www.ieb2013.fgua.es

50th Inner Ear Biology Workshop

Alcalá de Henares
Madrid / Spain
September 10th-13th
2013

&

Symposium

Hearing solutions: up to the ciliated cell and beyond

September 10th
2013

Program & Abstract Book

Organized by:

Universidad de Alcalá

Hospital Universitario Príncipe de Asturias
SaludMadrid
Comunidad de Madrid

CSIC
Consejo Superior de Investigaciones Científicas

UAM
Universidad Autónoma de Madrid

Abstract Book sponsored by:

amplifon

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Published by:

- Departamento de Cirugía, Ciencias Médicas y Sociales de la Universidad de Alcalá – Hospital Universitario Príncipe de Asturias de Alcalá de Henares (Madrid)
- Instituto de Investigaciones Biomédicas “Alberto Sols”, Consejo Superior de Investigaciones Científicas - Universidad Autónoma de Madrid

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Welcome

On behalf of the Organizing Committee it is our honour to welcome you to the 50th Workshop Inner Ear Biology (IEB).

The Workshop held at the University of Alcala, from 11th to 13th September 2013 is preceded by a clinically-oriented Symposium entitled "Hearing solutions: up to the ciliated and beyond", on 10th September.

IEB has been consolidated as a series of annual workshops that bring together world leaders in the field. In 2013 we celebrate the 50th IEB anniversary, and the inaugural and celebration conferences are designed to engage and inspire a new generation of inner ear researchers. The IEB Workshop is a consolidated forum for multidisciplinary interaction between basic research scientists and clinical scientists, working at the genetic, molecular, cellular, and whole-organ levels. Your participation, through the presentation of latest research results, developments, and applications in inner ear biology is instrumental for the success of this edition and of future IEB Workshops.

We are looking forward to share a memorable time at Alcalá de Henares.

On behalf of the Organizing Committee

Teresa Rivera & Isabel Varela-Nieto

IEB 50th Workshop

Chairs
Teresa Rivera Rodríguez
Isabel Varela-Nieto

Organising Committee
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Rafael Cediel
Julio Contreras Rodríguez
Francisco J. del Castillo
Ignacio del Castillo
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Marcelo Rivolta (Univ. Sheffield, UK)
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Sponsors and Collaborators

We would like to thank the following institutions and companies for their support to the IEB Workshop 2013:

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Sociedad Española de Bioquímica y Biología Molecular (SEBBM)
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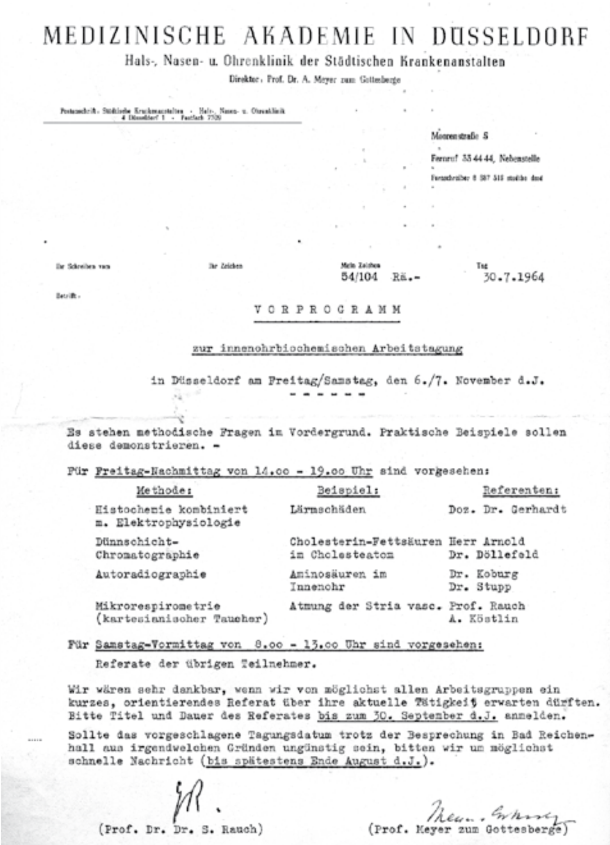


MEDIZINISCHE AKADEMIE IN DÜSSELDORF

Hals-, Nasen- u. Ohrenklinik der Städtischen Krankenanstalten

Direktor: Prof. Dr. A. Meyer zum Gottesberge

Moorenstraße 5

Fernruf 33 44 44, Nebenstelle

Ihr Schreiben vom	Ihr Zeichen	Mein Zeichen	Tag
		54/104 Rä.-	30.7.1964

Betrifft:

V O R P R O G R A M M

zur innenohrbiochemischen Arbeitstagung

in Düsseldorf am Freitag/Samstag, den 6./7. November d.J.

Es stehen methodische Fragen im Vordergrund. Praktische Beispiele sollen diese demonstrieren. -

Für Freitag-Nachmittag von 14.oo - 19.oo Uhr sind vorgesehen:

Methode:	Beispiel:	Referenten:
Histochemie kombiniert m. Elektrophysiologie	Lärmschäden	Doz. Dr. Gerhardt
Dünnschicht-Chromatographie	Cholesterin-Fettsäuren im Cholesteatom	Herr Arnold Dr. Döllefeld
Autoradiographie	Aminosäuren im Innenohr	Dr. Koburg Dr. Stupp
Mikrorespirometrie (kartesianischer Taucher)	Atmung der Stria vasc.	Prof. Rauch A. Köstlin

Für Samstag-Vormittag von 8.oo - 13.oo Uhr sind vorgesehen:

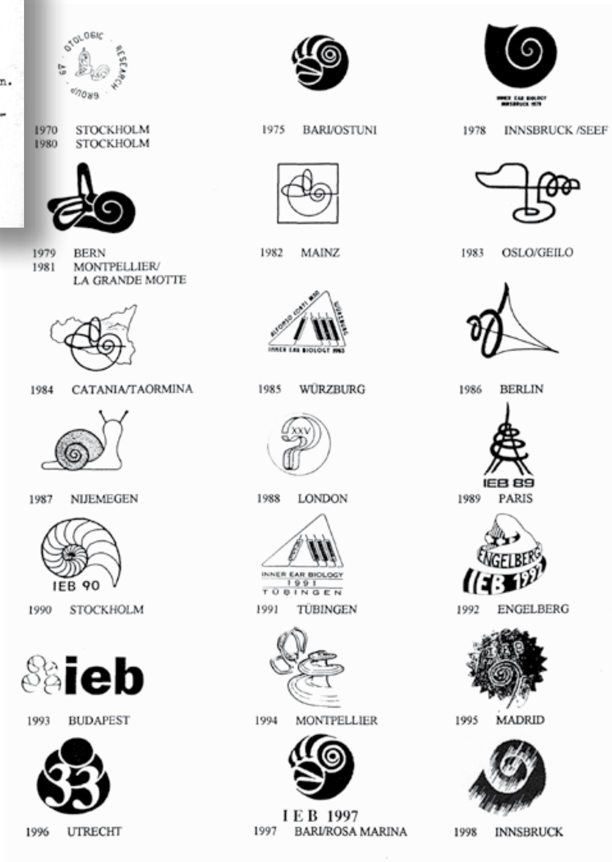
Referate der übrigen Teilnehmer.

Wir wären sehr dankbar, wenn wir von möglichst allen Arbeitsgruppen ein kurzes, orientierendes Referat über ihre aktuelle Tätigkeit erwarten dürften. Bitte Titel und Dauer des Referates bis zum 30. September d.J. anmelden.

Sollte das vorgeschlagene Tagungsdatum trotz der Besprechung in Bad Reichenhall aus irgendwelchen Gründen ungünstig sein, bitten wir um möglichst schnelle Nachricht (bis spätestens Ende August d.J.).

