcome by these improvements. Crucial for CI efficacy is preservation of a critical number of functional auditory neurons. In this perspective, cell-based therapy could provide benefits in conjunction with a CI. It has been shown that cell-based inner ear therapy is feasible, but in order to achieve sufficient auditory nerve recovery to complement CI performance it is essential that the majority of the implanted stem cells do survive and differentiate into neurons and glial cells as well as that they form appropriate connections. To gain insight into these processes, it is necessary to visualize these stem cells in the living animal after implantation into the (deafened) ear and monitor their survival and differentiation using advanced technologies such as bioluminescence imaging. The stem cells of choice are obtained from the hair follicle bulge (HFBCs). These stem cells are multipotent and can differentiate into neurons and glial cells and, importantly, they are good candidates for autologous transplantation circumventing stem cell graft rejection and graft-versus-host reactions. Since this type of stem cell is a novelty in the field of inner ear regeneration, a number of prerequisites had to be corroborated: (1) can HFBCs differentiate *in vitro* and *in vivo* into neurons and glial cells, (2) do HFBCs endure genetic modification, and (3) do HFBCs integrate into cochlear epithelia. In addition, we investigated if it is feasible to visualize HFBCs containing a bioluminescent reporter molecule in the guinea pig cochlea and, also, if ouabain can be used as an ototoxic agent to induce sensorineural hearing loss in guinea pigs. The results and consequences are presented.

P-92 Macrophages in the human cochlea - saviors or predators?**Wei Liu^{1*}, Helge Rask-Andersen¹**¹ Uppsala university

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Background

The human inner ear possesses resident macrophages within the connective tissue, neurons and hearing organ supporting cells. These cells are related to blood vessels within the innate and adaptive immune system. The macrophages can be recruited from blood-borne monocytes to damaged and dying hair cells induced by noise and ototoxic drugs, aging and diphtheria toxin induced hair cell degeneration. Precise monitoring may be crucial to avoid self-targeting. We analyzed Iba-1 expression cells in the human cochlea using super-resolution fluorescence microscopy (SR-SIM).

Material and methods

Four cochleae were dissected out during petro-clival meningioma surgery due to life-threatening tumor compression of the brain stem. Cochleae were fixed, decalcified and cryostat sectioned. Immunohistochemistry with antibodies against Iba-1 and type IV collagen. SR-SIM was performed with a Zeiss Elyra S.1 SIM system capable of achieving a lateral (X-Y) resolution of ≈ 100 nm and an axial (Z) resolution of ≈ 300 nm.

Results

SR-SIM demonstrated cell interaction of Iba-1 expressing cells in the human cochlea. In the lateral wall they were related to stria vessel endothelium. Over 50 microns long slender cells paralleled the central axons with pseudopodia extensions connecting several axons. Macrophage processes perforated the basal lamina with intercellular connections.

Discussion

Results suggest that human auditory neurons also show unexpected interaction between cells belonging to the innate immune system and the afferent neurons of the cochlea. It may suggest that related chemokine signaling exist in the human cochlea to protect SGNs after hair cell loss as recently described. These cells may act both as potential savior and predators in the human cochlea with relevance for both cochlear implantation and future stem cell-based therapy to replace human hair cells.

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P-93 Purification of otic progenitors from heterogeneous cell populations**Sarah Boddy^{1,*}, Paul Gokhale¹, Darrell Barrott¹, Ae-Ri Ji¹, Marcelo Rivolta¹***1 University of Sheffield*** Presenting author*

The progression of stem cell-derived products from lab to clinic is dependent on the reliable purification of specific cells from heterogeneous populations. Various methods for differentiating pluripotent cells along lineages associated with the developing auditory system have been reported; hence it is important to devise mechanisms to isolate the cells with potential to treat hearing impairment.

A cell surface antibody screen has been undertaken on otic progenitors generated from human embryonic stem cells (hESCs). Screen plates were imaged by automated microscopy thereby maintaining information relating to cell and colony morphologies. This information was used to narrow down potential hits based on known characteristics of otic progenitors.