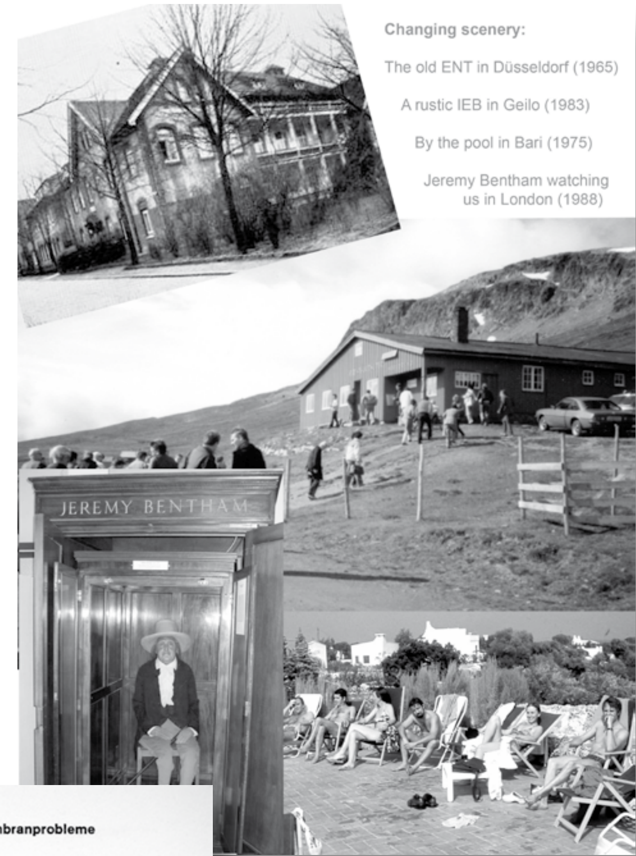
(Prof. Dr. Dr. S. Rauch) (Prof. Meyer zum Gottesberge)

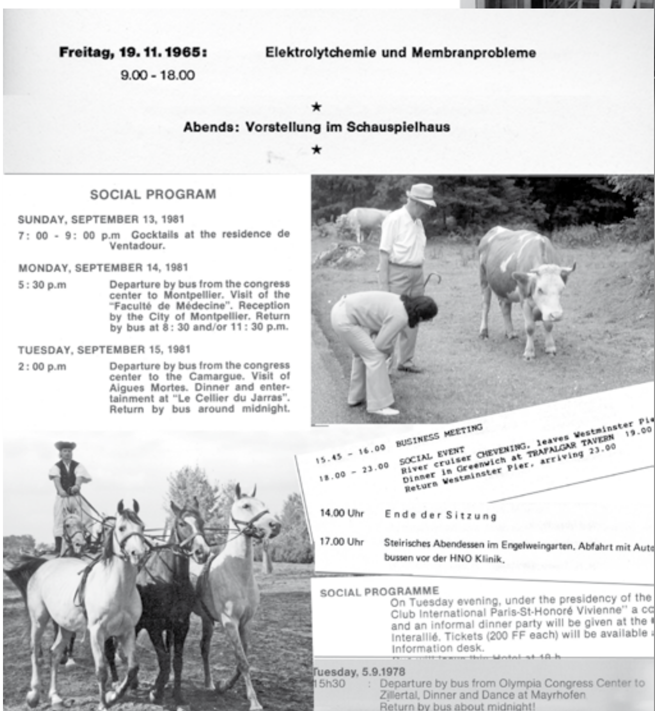
Inner Ear Biology started in 1964 in Düsseldorf as a small "Arbeitstagung". The first program featured methodological questions.



As the workshop grew, not only did the number of participants increase but the meetings moved out of Germany to other European venues. The logo of the cochlea in its various incarnations first appeared in the 1970s.

Different locations venue of the Workshop.



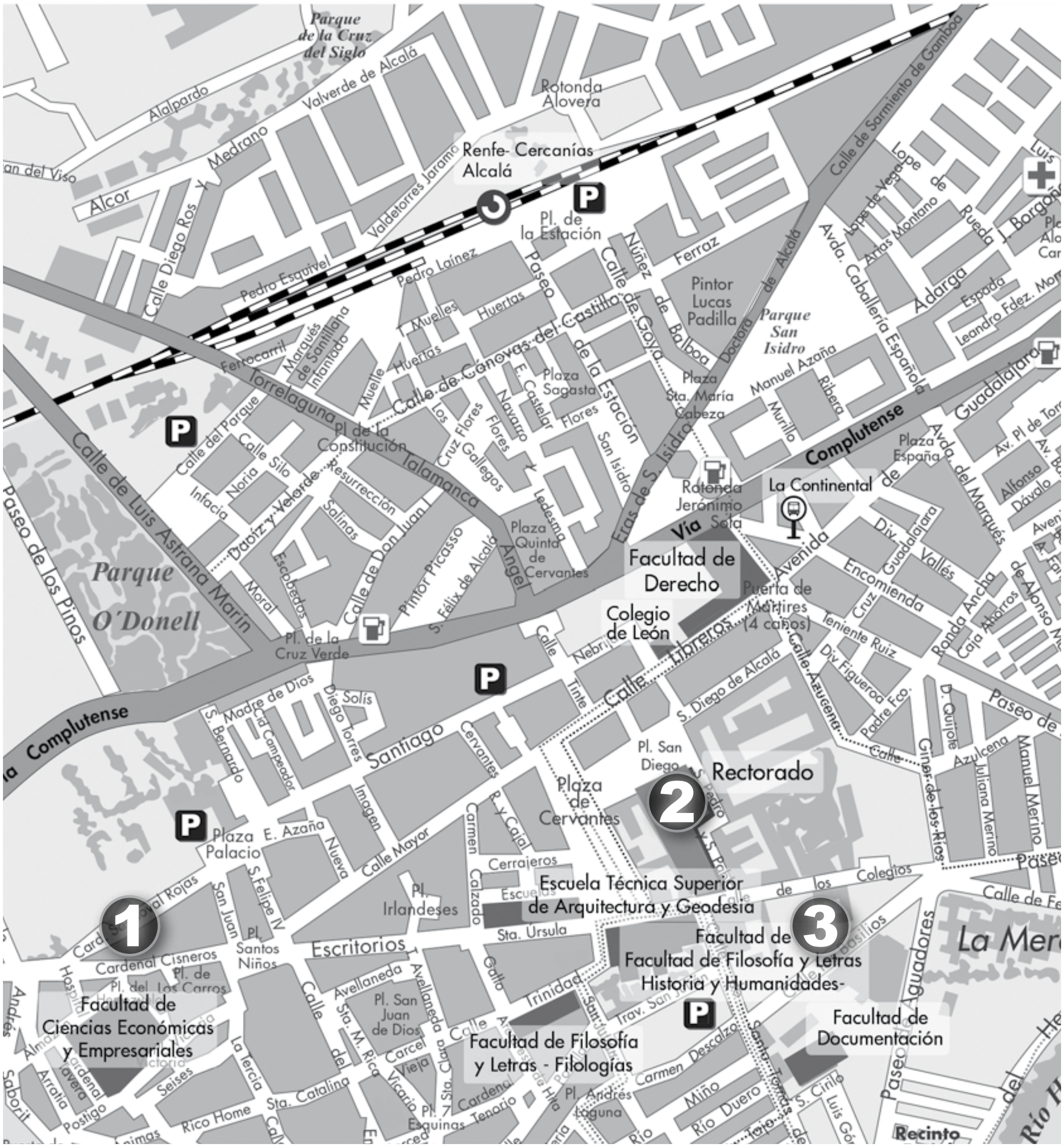


Although science was always in the foreground, social interactions were never neglected beginning with the first meetings.

General Information

A. Conference Venue

The Workshop and Symposium will be held in the Meeting Room of the “Facultad de Ciencias Económicas y Empresariales” of the “Universidad de Alcalá”, located within 10 minutes walking distance from the city centre and from most accommodation.



Map of the city center of Alcalá de Henares

1. **Registration desk Meeting Room Start of the guided visit to the old historic center of Alcalá de Henares.**
Facultad de Ciencias Económicas y Empresariales, Universidad de Alcalá
Victoria Square, 2

2. **Inauguration and Plenary Conference**
Start of the guided visit to the old University of Alcalá
Welcome cocktail
Universidad de Alcalá, Colegio San Ildefonso (Rectorado)
San Diego Square

3. **Workshop dinner**
Parador de Alcalá de Henares
Colegios Street, 10

B. Registration

The registration desk is located in the hall of the Facultad de Ciencias Económicas y Empresariales, Universidad de Alcalá.
Victoria Square, 2

It will be open during the following hours:

- **Tuesday, September 10th:** from 08:00 to 10:00 and 16:00 to 18:00 hours
- **Wednesday, September 11th:** from 08:00 to 13:30 and 15:00 to 18:30 hours
- **Thursday, September 12th:** from 08:00 to 13:30 and 15:00 to 18:30 hours
- **Friday, September 13th:** 08:00 to 13:30 hours.

c. Lunches and coffee breaks

Lunches and Coffees will be provided from Wednesday to Friday free of charge to registered participants.

Coffee breaks will be held in the "*Patio de Columnas*" (Cloister) of the Facultad de Ciencias Económicas y Empresariales, from Wednesday to Friday, according to the Workshop programme.

Lunches will be held at the Restaurant of the Facultad de Ciencias Económicas y Empresariales, Wednesday and Thursday, according to the Workshop programme.

Finally, the **Workshop dinner** will be held in "*Parador de Alcalá de Henares*" at 21:00 on Thursday 12th. A limited number of tickets for the **Workshop Dinner** will be available for purchase at the Registration Desk.

D. Internet Access

Internet access will be available at the "Room O4" in the Facultad de Ciencias Económicas y Empresariales. Three laptops will be provided by the Workshop Organization for the registered participants.

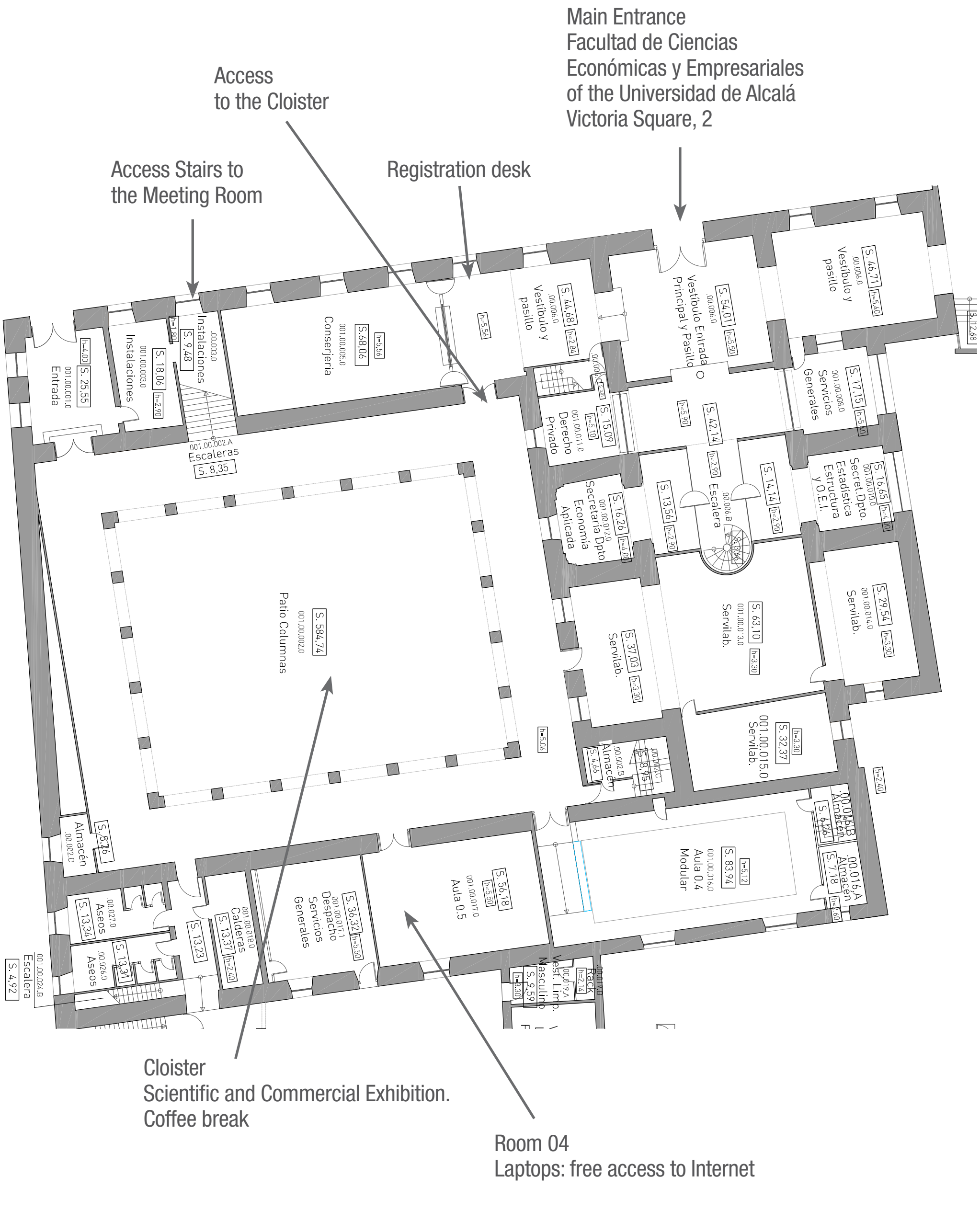
There will be free WIFI:
SSID Name: IEB2013 Workshop
Password: ALCALAIEB2013

E. Guided visits

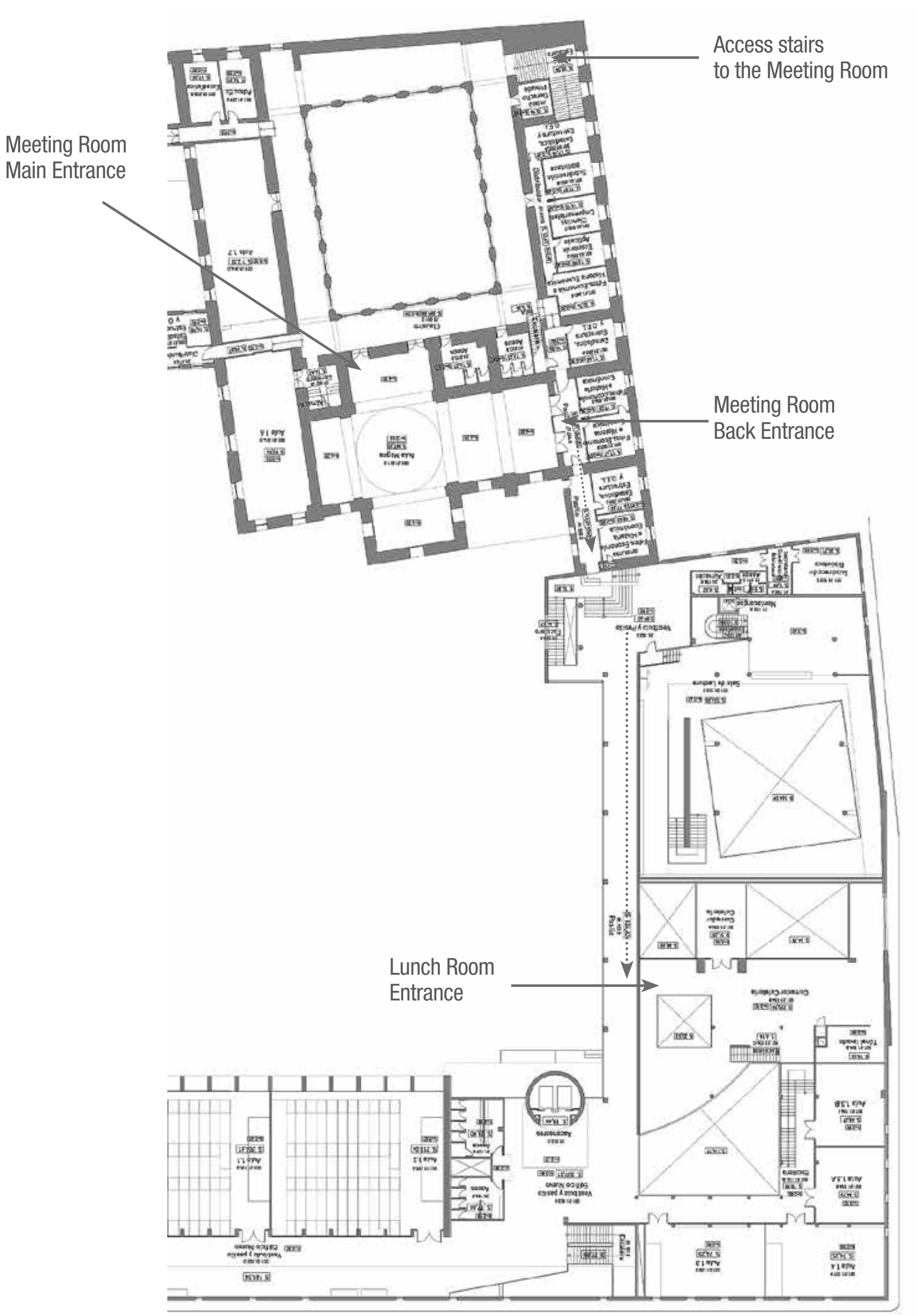
The **guided visit to the old Universidad de Alcalá** will be held on Tuesday, September 10th free of charge to registered participants. The visit will start at 19:00 from the front of the Colegio San Ildefonso (Rectorado), San Diego Square.