The top antibodies revealed by the screen have been tested for co-expression with markers associated with inner ear development, and used to separate out subpopulations of otic progenitors from heterogeneous cultures. These subpopulations have been further differentiated along sensory hair cell or neuronal lineages to verify the effectiveness of fluorescence-activated cell sorting (FACS) with the screen antibodies as a mechanism for selecting key otic progenitor cell types. In addition, expression of the antibodies has been sought in both fixed tissue from human foetuses and in cell lines derived from the developing human ear. These data have revealed overlap between cell surface antigen expression in hESC-derived otic progenitors, human foetal auditory stem cells and in situ cells of the developing auditory and vestibular systems.

Results from all of the methods used to verify the usefulness and relevance of the antibodies highlighted in the original screen will be presented. Collectively they support the use of the chosen antibodies and FACS system in selecting otic progenitors for use as cellular therapeutics.

P-94 Identification and characterisation of a neural crest stem cell-derived population in dental pulp cells: It's potential for auditory regeneration**Oscar O. Solis Castro^{1,*}, Fiona Boissonade¹, Marcelo N. Rivolta¹**¹ *The University of Sheffield** *Presenting author*

Dental pulp cells (DPCs) are known to contain adult stem cell properties and have been widely described as mesenchymal stem cells. However, due to their neural crest origin during embryonic development, DPCs are also termed ecto-mesenchymal stem cells (EMSCs). EMSC from different adult sources have been proposed as ideal for deriving ectodermic cell lineages, including neurons and glia. Furthermore, previous research in DPCs has suggested the presence of cells with neural crest-like phenotypic characters (NCSCs). The aim of this research is to enrich and evaluate a particular NCSC from DPCs (DPC-NCSC) to differentiate them into sensory neurons that could be used to treat auditory neuropathies causing deafness. Different approaches were tested to enrich a NCSC population from DPCs, such as i) Defined culture conditions for neurogenic and NCSC cell growth, ii) direct isolation by fluorescence activated cell sorting (FACS), and iii) neurosphere formation. The cells obtained from these strategies were characterised and different neuralisation protocols tested. The obtained enriched DPC-NCSCs were driven to a neural fate by dcAMP induction, and its capacity to form neurons in co-culture with gerbil cochlear explants was investigated. Initial results showed that DPCs are heterogeneous cultures that present NCSC markers. One of our defined culture conditions seemed to be more neurogenic than the standard medium used broadly. Importantly, neurosphere formation can yield a NCSC-like phenotype able to neuralise and interact with cochlear explants. The results suggest for the first time that human dental pulp cells have the potential to be used for auditory regeneration, and such potential should be explored further.

P-95 The effective differentiation and proliferation of iPS cells into Connexin26 gap junction plaque forming cells**Ayumi Fujimoto^{1,*}, Ichiro Fukunaga¹, Kaori Hatakeyama¹, Katsuhisa Ikeda¹, Kazusaku Kamiya¹***1 Juntendo University Faculty of Medicine*** Presenting author***Introduction**

Hereditary deafness affects about 1 in 1600 children and GJB2 gene mutation is most frequent cause for this disease. GJB2 encodes connexin26 (Cx26), a component in cochlear gap junction. Cx26 is mainly expressed in cochlear supporting cells and fibrocytes, and forms large gap junction plaque (GJP) macromolecular complex (Ahmad, Biochem. Biophys. Res. Commun., 2003). Recently, we have developed a method for preparing inner ear precursor cells with Cx26GJP from mouse iPS cells (Fukunaga, Stem Cell Reports, 2016 Vol.7(6)1023-1036). In this study, we aimed for separation of inner ear progenitor cells and mass culture of the cells by simplification of the method.

Methods

The cochlear feeder cells were developed from adult cochlear tissue to support cochlear differentiation. Undifferentiated mouse iPS cells were seeded on the cochlear feeder cells directly, and cultured in mediums which contain several reagent cocktails. GFP signals controlled by Nanog promotor (Nanog-GFP) were monitored as an undifferentiated state marker. To investigate the characterization of cochlear feeder cells, surface antigens were identified by flow cytometry.

Results

The iPS cells proliferated on cochlear feeder cells and showed gradual decrease in expression of Nanog-GFP in all conditions. Remarkable morphological changes among the reagents were observed in about 1-2 weeks. In one of these conditions, a number of the cells forming Cx26-gap junction plaques were observed. Surface antigens expressed in cochlear feeder cells were similar to those of mouse mesenchymal stem cells.