The **guided visit to the old historic center of Alcalá de Henares** will be held on Wednesday, September 11th free of charge to registered participants. The visit will start at 19:30 from the front of the Facultad de Ciencias Económicas y Empresariales, Universidad de Alcalá (Main Entrance), De la Victoria Square, 2.

Ground floor of the Facultad de Ciencias Económicas y Empresariales of the Universidad de Alcalá



First floor of the Facultad de Ciencias Económicas y Empresariales of the Universidad de Alcalá



Oral presentations

Time allotted to speakers is 12 minutes with an additional 3 minutes for discussion. Adhesion to the allocated speaking time is strongly recommended; session chairpersons will be urged to consider time management.

Presentations are only accepted in PowerPoint or PDF formats. Please bring your presentation on a CD or a USB memory stick (do not protect it with software). For video and audio files please submit AVI, WMV and MPG files only, as a separate file. Please make sure that any required CODEC files for the videos are also submitted. You may check and edit, if necessary, your presentation during the congress. There will be a notebook with MS PowerPoint 2007 and 2010 at the venue.

The projection data of your presentations before and during the whole congress will be recorded and managed centrally. Speakers should contact the Organising Secretariat to hand in their presentations for uploading at least two hours before the beginning of the session or in the late afternoon of the day before, in case of early morning presentations.

Posters

Posters Nº P-01 to P-38 should remain posted from Tuesday to Wednesday evening, whilst posters Nº P-39 to P-97 should remain posted from Thursday to Friday lunch time. Presenting authors should be at their posters during the indicated presentation times. Poster board dimensions are 80 cm (32-inch) in width and 100 cm (40-inch) in height (portrait orientation).

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SYMPOSIUM PROGRAM

50th
Inner Ear Biology
Workshop

Alcalá de Henares
Madrid / Spain
September 10th - 13th
2013

Symposium: *Hearing solutions: up to the ciliated cell and beyond*

Tuesday, September 10th

Venue: Facultad de Ciencias Económicas y Empresariales
Universidad de Alcalá
De la Victoria Square, 2
Alcalá de Henares (Madrid), Spain

Symposium Chair: Teresa Rivera MD PhD

08:00—10:00 Registration

10:00—11:30 ***Auditory pathway and cochlear implants***
Chair: Manuel Manrique MD PhD

Learning to preserve the inner ear function during cochlear implantation
Manuel Manrique

Drug delivery through cochlear implants
Claude Jolly

P*ET scan, an alternative t*o discover the effects of auditory deprivation
Javier Arbizu

Awa*king the central auditory sy*stem with cochlear implants
Karen Gordon

11:30—12:00 Coffee break

12:00 ***Indications in auditory implants***
Chair: Luis Lassaletta MD PhD

12:00—12:30 Conference: ***From hearing aids to ABI (auditory brainstem implant): a world of options***
Luis Lassaletta MD PhD

12:30—14:00 Round table: ***Hearing solutions in different clinical situations***
Chair: Javier Gavilán
Participants:
- Carlos Cenjor
- Martin Kompis
- Georg Sprinzl
- Ángel Ramos
- Luis Lassaletta

WORKSHOP PROGRAM OVERVIEW

50th
Inner Ear Biology
Workshop

Alcalá de Henares
Madrid / Spain
September 10th - 13th
2013

50th Inner Ear Biology Workshop

Tuesday, September 10th

Venue: Facultad de Ciencias Económicas y Empresariales
Universidad de Alcalá
De la Victoria Square, 2
Alcalá de Henares (Madrid), Spain

17:00—18:00 Registration
Poster Session I
Stands

Venue: Facultad de Ciencias Económicas y Empresariales
Universidad de Alcalá
De la Victoria Square, 2
Alcalá de Henares (Madrid), Spain

18:00—18:45 Inauguration

Plenary Conference: ***Celebrating IEB 50th Anniversary:***
Chair: Jose Manuel Juiz

Inner ear biology at fifty
Jochen Schacht (UMICH, Ann Arbor, USA)

Venue: Colegio San Ildefonso (Rectorado)
Universidad de Alcalá
San Diego Square
Alcalá de Henares

19:00—20:00 Guided visit to the old University of Alcalá

Venue: Colegio San Ildefonso (Rectorado)
Universidad de Alcalá
San Diego Square
Alcalá de Henares

20:00 Welcome Cocktail

Venue: Patio de Filósofos
Colegio San Ildefonso (Rectorado)
Universidad de Alcalá
San Diego Square
Alcalá de Henares

Wednesday, September 11th

08:00	Registration **Poster Session I**
08:30	**Welcome to the participants**
08:45	**Session 1**: ***Molecular and anatomical bases of hearing and hearing loss (I)*** **Chairs*:*** Miguel Merchán and Angela Meyer zum Gottesberg
08:45—09:15	Target Lecture: *Behavioral responses in a complex auditory discrimination task depends on the sequence of cues* Alessandro E.P. Villa (UNIL, Lausanne, CH)
09:15—10:45	Oral presentations: from O1 to O-06
10:45—11:15	Coffee break **Poster Session I**
11:15	**Session 2: *Molecular and anatomical bases of hearing and hearing loss (II)*** **Chairs:** Barbara Canlon and Anthony W. Gummer
11:15—11:45	Target Lecture: *Myosins as system integrators for the regulation of mechanotransduction in the inner ear* Bechara Kachar (NIDCD, Bethesda, USA)
11:45—13:30	Oral presentations: from O-07 to O-11
13:30—15:00	Lunch
15:00—16:00	**Poster Session I: from P-01 to P-38**
16:00	**Sessions 3 and 4: *Genetics of Hearing Impairment in Human and Mouse*** **Chairs:** Karen P. Steel and Hannie Kremer Target Lecture 1: *Genetics of monogenic deafness unraveled at fast pace* Hannie Kremer (UMC, Nijmegen, NL)
16:30—17:00	Target Lecture 2: *Mouse models for human hereditary deafness DFNA8/12 and DFNB21* Guy Richardson (US, Sussex, UK)
17:00—18:30	Oral presentations: from O-12 to O-17
18:30	End of Poster Session I
19:30—21:00	Guided visit to the old historic center of Alcalá de Henares Starting from Facultad de Ciencias Económicas y Empresariales Universidad de Alcalá De la Victoria Square, 2 Alcalá de Henares

Thursday, September 12th

08:00	Registration **Poster Session II**
09:00	**Session 5:** ***Ototoxic damage and Vestibular diseases*** **Chairs:** Agnieszka J. Szczepek and Giovanni Paludetti
09:00—09:30	Target Lecture: *Deep phenotyping of vestibular disorders for next generation sequencing* José Antonio López Escámez (UGR, Granada, SP)
09:30—11:00	Oral presentations: from O-18 to O-23
11:00—11:30	Coffee break **Poster Session II**
11:30	**Session 6:** ***Noise-induced hearing loss*** **Chairs**: Lukas Rüttiger and Anna Fetoni
11:30—12:00	Target Lecture: *ACEMg in the prevention and treatment of hearing impairment* Josef M. Miller (UMICH, Ann Arbor, USA)
12:00—13:30	Oral presentations: from O-24 to O-29
13:30—13:45	Group photo Venue: Cloister of the Facultad de Ciencias Económicas y Empresariales
13:45—15:00	Lunch
15:00—16:00	**Poster Session II: from P-39 to P-97**
16:00	**Session 7:** ***Novel therapies and diagnostic tools*** **Chair:** Allen Ryan
16:00—16:30	Target Lecture: *Exploring disrupted cochlear homeostasis: a second youth for "old" diagnostic tools?* Paul Avan (UdA, Clermont-Ferrand, FR)
16:30—18:00	Oral presentations: from O-30 to O-35
18:00—18:45	Plenary Conference: ***50 years of IEB Science*** **Chair:** Ignacio del Castillo *Inner ear biology at fifty—a science timeline* Jonathan Ashmore (UCL, London UK)
18:45—19:15	Spoendlin Award Lecture **Chair**: Marcelo N. Rivolta
19:15—19:30	Spoendlin Award
21:00	Workshop dinner Venue: Parador de Alcalá de Henares Colegios Street, 8 Alcalá de Henares