Conclusions

In this study, iPS cells differentiated into neural or non-neural cells depending on the reagent cocktails on cochlear feeder cells. Our method is thought to be effective to establish Cx26 gap junction forming cells similar to cochlear fibrocytes and supporting cells. By establish of this method, it is expected to make disease model cells of patient with hereditary deafness caused by GJB2 gene mutation for the drug screening.

P-96 Adenosine A2a receptor agonist CGS 21680 mitigates deleterious effects on hearing and cognition due to noise**Manish Shukla^{1,*}, Kaustav Roy¹, Neeru Kapoor¹***1 Defence Institute of Physiology & Allied Sciences, DRDO, S. K. Mazumdar Road, Delhi 110054*** Presenting author***Introduction**

Hearing impairment is a potential risk factor, and even mild or moderate hearing loss associated with cognitive decline. This study was performed to investigate the effect of hearing loss on cognitive function to evaluate neural degeneration and DCX, NeuN expression in the hippocampus, of rat brain to show more direct evidence of the relationship between hearing and cognition. The aim of this study was to evaluate the protective effect of CGS 21680 against NIHL.

Methods

Sprague Dawley rats (200-220 g) were used in this study. Rat of the noise-induced hearing loss (NIHL) group were exposed to 100 dB SPL white noise for 2 hour for 15 days. Of these, a group was treated with CGS 21680 (i.p. 100µg/Kg body wt. at 1hr prior for 15 consecutive days). The loss of hearing function was measured by the auditory brainstem response (ABR) the behavioral assessments were performed using morris water maze (MWM). The extent of damage with rhodamine-phalloidin (Rh-Ph) staining, myosin VII expression in cochlea; DCX, NeuN expression in the hippocampus of the rat brain was investigated by immune-histochemical staining study.

Results

There was significant difference of mean ABR thresholds among groups. The rats treated with CGS21680 showed significantly short mean latency and path-length to reach the platform compared to the noise group rats. Consistent with these findings, CGS21680 treated group was found to have significantly increased the number of NeuN, doublecortin (DCX) positive cells & decrease hair cell loss as shown by Rh-Ph staining.

P-97 Functionalization of polymer fibers with extracellular matrix components**Inga Wille¹, Peter Behrens¹, Jennifer Schulze², Athanasia Warnecke²***1 Leibniz Universität Hannover**2 Hannover Medical School*

The application of polymer fibers consisting of poly- ϵ -caprolactone (PCL) and polyglycolide (PGA) has already been widely studied in the field of biomedical sciences. Both polymers have been used for example as basic material for drug delivery systems or 3D-scaffolds in tissue engineering. They allow an efficient modification of their physical, chemical and mechanical properties by copolymerization or blending with many other polymers as well as by surface functionalization. Moreover they have shown a good biocompatibility and are biodegradable.^[1] The aim of this work is to modify the surface of fibers made from PCL/PGA copolymers in order to test their applicability as components in scaffolds for tissue regeneration. For this purpose, the biodegradable fibers were coated with extracellular matrix components; as one representative there is heparan sulfate. It is also an anticoagulant and could enhance wound healing after implantation.^[2] Heparan sulfate is well-known in clinical applications and

is, for example, used in the eyedrops Cacicol® to regenerate and heal the cornea. Another promising agent is laminin, which belongs to collagen like glycoproteins. It is proven that the attachment of laminin to polymer substrates increases the affinity for directing peripheral nerve regeneration.^[3]

In this work the polymer fibers are functionalized with aminogroups via aminolysis using ethylenediamine. Afterwards the extracellular matrix components are linked to the functionalized fibers. Toluidine blue was used in a colorimetric assay to validate the presence of heparan sulfate on the fibers. The attachment and release of laminin was proven by an ELISA. Furthermore, cell culture investigations with fibroblasts showed a good cytocompatibility.

This work was supported by the Cluster of Excellence Hearing4all.

P-98 Immunocytochemical and electrophysiological comparison of native and 3D-cultured cochlear fibrocytes**Anya Osborn¹, David Furness¹, Shanthini Mahendrasingam¹, Michael Evans¹, Douglas Caruana¹***¹Keele University*

Fibrocyte degeneration in the cochlear lateral wall is one possible pathology of age-related metabolic hearing loss (presbycusis). Fibrocytes are vital for potassium recycling and maintenance of the endocochlear potential. It has been proposed that a cell replacement therapy could prevent fibrocyte degeneration in the CD/1 mouse model of hearing loss (Mahendrasingam et al., 2011). One source of replacement cells is cultured spiral ligament fibrocytes. Fibrocytes cultured as monolayers or on 3-D collagen I gels were compared with fibrocytes in cochlear slices or micro-dissected spiral ligament from ~P7 cochlea of CD/1 mice using immunolabelling, electrophysiology, and electron microscopy techniques. Fibrocytes successfully grown for short periods on a 3D collagen I matrix resembled the native appearance, having rounded cell bodies with processes extending from them, compared to a flatter morphology when grown in a monolayer. These cells expressed aquaporin 1 (AQP1) which, in the cochlea, is confined to type III cells, and to a lesser extent S-100 found in other fibrocyte types. They also expressed the inwardly-rectifying potassium channel Kir5.1, known to be present in native fibrocytes, as well as labelling for the gap junction proteins connexin 26 and 31. Finally, whole-cell voltage clamp recordings from the cells on gels revealed inwardly and outwardly-rectifying potassium currents comparable with putative native fibrocytes, with similar median resting membrane potentials of -8 mV in cultured and -6 mV in native fibrocytes. To conclude, the 3-D cultured fibrocytes show native-like morphology and possess functional potassium channels. Further characterisation of this potassium current using targeted blockers and immunogold labelling of protein expression will be performed. If successful this would provide evidence that these cells are suitable for transplantation into the lateral wall of the cochlea in a cell therapy to treat metabolic presbycusis.

P-99 A small molecule regenerative therapy approach to treat sensorineural hearing loss by the REGAIN Horizon 2020 consortium**Anne Schilder^{1,*}, Helmuth van Es², Leonie Middelink², Joanne Palmer¹, Helen Blackshaw¹, Matthew Topping¹, Katherine Lazarides², John Gill², Thanos Bibas³, Dimitris Kikidis³, Stephan Wolpert⁴, Shakeel Saeed⁵***1 University College London**2 Audion Therapeutics**3 University of Athens**4 University of Tübingen**5 Royal National Throat, Nose and Ear Hospital*** Presenting author*

Hearing loss affects 330 million adults worldwide; its' most common cause is sensorineural hearing loss (SNHL) due to auditory hair cell loss. Treatment is limited to hearing aids and cochlear implants. Neither device restores natural hearing and further deterioration is common. Given this, there is a clear unmet need for novel hearing therapies.

Preclinical studies have demonstrated that inhibition of Notch signalling with a gamma-secretase inhibitor (GSI) can regenerate outer hair cells and partially restore hearing. Supported by a €5.8 million EU Horizon 2020 grant, the international REGAIN (REgeneration of inner ear hair cells with GAMma-secretase INhibitors) Consortium seeks to translate these preclinical findings into a potential hearing therapy.

Preclinical development has been completed, including GSI candidate selection, drug synthesis optimisation, development of a suitable formulation, inner ear pharmacokinetics (PK) and toxicology studies.

A preclinical data package has been submitted for regulatory approval of a phase I safety study, followed by a phase II efficacy study in the UK, Germany and Greece. The GSI will be administered locally to the worst hearing ear of adults with mild to moderate SNHL in three doses, one week apart. Trial outcomes include systemic and local safety assessed by a range of hearing, tinnitus and balance tests.

Preclinical inner ear PK studies with the formulated drug product revealed good cochlear drug exposure and provided the data for dosing in patients. Genotoxicity, ototoxicity, systemic toxicity tests and dermal irritation studies showed no evidence of toxicity. Regulatory submissions are in the process of being completed as per June 2017; the safety study is planned for later in 2017.

Small molecule drugs safely targeting the underlying biological causes of SNHL have the potential to meet a significant need for millions of patients. The REGAIN consortium is progressing the pharmaceutical treatment of SNHL through rigorous clinical testing.