Friday, September 13th

08:00	Registration **Poster Session II**
09:00	**Session 8:** ***Molecular bases of inner ear development and regeneration*** **Chairs:** Brigitte Malgrange and Takayuki Nakagawa
09:00—09:30	Target Lecture: *Translating development into regeneration for the ear* Marcelo N. Rivolta (UoS, Sheffield, UK)
09:30—11:00	Oral presentations: from O-36 to O-41
11:00—11:30	Coffee break **Poster Session II**
11:30—12:15	Closing conference: **Chair:** Miguel Ángel Moreno *Insights into deafness from new mouse mutants* Karen P. Steel (King´s College, London, UK)
12:30—13:30	Business Meeting Closing Ceremony End of Poster Session II End of Workshop

DETAILED WORKSHOP PROGRAM

50th
Inner Ear Biology
Workshop

Alcalá de Henares
Madrid / Spain
September 10th - 13th
2013

Tuesday, September 10th

16:00—18:00 Registration
Poster Session I
Stands

Venue: Facultad de Ciencias Económicas y Empresariales
Universidad de Alcalá
De la Victoria Square, 2
Alcalá de Henares

18:00—18:45 Inauguration
Plenary Conference: ***Celebrating IEB 50th Anniversary***:
Chair: Jose Manuel Juiz

Inner ear biology at fifty
Jochen Schacht (UMICH, Ann Arbor, USA)

Venue: Colegio San Ildefonso (Rectorado)
Universidad de Alcalá
San Diego Square
Alcalá de Henares

19:00—20:00 Guided visit to the old University of Alcalá

Venue: Colegio San Ildefonso (Rectorado)
Universidad de Alcalá
San Diego Square
Alcalá de Henares

20:00 Welcome Cocktail

Venue: Patio de Filósofos
Colegio San Ildefonso (Rectorado)
Universidad de Alcalá
San Diego Square
Alcalá de Henares

Wednesday, September 11th

08:00 Registration
Poster Session I

08:30 **Welcome to the participants**

08:45 **Session 1:** ***Molecular and anatomical bases of hearing and hearing loss (I)***
Chairs: Miguel Merchán and Angela Meyer zum Gottesberg

08:45—09:15 Target Lecture:
Behavioral responses in a complex auditory discrimination task depends on the sequence of cues
Alessandro E.P. Villa (UNIL, Lausanne, CH)

09:15—10:45 Oral presentations:

O-01 *Sensorineural hearing loss and ischemic injury: development of animal models to assess vascular and oxidative effects*
Elena Olivetto, Valeria Guaran, EdiSimoni, Pietro Giordano, Rina Bragagnolo, Laura Astolfi and Alessandro Martini

O-02 *Dexamethasone prevents electrode insertion trauma-initiated inflammatory response and fibrosis*
Esperanza Bas, Chhavi Gupta, Adrien A. Eshragui and Thomas R. Van De Water

O-03 *The inferior colliculusis not an obligatory relay in the ascending auditory pathway*
Enrique Saldaña, F. Javier Bernardo, Yongming Jin, Marcelo Gómez-Álvarez, M. Auxiliadora Aparicio, Emmanuel Márquez, David Sloan and Albert S. Berrebi

O-04 *On the controversy about the sharpness of human cochlear tuning*
Enrique A. López-Poveda *and Almudena Eustaquio-Martin*

O-05 *Voltage under fire: fast IR laser-induced shifts in prestin's Boltzmann distribution along the voltage axis*
Rotimi Okunade and Joseph Santos-Sacchi

O-06 *Intravital calci*um imaging of cochlear hair cells upon sound stimulation
Yu Matsumoto, Shotaro Karino, Shigeyuki Namiki, Kazunori Kataoka and Tatsuya Yamasoba

10:45—11:15 Coffee break

11:15 **Session 2:** ***Molecular and anatomical bases of hearing and hearing loss (II)***
Chairs: Barbara Canlon and Anthony W. Gummer

11:15—11:45 Target Lecture:
Myosins as system integrators for the regulation of mechanotransduction in the inner ear
Bechara Kachar (NIDCD, Bethesda, USA)

11:45—13:30 Oral presentations:

O-07 Identification of tympanic border cells as slow-cycling cells in the cochlea
Norio Yamamoto, Mirei Taniguchi, Takayuki Nakagawa, Eriko Ogino and Juichi Ito

O-08 *A voltage sensitive dye assessment of electrical coupling in cell networks of the inner ear*
Federico Ceriani and Fabio Mammano

O-09 *Circadian rhythms in the auditory system*
Inna Meltser, Vasiliki Basinou, Sergey Savelyev, Christopher R. Cederroth, Gabriella Schmitz Lundkvist and Barbara Canlon

O-10 *The role of transmembrane channel-like proteins in hair cell mechanotransduction*
Robert Fettiplace, Kyunghee X. Kim, Shanthini Mahendrasingam, Carole M. Hackney and David N. Furness

O-11 *Inner hair cell membranes in three dimensions: links between membranes, mitochondria and synaptic vesicles*
Anwen Bullen, Roland Fleck, Jonathan Ashmore, Carolyn Moores and Andrew Forge

13:30—15:00 Lunch

15:00—16:00 **Poster Session I**

P-01 *IGF-I deficiency causes upregulation of glutamatergic neurotranmission in the mouse cochlear nuclei*
Verónica Fuentes-Santamaría, Julio Contreras, Juan C. Alvarado, Lourdes Rodríguez-de la Rosa, Silvia Murillo-Cuesta, José M. Juiz and Isabel Varela-Nieto

P-02 *Polyphenols effect on oxidative stress and apoptosis in cochleas of experimental animal associated with age*
C. Sánchez-Rodríguez, Ricardo Sanz-Fernández, J. Esteban-Sánchez, E. Martín-Sanz, L. Rodrigáñez, L. De Toro Gil and E. Durio-Calero

P-03 *Sources of input to the lateral superior olive*
Marcelo Gómez-Álvarez, Joseba Miranda, Emmanuel Márquez and Enrique Saldaña

P-04 *The posterior limitans nucleus is the main thalamic target of the external cortex and the nucleus of the brachium of the inferior colliculus*
Emmanuel Márquez, Marcelo Gómez and Enrique Saldaña

P-05 *Olivary Complex and inner ear plastic reorganization after auditory cortex ablation*
Verónica Lamas, Elena Morillo and Miguel Merchán

P-06 *Changes of the olivocochlear efferent system in the hamster GASH:Sal*
Sonia Hernández Noriega, David Sánchez Benito, Miguel Angelo Hyppolito, Ricardo Gómez-Nieto, Orlando Castellano Benítez, Luis J. Muñoz, Adriana de Andrade Batista Murashima, Norberto Garcia-Cairasco and Dolores E. López García

P-07 *Voltage-dependent inhibition of N-type calcium channels by activation of ORL-1 receptor in the vestibular afferent neurons of the rat*
Rosario Vega, Emmanuel Seseña, Aída Ortega and Enrique Soto

P-08 *Folate deficiency alters homocysteine cycle in the cochlea and causes premature hearing loss in mice*
Raquel Martínez-Vega, Francisco Garrido, Gregorio Varela-Moreiras, Teresa Partearroyo, Rafael Cediel, Néstor Vallecillo, María A. Pajares and Isabel Varela-Nieto

P-09 *Burst activity and ultrafast activation kinetics of CaV1.3 Ca^{2+} channels support presynaptic activity in adult gerbil hair cell ribbon synapses*
Sergio Masetto, Valeria Zampini, Stuart L. Johnson, Christoph Franz, Marlies Knipper, Matthew C. Holley, Jacopo Magistretti and Walter Marcotti

P-10 *Subnuclear endocytic activity of the outer hair cell*
Csaba Harasztosi, Emese Harasztosi and Anthony W. Gummer

P-11 *3D imaging of the guinea pig cochlea and spiral ganglion neuron quantification*
Antonina Wrzeszcz, Verena Scheper, Thomas Lenarz and Günter Reuter

P-12 *Formation and properties of the acoustic field in the cochlear duct I. The diapason of perceived sound frequencies*
Evgeny L. Ovchinnikov, V.V. Ivanov, Yu V. Ovchinnikova, K.A. Adishirin-zade and T.I. Minaeva

P-13 *Formation and properties of the acoustic field in the cochlear duct II. The dispersion of acoustic waves and relaxation of the sound field*
Evgeny L. Ovchinnikov, V.V. Ivanov, Yu V. Ovchinnikova, K.A. Adishirin-zade and T.I. Minaeva

P-14 *Morphological changes of cochlear gap junction plaques in Brn4 deficient mouse, a mouse model of DFN3 non-syndromic deafness*
Kidokoro Yoshinobu, Kamiya Kazusaku, Minowa Osamu and Ikeda Katsuhisa

P-15 *sTSLIM imaging of rodent and human inner ears*
Peter A. Santi, Shane Johnson and Sebahattin Cureoglu

P-16 *Cell therapy for hereditary deafness with bone marrow mesenchymal stem cell and the activation of stem cell homing*
Kazusaku Kamiya, *Keiko Karasawa, T*akashi Anzai, Osamu Minowa and Katsuhisa Ikeda

P-17 *Deafening guinea pigs using locally applied kanamycin and furosemide*
Peter Bako*, Hubert Löwenheim and Marcus Müller*

P-18 B*DNF mediated neurite outgrowth in a mouse spiral ganglion cell model*
Marcus Müller*, Anke Tropitzsch, Benedikt Kramer, Claudia Frick and Hubert Löwenheim*

P-19 *Aquaporins constitute a molecular water shunt in the cochlear apex – Implications for longitudinal endolymph flow and Menière's disease*
Hubert Löwenheim, Bernhard Hirt, Corinna Gleiser, Alec N. Salt, Helge Rask-Andersen, Jean Smolders, Marcus Müller and Andreas Eckhard

P-20 *Expression and distribution of Connexin 26 and 30 in the Human Cochlea-A confocal laser immunohistochemistry study*
Wei Liu and Helge Rask-Andersen

P-21 *Morphologic change in the inner ear of the Aquaporin (AQP)-11 knockout mice*
Rie Nishioka, Taizo Takeda, Masahiro Komori, Taisuke Kobayashi and Masamitsu Hyodo

P-22 *Functional and morphological characterization of NOD-SCID inner ear*
Edi Simoni, Elena Olivetto, Valeria Guaran, Filippo Valente, Pietro Giordano, Laura Astolfi and Alessandro Martini

P-23 *Hearing impairments in diet-induced obesity in mice*
Takeshi Fujita, Daisuke Yamashita, Shingo Hasegawa, Sayaka Katsunuma, Hitoshi Tanimoto and Ken-ichi Nibu

P-24 *Morphological and electrophysiological cochlear changes in two models of epilepsy, the WAR and GASH:Sal strains, after audiogenic kindling*
Dolores E. López García, José Antônio Côrtes de Oliveira, Flávio Del Vecchio, Adriana de Andrade Batista Murashima, Maria Rossato, Teddy Talbot, Norberto Garcia Cairasco and Miguel Angelo Hyppolito

P-25 *Transepithelial K^+ secretion and Na^+ absorption in human endolymphatic sac epithelium: ex vivo study for understanding net physiologic current of endolymphatic sac epithelium*
Sung Huhn Kim, Mia Ghi, Jin Young Kim, Won-Sang Lee and Jae Young Choi

P-26 *Effect of reduced frequency selectivity on hearing impaired listener in perceiving nonlinearly distorted speech and music*
Stefania Goncalves, Tan Chin-Tuan, Brian C.J. Moore and Mario Svirsky

P-27 *A multiscale computational model of guinea pig cochlea to probe neuropathy mechanisms*
Jérôme Bourien, Régis Nouvian, Antoine Huet, Gilles Desmadryl and Jean-Luc Puel

P-28 *Coupling between inner hair cells in the adult mouse cochlea*
Antonio M. García de Diego, Piotr Sirko and Jonathan Ashmore

P-29 *NMII forms a contractile transcellular sarcomeric network to regulate apical cell junctions and tissue geometry*
Seham Ebrahim and BecharaKachar

P-30 *Molecular genetic diagnosis of Usher syndrome*
José M. Millán, Elena Aller, María J. Aparisi, Gema García-García, Regina Rodrigo, Thomas Besnard, Carmen Ayuso, Anne-Françoise Roux and Teresa Jaijo

P-31 *Hearing impairment and human inner ear degeneration caused by missense mutation in WFS1 gene*
Rudolf Glueckert, Mario Bitsche, Michael Blumer, Helge Rask-Andersen, Wei Liu, Irina Alafuzoff, Anneliese Schrott-Fischer and Lisbeth Tranebjaerg

P-32 *A novel splice-site mutation in the GJB2 gene causing mild postlingual hearing impairment*
Marta Gandía, Francisco J. del Castillo, Francisco J. Rodríguez-Álvarez, Manuela Calderón, Gema Garrido, Miguel A. Moreno-Pelayo, Manuela Villamar, Felipe Moreno and Ignacio del Castillo

P-33 *EPS8 is necessary for the normal expression of cochlear, but not vestibular hair cell K^+ channels*
Ivo Prigioni, Elisa Tavazzani, Giancarlo Russo, Jacopo Magistretti, Donatella Contini, Paolo Spaiardi, Teresa Soda and Sergio Masetto

P-34 *Different expression of histone modification in the spiral ganglion of Mn-SOD knock out mice*
Ken-ichi Watanabe and Wilhelm Bloch

P-35 *WBP2-deficient mice show progressive high-frequency hearing loss and abnormal cochlear innervation*
Annalisa Buniello, Andreea Huma, Raquel Martinez-Vega, Neil Ingham and Karen Steel

P-36 *Is a connexin delition associated hearing loss treatable?*
Ryosei Minoda, Toru Miwa, Takao Yamada, Momoko Ise and Eiji Yumoto

P-37 *Mouse models of deafness that suggest impaired calcium mobilization in inner ear cells: possible pathways that differentiate the phenotype according to the molecular nature of the mutations*
Osamu Minowa, Hiromi Motegi, Hideaki Toki, Maki Inoue, Yoichi Gondo and Tetsuo Noda

P-38 *Hearing phenotype of the conditional inducible mapk14-null mouse*
Lourdes Rodríguez- de la Rosa, Silvia Murillo-Cuesta, María Arechederra, Ana Cuenda, Almudena Porras and Isabel Varela-Nieto

16:00 **Sessions 3 and 4: *Genetics of Hearing Impairment in Human and Mouse***
Chairs: Karen P. Steel and Hannie Kremer

16:00—16:30 Target Lecture 1:
Genetics of monogenic deafness unraveled at fast pace
Hannie Kremer (UMC, Nijmegen, NL)

16:30—17:00 Target Lecture 2:
Mouse models for human hereditary deafness DFNA8/12 and DFNB21
Guy Richardson (US, Sussex, UK)

17:00—18:30 Oral presentations:

O-12 *A nonsense mutation in CLIC5 causes autosomal recessive sensorineural hearing impairment with vestibular dysfunction*
Celia Zazo Seco, Anne Oonk, Jaap Oostrik, Ronald Admiraal, Hannie Kremer and Margit Schraders

O-13 *Novel genetic tools to screen for deafness-causing mutations in the tandemly-duplicated region that contains the STRC (stereocilin) gene and its pseudogene*
Laura Ruiz-Palmero, Marta Gandía, Elena Gómez-Rosas, Manuela Villamar, Felipe Moreno, Ignacio del Castillo and Francisco J. del Castillo

O-14 *Identification of rare variants in Mt-ND1 and LARS human genes in Meniere's disease suggest a two-hit model*
Teresa Requena, Sonia Cabrera, Irene Gázquez and José Antonio López-Escámez

O-15 *Disturbance of axonal microtubules in the auditory nerve and outer hair cell degeneration accompanied by progressive hearing loss in the Pmn/Pmn mouse*
Kristen Rak, Silke Frenz, Johannes Völker, Andreas Radeloff, Rudolf Hagen, Sibylle Jablonka and Robert Mlynski

O-16 *Correction of hearing in the mouse model of hereditary deafness by Gjb2 gene transfer using adeno-associated viral vectors*
Katsuhisa Ikeda, Takashi Iizuka, Kazusaku Kamiya and Osamu Minowa

O-17 *Developmental changes of epithelial Na^+ channel expression and function in the inner ear of pendrin knock-out mouse as a perspective of development of endolymphatic hydrops*
Sung Huhn Kim, Bo Gyung Kim, Jin Young Kim and Jae Young Choi