P-100 Peroxisome proliferator-activated receptor (PPAR) agonists protect auditory hair cells from gentamicin-induced oxidative stress and apoptosis**Marijana Sekulic-Jablanovic^{1,*}, Vesna Petkovic¹, Matthew B. Wright², Krystsina Kucharava¹, Nathan Huerzeler¹, Alessia Ramseier¹, Alexander Bausch², Daniel Bodmer¹***1 University Hospital Basel, Basel, Switzerland**2 Strekin AG, Basel, Switzerland*** Presenting author*

The thiazolidinedione (TZDs, e.g. pioglitazone) and fibrate (e.g. fenofibrate) drugs are used to treat type 2 diabetes and lipid disorders. TZDs and fibrates are agonists for the peroxisome proliferator-activated receptors, PPAR γ and PPAR α . PPARs are potential targets in several indications involving oxidative stress such as renal and cardiac ischemia-reperfusion injury.

We explored the ability of diverse PPAR agonists to prevent gentamicin-induced auditory hair cell apoptosis. Organ of Corti (OC) from 5-day old mice were exposed to gentamicin +/- either pioglitazone, tesaglitazar, muraglitazar or fenofibric acid in vitro, followed by detection and counting of hair cells (HCs). Immunohistochemistry and Western blots were performed to study the expression of PPAR γ and PPAR α protein in mouse cochlea. Finally, we measured effects of treatments on PPAR target gene and redox gene expression, levels of ROS, lipid peroxidation products and activated caspases.

Gentamicin (50 mM) led to reproducible losses of 50% of hair cells. Pioglitazone, tesaglitazar, and fenofibric acid all provided >90% protection of HCs from gentamicin toxicity. Western blots detected high levels of PPAR γ and PPAR α protein in mouse OC lysates. Immunofluorescence experiments detected PPAR γ and PPAR α protein in auditory HCs and other cell types in the organ of Corti. Gentamicin treatment increased ROS, lipid peroxidation, and altered redox gene expression in mouse OCs. Pioglitazone treatment almost completely prevented the increase in ROS induced by gentamicin, inhibiting subsequent formation of 4-HNE (4-hydroxy-2-nonenal) and caspase activation. Gene expression analysis demonstrated that each drug had a unique gene signature. Notably, pioglitazone upregulated expression of SOD, GSH, catalase and UCP2, important antioxidant enzymes.

Together these data suggest that PPAR agonists may be effective treatments for hearing loss by opposing oxidative stress, lipid peroxidation and hair cell death.

P-101 Comparison of salicylate-induced and noise-induced tinnitus rat models

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Tinnitus, the perception of a “phantom” sound in the absence of external stimulation, is a common consequence of damage to the auditory periphery. It affects around 15% of the population and may induce intolerable discomfort. Whereas some drug candidates are in the process of being developed, nowadays no effective treatment exists to cure tinnitus. Because it remains very difficult to detect tinnitus objectively in animal models, carry out new quantitative methods become the key step to develop new compounds involved in tinnitus treatment.

Aim

The objective of this study is to compare the salicylate-induced and the noise-induced tinnitus rat models using three robust and validated experimental *in vivo* techniques: behavioral tests, auditory cortex electrophysiology and brain *in vivo* imaging.

Material and methods

For salicylate-induced tinnitus model, salicylate is administered by intraperitoneal injection at 300mg/kg/day. For acoustic trauma-induced tinnitus model, animals are exposed to a unilateral acoustic trauma of 116 to 118dB SPL of two octaves (8-24KHz) band noise centered at 16 kHz during 1h. Two hours after salicylate administration or 30 days after acoustic trauma the presence of tinnitus is determined using gap prepulse inhibition test, unicellular electrophysiology of primary auditory cortex and *in vivo* manganese enhanced MRI.

Conclusion

The combination of behavioral test, electrophysiology recording and *in vivo* imaging allows to measure putative signs of tinnitus in both rat models. Similar results were observed in electrophysiology and MEMRI imaging read-outs for salicylate and noise induced tinnitus model. However, using gap prepulse inhibition test, we observed that salicylate induced tinnitus at the broadband noise (bbn) whereas the acoustic trauma induced tinnitus at 12, 16 and 24 kHz but not at the bbn. Taken together, these data open the door for screening and characterization of new drug efficacy on tinnitus disorder.