18:30 End of Poster Session I

19:30—21:00 Guided visit to the old historic city center of Alcalá de Henares

Starting from
Facultad de Ciencias Económicas y Empresariales
Universidad de Alcalá
De la Victoria Square, 2
Alcalá de Henares

Thursday, September 12th

08:00 Registration
Poster Session II

09:00 **Session 5: *Ototoxic damage and Vestibular diseases***
Chairs: Agnieszka J. Szczepek and Giovanni Paludetti

09:00—09:30 Target Lecture:
Deep phenotyping of vestibular disorders for next generation sequencing
José Antonio López Escámez (UGR, Granada, SP)

09:30—11:00 Oral presentations:

O-18 *Modulation of copper transporters in protection against cisplatin-induced cochlear hair cell damage*
Dalian Ding and Richard Salvi

O-19 *Effect in vivo of dimethyl sulphoxide (DMSO) in the ototoxicity by cisplatin, an animal model*
Amaya Roldán-Fidalgo, Almudena Trinidad Cabezas and Rafael Ramírez-Camacho

O-20 *Co-treatment with ototoxic nitriles and CYP2E1 inhibitors: a new mouse model for vestibular hair cell loss with limited systemic toxicity*
Jordi Llorens, Sandra Saldaña-Ruíz, Lara Sedó-Cabezón and Pere Boadas-Vaello

O-21 *Ubiquinone rescues auditory hair cells from the cisplatin-induced toxicity*
Agnieszka J. Szczepek*, Elisabeth Gerschner, Heidi Olze, Olga Hegend and Birgit Mazurek*

O-22 *Local delivery of brain-derived-neurotrophic factor on the perforated round window membrane: a possible clinical application*
Sarah Havenith, HuibVersnel, Sjaak F.L. Klis and Wilko Grolman

O-23 *Aminoglycoside modulation of the acid sensing ion channels in the cochlear afferent neurons*
Enrique Soto, Rosario Vega and Antonia González

11:00—11:30 Coffee break
Poster Session II

11:30 **Session 6: *Noise-induced hearing loss***
Chairs: Lukas Rüttiger and Anna Fetoni

11:30—12:00 Target Lecture:
ACEMg in the prevention and treatment of hearing impairment
Josef M. Miller (UMICH, Ann Arbor, USA)

12:00—13:30 Oral presentations:

O-24 *Acute antioxidant response and apoptosis regulation in rats after noise exposure*
Pedro Melgar-Rojas, Juan Carlos Alvarado, Verónica Fuentes-Santamaría, Cruz Gabaldón and José Manuel Juiz

O-25 *Adenosine amine congener as a cochlear rescue agent: an update*
Srdjan M. Vlajkovic, Song Y. Paek, Howard H.T. Chi, Hao Chang and Peter R. Thorne

O-26 *Autophagy is a defense mechanism against noise-induced hearing loss*
Su-Hua Sha and Hu Yuan

O-27 *The early onset of oxidative stress processes in the organ of Corti after intense noise exposure: the role of membrane fluidity*
A.R. Fetoni, G. Maulucci, S.L.M. Eramo, M. De Spirito, F. Paciello, D. Troiani and G. Paludetti

O-28 *Hyperacusis and tinnitus studies in the aging rat*
Dorit Möhrle, Ksenia Varakina, Marlies Knipper and Lukas Rüttiger

O-29 *C-RAF deficiency causes cochlear abnormalities and profound sensorineural deafness in the mouse*
Marta Magariños, Rocío de Iriarte, Ulf R. Rapp and Isabel Varela-Nieto

13:30—13:45 Group photo
Venue: Cloister of the Facultad de Ciencias Económicas y Empresariales

13:45—15:00 Lunch

15:00—16:00 **Poster Session II** :

P-39 *Temporary dysfunction of outer hair cells after intratympanic application of Pseudomonas aeruginosa exotoxin A*
Adnan Lidian, Birgitta Linder, Barbara Canlon and Matti Anniko

P-40 *Ototoxic effects of Mefloquine in cochlear organotypic cultures*
Haiyan Jiang, Dalian Ding and Richard Salvi

P-41 *Otoacoustic emissions and cochlear functionality in workers exposed to styrene*
R. Sisto, L. Cerini, M.P. Gatto, M. Gherardi, A. Gordiani, E. Paci, F. Sanjust, T. Botti, G. Tranfo and A. Moleti

P-42 *Afferent pathology and repair associate with vestibular dysfunction and recovery during chronic ototoxicity in the rat*
Lara Sedó-Cabezón and Jordi Llorens

P-43 *Oncostatin M—induced protection against cisplatin ototoxicity is mediated via STAT3*
Elisabeth Gerschner, Olga Hegend, Heidi Olze, Birgit Mazurek and Agnieszka Szczepek

P-44 *Protective role of edaravone against neomycin-induced ototoxicity in zebrafish*
Jiwon Chang, Kang Woo Kim, Gi Jung Im, Hak Hyun Jung and June Choi

P-45 *Systemic treatment with resveratrol and N-acetylcysteine prevents from local ototoxicity with kanamycin and furosemide in rats*
F. García-Alcántara, S. Murillo-Cuesta, R. Martínez-Vega, J. Contreras, L. Sanz, I. Varela-Nieto and T. Rivera

P-46 *Ear in a dish: development of an in vitro assay for ototoxic and otoprotective drugs screening*
Aurélie Dos Santos, Andreas Eckhard, Ngoc-Nhi Luu, Marcus Müller and Hubert Löwenheim

P-47 *Deiters cells regulate the remodelling of aminoglycoside-injured organ of Corti, through the release of HMGB1*
Sabine Ladrech, Marc Mathieu, Hassan Boukhaddaoui, Jean-Luc Puel and Marc Lenoir

P-48 *Preliminary results with the NMDA antagonist memantine in salicylate-induced tinnitus*
M. Ralli, R. Salvi, F. Paciello, S.L.M. Eramo, D. Troiani, A.R. Fetoni and G. Paludetti

P-49 *Effects of the calyx on the apparent properties of vestibular type I hair cells K^+ currents*
Elisa Tavazzani, Giancarlo Russo, Jacopo Magistretti, Paolo Spaiardi, Teresa Soda, Ivo Prigioni and Sergio Masetto

P-50 *Proteomic analysis of vestibular schwannoma*
Jae-Hyun Seo, Kyoung-Ho Park and Seong-Cheon Bae

P-51 *Sudden sensorineural hearing loss, inflammatory disease & TNF-alpha*
Elias Q. Scherer, Andreas Knopf and Elmar Oestreicher

P-52 *Polymorphisms in genes involved in the free-radical process in patients with Ménière's disease and sudden sensorineural hearing loss*
Masaaki Teranishi, Yasue Uchida, Naoki Nishio, Ken Kato, Hironao Otake, Tadao Yoshida, Michihiko Sone, Saiko Sugiura, Fujiko Ando, Hiroshi Shimokata and Tsutomu Nakashima

P-53 *Reducing noise-induced hearing loss by peptides targeting transforming growth factor-β1*
Silvia Murillo-Cuesta, Lourdes Rodríguez-de la Rosa, Lorena Sanz, Carlos Avendaño, Teresa Rivera and Isabel Varela-Nieto

P-54 *IGF-I deficit predisposes to noise-induced hearing loss in mice*
Adelaida Celaya, Silvia Murillo-Cuesta, Lourdes Rodríguez-de la Rosa, Rafael Cediel, Carlos Avendaño, Julio Contreras and Isabel Varela-Nieto

P-55 *Functional and anatomical correlation in noise-induced hearing loss mouse model*
L. Sanz, C. Avendaño, S. Murillo-Cuesta, R. Cediel, F. García-Alcántara, P. Cobo, I. Varela-Nieto and T. Rivera

P-56 *Protective effect of rosmarinic acid in noise induced hearing loss: a functional and morphological study*
F. Paciello, S.L.M. Eramo, R. Rolesi, A.R. Fetoni, D. Troiani and G. Paludetti

P-57 *Role of p66shc in noise induced hearing loss: a functional and morphological study in mice and rats*
S.L.M. Eramo, A.R. Fetoni, F. Paciello, D. Troiani, G. Paludetti and G. Pani

P-58 *Analysis of vulnerability of hearing loss over age subsequent to the deletion of BDNF or Cav1.2 in the cochlea*
S.C. Lee, D. Campanelli, K. Varakina, D. Bing, A. Zuccotti, W. Singer, L. Rüttiger, T. Schimmang and M. Knipper