P-102 Characteristic molecular and functional biomarkers for tinnitus in humans

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Tinnitus is a widespread auditory disorder affecting approximately 10-15% of the population, often with debilitating consequences. The exact neurophysiological basis of chronic tinnitus remains unknown and in various aspects highly controversial. While some studies link a pathological increase in central responsiveness subsequent to cochlear damage with tinnitus other suggest that hearing loss is not necessarily causally involved in tinnitus and a failure to generate central hyperactivity is rather associated with tinnitus. We here present the results from preclinical pilot studies that aimed to investigate characteristic functional and molecular biomarkers in homogenous matched hearing impaired human subjects with and without tinnitus. Using a combination of investigations from biomarkers in body fluids, from audiometry fine structure analysis and resting or evoked fMRI in distinguished patient and control groups we present new characteristic features for the tinnitus group. We will discuss the findings in the context of urgent needs for using objective biomarkers for future therapeutic interventions in tinnitus patients.

Acknowledgments:

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P-103 Selective removal of type I hair cell in vestibular end organs using a novel transtympanic gentamicin administration in guinea pigs**Makoto Chiba^{1,*}, Tsukasa Ito¹, Chikako Shinkawa¹, Yutaka Koizumi¹, Seiji Kakehata¹***¹ Yamagata University Faculty of Medicine*** Presenting author***Objective**

To determine whether a vestibular system animal model can be developed with gentamicin which causes total loss of type I hair cells with no loss of type II hair cells and supporting cells. This model should serve to facilitate future research on the function of type I hair cells and type II hair cells in the vestibular system.

Material and methods

We divided animals into four groups: two transtympanic injection of gentamicin groups, an intraperitoneal injection of gentamicin group and a control group. The transtympanic groups were injected with gentamicin via the left tympanic cavity, and the gentamicin was maintained in contact with both the round window membrane and oval window for either 2 h (TT-2 Group) or 6 h (TT-6 Group). The intraperitoneal injection group received gentamicin via an intraperitoneal injection administered to each animal for 10 consecutive days (IP Group). We performed no treatment on the Control Group. We compared mortality rate, suppression of body weight gain, damage to vestibular function and damage to the vestibular epithelium based on an examination of the total hair cell density and the number of type I hair cells, type II hair cells and supporting cells.

Results

The TT-2 Group exhibited a nearly total loss of type I hair cells with almost no loss of type II hair cells and supporting cells. In addition, while the TT-2 Group exhibited a significantly lower loss of type I hair cells in comparison to the Control Group, no significant difference was observed in loss of type II hair cells between the two groups.

Conclusion

Our novel transtympanic gentamicin injection method produced nearly total loss of type I hair cells without loss of type II hair cells and supporting cells as well as a mortality rate of zero and no significant suppression of body weight gain.

P-104 Ultrastructural study on the morphology of the vestibular labyrinth**Silvia Palma¹, Paola Boldrini², Raoul Nucci³, G. Cenacchi⁴, A. Martini⁵***1 ENT, ASL, Modena,**2 Center of Electron Microscopy, University of Ferrara**3 ENT Dep, Ospedale Civile of Legnano**4 Department of Biomedical and Neuromotor Sciences, Alma Mater Studiorum University of Bologna, Italy**5 Institute of Otolaryngology, Department of Neurosciences, Padova University, Italy*

Schwannomas of the 8th cranial nerve account for approximately 10% of all intracranial tumors; they arise in more than 90% of cases in the inferior vestibular ramus and several aspects of the disease's pathophysiology remain to be understood. Schuknecht hypothesized three possible mechanisms underlying the condition: (a) destruction of the cochlear and vestibular nerve fibers; (b) ischemic damage to the sensory organs due to a compressive effect; and (c) alterations in the biochemical composition of inner ear fluids. All of these variables are probably involved and their effect on currently available therapies as well as the different clinical pictures characterizing the tumor needs to be clarified.

Ultrastructural findings in the vestibular organs removed via the translabyrinthine approach are discussed with the aim to investigate the underlying mechanisms of hearing loss and balance disorders in patients affected by vestibular schwannoma. The sample included 3 males and 7 female patients, the neoplasms varied from 5 to 28 mm in size.

The absence of correlation between patients' clinical status and ultrastructural findings of the sensory epithelium confirms that the degenerative aspects that have been identified are largely due to artefacts or are age-related. The morphological features of the non-sensory epithelium suggest that the potassium circulation system is preserved in patients with vestibular schwannoma even when they present clearly evident cochlear and/or vestibular symptoms.

The damage caused by the neoplasm during the early stage of its development could originally be of a neurotoxic nature and subsequently become mechanical. The irritative stimulus could originate from an altered calcium homeostasis in the endolymph, which could, in turn, be linked to the activity of the hair cells; the latter could be affected at a biochemical level by the dysfunction of the nerve fibers with which they are connected in a cascading mechanism.

P-105 Suggestion of animal models in space motion sickness by a changeable gravity system - A preliminary study

Hyun Ji Kim^{1,*}, Eun Hae Jeon¹, Kyu-Sung Kim¹

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Background

Motion sickness occurs as a result of a mismatch or conflict between the information arising from the vestibular system, the visual and proprioceptive inputs. In previous study, there have been introduced motion-induced pica model for an index of motion sickness in rats. However, there have been insufficient further studies on motion sickness or space motion sickness using animal models. In this study, we verified the motion-induced pica model in altered gravitational conditions and evaluated the effect of hypergravity on vestibular system.

Methods

We observed the change of kaolin(hydrated aluminum silicate) consumption during the exposure of various gravitational stimuli(2G for 4hrs, 8hrs, 24hrs and 4G for 2hrs, 4hrs, 24hrs) in rats(aged 7~8weeks, weighing 250-300g). Using an animal rotator, we assessed the vestibular function (recording the eye movement) after hypergravity load. We estimated the gain at 0.04, 0.08, 0.16, 0.32Hz on earth vertical axis rotation.

Results

Kaolin consumption was increased after load of hypergravity, in comparison with normal control group. There was a pattern to increasing kaolin intake by hypergravity load day by day. Kaolin intake was increased 2days after the start of hypergravity reaching a peak 3-5 days afterward. Then the intake gradually declined for 3-4days. Kaolin intake was correlated with degree of gravitational stimulus. Under 4G for 8hrs, the gain at 0.04, 0.08, 0.16, 0.32 HZ were 0.572 ± 0.125 , 0.638 ± 0.097 , 0.660 ± 0.083 , 0.756 ± 0.087 respectively. Under 2G for 8hrs, the gain at 0.04, 0.08, 0.16, 0.32 HZ were 0.508 ± 0.113 , 0.66 ± 0.455 , 0.718 ± 0.093 , 0.832 ± 0.198 respectively. There were no significant differences between two groups.

Conclusion

By observing the data, hypergravity stimulation affect the kaolin intake and it was correlated with the degree of gravitational stimuli and exposure time. We can induce the results that short-term hypergravity stimulation cause not remarkable changes on VOR gain in rats.

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P-106 Polymorphisms in genes in patients with Meniere's disease

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Introduction

Although the etiologies of Ménière's disease (MD) remain unclear, genetic factors could contribute, at least in part. Recently, accumulating evidence has demonstrated that inflammatory responses are related to the pathology of inner ear disease. We investigated the association between genetic polymorphisms located in genes related to the inflammatory process and susceptibility to MD in the present study.

Methods

Patients affected by MD, who attended the Department of Otorhinolaryngology of the Nagoya University Hospital between November 2007 and March 2011, were enrolled in the study. The subjects of the control group were selected from the comprehensive Longitudinal Study of Aging (NILS-LSA), an ongoing population-based study with a two-year follow-up, conducted by the National Institute for Longevity Sciences. Polymorphisms in the genes: tumor necrosis factor α (*TNF α* ; rs1800630); interleukin-1 receptor-associated kinase 1 (*IRAK1*; rs1059702); interleukin 4R (*IR4R*; rs1801275); c-reactive protein (*CRP*; rs1130864); TNF receptor super family 1B (*TNFRSF1B*; rs1061624); cyclooxygenase 2(*COX2*; rs20417); protein kinase C, eta (*PRKCH*; rs2230500); endothelin 1 (*EDN1*; rs5370); uncoupling protein 2 (*UCP2*; rs660339); vascular endothelial growth factor (*VEGF*; rs3025039; rs699947; rs1570360); complement factor H (*CFH*; rs1061170); Interleukin 6 (*IL6*; rs1800796); Interleukin 10 (*IL10*; rs1800872); intercellular adhesion molecule1 (*ICAM1*; rs5498); platelet glycoprotein Ia (*GPIa*; rs1126643); matrix metalloproteinase 3 (*MMP3*; rs3025058) and matrix metalloproteinase 12 (*MMP12*; rs2276109) were investigated for statistical analysis.

Results

The *GPIa* polymorphism was significantly associated with a risk of MD; in addition, the OR for the *GPIa* polymorphism and MD risk was 1.435 (CI: 1.035–1.990) with adjustment for age and sex. The severity of hearing loss in patients with MD were not related to the genotypes. The remaining polymorphisms failed to show any associations with the risk of MD.

Conclusion

In conclusion, the *GPIa* polymorphisms were significantly associated with the risk of MD.

P-107 Mitochondrial calcium overload mediates sensitivity to noise-induced hearing loss**Su-Hua Sha^{1,*}, Xianren Wang¹, Hao Xiong¹, Haishan Long¹, Kayla Hill¹***1 Medical University of South Carolina, USA*** Presenting author*

Mitochondria play a key role in cellular calcium homeostasis. This action is mediated in part by the mitochondrial calcium uniporter (MCU), a selective channel that regulates calcium entry into mitochondria, coupled with calcium extrusion from mitochondria via the sodium calcium exchanger (NCLX). In this study, we investigated mitochondrial regulation of cellular calcium levels via MCU and NCLX in noise-induced hearing loss (NIHL) and cochlear synaptopathy using adult CBA/J mice. Immunolabeling for MCU increased in cochlear cells, including sensory hair cells, spiral ganglion cells, marginal cells, and fibrocytes, while immunolabeling for NCLX decreased after noise exposure. Inhibition of calcium channels via treatment with verapamil reversed noise-induced changes in MCU and NCLX in sensory hair cells and attenuated NIHL and synaptic ribbon loss. Furthermore, inhibition of MCU activity via MCU siRNA silencing or the specific pharmacological inhibitor Ru360 attenuated noise-induced loss of outer hair cells (OHCs) and synaptic ribbons, wave I amplitudes, and NIHL. This protection was afforded, at least in part, through reduced cleavage of caspase 9. These results suggest that noise exposure leads to mitochondrial calcium overload via MCU and NCLX. Mitochondria, as calcium stores, regulate cytosolic calcium levels. Mitochondrial calcium overload initiates cell death pathways and subsequent loss of hair cells and synaptic connections.

Author keywords: Mitochondrial Calcium Uniporter / Mitochondrial Sodium Calcium Exchanger / Noise-induced Hearing Loss

P-109 Activation of miR-34a impairs autophagic flux and promotes cochlear cell death via upregulating ATG9A: implication for age-related hearing loss

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Age-related hearing loss (AHL) is a major unresolved public health problem. We have previously elucidated that the activation of cochlear miR-34a is correlated with AHL in C57BL/6 mice. A growing body of evidence points that aberrant autophagy promotes cell death during the development of multiple age-related diseases. The aim of this study was to investigate the role of miR-34a-involved disorder of autophagy in the pathogenesis of AHL. Our results showed that miR-34a expression was markedly up-regulated in the aging cochlea accompanied with blockage of autophagic flux. In the inner ear HEI-OC1 cell line, miR-34a overexpression resulted in an accumulation of autophagosomes and impaired autophagosome-lysosome fusion, and led to cell death subsequently. Notably, ATG9A, an autophagy protein, is significantly decreased after miR-34a overexpression. Knockdown of ATG9A inhibited autophagy flux, which is similar to the effects of miR-34a overexpression. Moreover, ursodeoxycholic acid significantly rescued miR-34a-induced HEI-OC1 cell death by restoring autophagy activity. Collectively, these findings provide a new understanding of the biological effects of miR-34a in the development of AHL and highlight miR-34a as a potential target for AHL treatment.

Key words: miR-34a / autophagy / age-related hearing loss / ATG9A / ursodeoxycholic acid



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