P-59 *Analysis of acute stress on long-term vulnerability after an acoustic injury in a mature rat model*
K. Kasini, I. Köpschall, K. Rohbock, L. Rüttiger, M. Knipper and M. Jaumann

P-60 *Lack of BDNF in the cochlea but not in the brain influences sound processing*
Dario Campanelli, Sze Chim Lee, Annalisa Zuccotti, Wibke Singer, Katja Gutsche, Lukas Rüttiger, Thomas Schimmang and Marlies Knipper

P-61 *Hearing and comorbidities in patients with professional hearing loss*
Natalia N. Petrova and Viya S. Panshina

P-62 *Synergistic effect of Styrene exposure and acoustic trauma: a functional and morphological study in a model of noise induced hearing loss (NIHL)*
R. Rolesi, F. Paciello, S.M.L. Eramo, D. Troiani, A.R. Fetoni and G. Paludetti

P-63 *Review of TNF blockers and other biological therapy agents in autoimmune inner ear disease*
David Lobo, José Ramón García-Berrocal, José María Verdaguer and Rafael Ramírez-Camacho

P-64 *Efficacy of etanercept in an animal model of autoimmune labyrinthitis*
David Lobo, José Ramón García-Berrocal, Almudena Trinidad and Rafael Ramírez-Camacho

P-65 *Guidance of spiral ganglion cell neurites using a nanomatrix in vitro*
Claudia Frick, *Marcus Müller, Ute Wank, Karl-Heinz Wiesmüller and Hubert Löwenheim*

P-66 *Methylprednisolone injection to the inner ear: an alternative to the systemic therapy*
Jeanette Sáenz-Piñones, José Ramón García-Berrocal, Ithzel Villarreal, Emilia T. Martin-Mata, Almudena Trinidad and Rafael Ramírez-Camacho

P-67 *Fibrin-collagen coating of cochlea implant electrodes for transplantation of adipose-derived stem cells*
Philipp Schendzielorz, Agmal Scherzed, Kristen Rak, Johannes Völker, Thomas Gehrke, Christian Ginzkey, Rudolf Hagen and Andreas Radeloff

P-68 *A novel method for transplantation of terminally differentiated neurons derived from pluripotent stem cells into the cochlea*
Hiroe Ohnishi, Takayuki Nakagawa, Masaaki Ishikawa, Yosuke Tona, Nakarin Asaka and Juichi Ito

P-69 *Survival of human iPS cell-derived neurons after transplantation into guinea pig cochleae*
Masaaki Ishikawa, Takayuki Nakagawa, Hiroe Onishi, Yosuke Tona, Nakarin Asaka, Yoshitaka Kawai and Juichi Ito

P-70 *Molecular optical imaging enables visualization of exogenous stem cells in the intact guinea pig cochlea*
John C.M.J. de Groot, Laura Mezzanotte, TimoSchomann, Clemens W.G.M. Löwik, Johan H.M. Frijns and Margriet A. Huisman

P-71 *Mondini dysplasia: conceptual new and new electrode designed for cochlear implantation*
Mª José Lavilla Marti de Valmaseda, Silvia V. Domínguez Ovejas, Daniella Laguado Bulgheroni, Javier Cervera Escario, José Marcos Calle and M. Aparicio

P-72 *Application of new generation technologies (NGS and aCGH) to the integral diagnosis and investigation of inherited hearing loss*
Matías Morín, Lucia Borreguero, Maria Palomares, Ángeles Mencía, Luciana S. Serrao de Castro, Fernando Mayo, Julián Nevado, Pablo Lapunzina, Ignacio del Castillo, Javier Santoyo, Felipe Moreno and Miguel Ángel Moreno-Pelayo

P-73 *Highly efficient diagnostic testing in patients with hereditary hearing loss using Panel-based Next Generation Sequencing*
Sarah Fehr, Moritz Menzel, Saskia Biskup, Konstanze Hörtnagel, Tim Scheurenbrand, Andrea Sprecher, Anke Tropitzsch and Hubert Löwenheim

P-74 *SVT-AP-99: Inhibition of Apaf-1 as a potential therapeutic strategy to prevent cisplatin-induced hearing loss*
Sandra Marchán, Celia Casares, Estefanía Traver, Almudena Trinidad, José Ramón García Berrocal, Natividad García Villar, Javier Sanagustín, Carmen Herrero, Carmen Lagunas and Rafael Ramírez-Camacho

P-75 *Plucked human scalp hair follicles may serve inner ear cell- based therapy*
Coen Gho*, Suzy Varderesyan, Johan H.M Frijns, Marcelo N. Rivolta and* Margriet A. Huisman

P-76 *Analysis of the effect of stimulated cGMP cascade on the vulnerability for hearing loss after noise exposure during aging*
Ksenya Varakina, Mahdieh Alinaghikhani, Dan Bing, Sze Chim Lee, Iris Köpschall, Marlies Knipper and Lukas Rüttiger

P-77 *Preventive effect of polyphenols on hearing loss comparing evoked potentials hearing of brain stem responses and stable state hearing in experimental animals age-related*
R. Sanz-Fernández, Carolina Sánchez-Rodríguez, J. Esteban-Sánchez, E. Martín-Sanz, L. Rodrigáñez, L. De Toro Gil and E. Durio-Calero

P-78 *Autoantibodies and sudden hearing loss*
Johanna Rebholz, Kerstin Ratzlaff, Reinhild Klein and Mark Praetorius

P-79 *Methods for hearing threshold measurements with ABR in guinea pigs*
Johannes Fischer, Jochen Steinhoff and Thomas Stark

P-80 *Influence of cochleostomy and implant insertion on drug gradients following intratympanic application in guinea pigs*
E.B. King, J.J. Hartsock, S.J. O'Leary and A.N. Salt

P-81 *Change in endocochlear potential during experimental insertion of a simulated cochlear implant electrode in the guinea pig*
Hidetoshi Oshima, Ryoukichi Ikeda, Kazuhiro Nomura, Muneharu Yamazaki, Hiroshi Hidaka, Takeshi Oshima, Tetsuaki Kawase and Toshimitsu Kobayashi

P-82 *Functional and histological outcomes following cochlear implantation in the mouse*
Nina Mistry, Lisa S. Nolan, Andrew Forge, Shakeel R. Saeed and Ruth R. Taylor

P-83 *Laser assisted cochleostomy in guinea pigs*
Digna M.A. Kamalski, Jeroen P.M. Peters, Tjeerd de Boorder, Rudolf M. Verdaasdonk, Sjaak F.L. Klis and Wilko Grolman

P-84 *A comparison of two vasoactive/vasodilative agents in combination with corticosteroid for treatment of sudden sensorineural hearing loss*
Špela Kordiš, Miha Žargi and Saba Battelino

P-85 *High speed imaging in laser-assisted stapedotomy*
Digna M.A. Kamalski, Tjeerd de Boorder, H.P.M. (Jeroen) Peters, Huib Versnel, Rudolf M. Verdaasdonk and Wilko Grolman

P-86 *Characterization of neural stem cells in the rat cochlear nucleus*
J. Voelker, K. Rak, R. Hagen and R. Mlynski

P-87 *Study of the role of miRNAs in the development of the auditory portion of the inner ear*
P. Van Den Ackerveken, A. Huyghe, R. Sacheli, F. Rogister, P.P. Lefebvre, L. Nguyen and B. Malgrange

P-88 *First steps towards a gapless interface between auditory neurons and multi-electrode arrays in vitro*
Stefan Hahnewald, Marta Roccio, Pascal Senn, Anne Tscherter, Jürg Streit, Julien Brossard, Herbert Keppner and Hans Rudolf Widmer

P-89 *Autophagy is required for apoptotic cell clearance and neural differentiation in early otic development*
Rocío de Iriarte, Marta Magariños, María Rodríguez Aburto, Hortensia Sanchez-Calderón, Juan M. Hurlé and Isabel Varela-Nieto

P-90 *TIS21 is involved in the development of spiral ganglion cells*
Takao Yamada, Ryosei Minoda, Momoko Ise and Eiji Yumoto

P-91 *Inhibition of matrix metalloproteinase-2 but not metalloproteinase-9 influences spiral ganglion neurons in vitro*
Cristian Setz, Eric Wei, Michael Sung, Soledad Levano, Daniel Bodmer and Yves Brand

P-92 *The regulation of Hes/Hey factors during sensory development of the chicken inner ear*
Jelena Petrovic, Gina Abelló, Héctor Gálvez, Joana Neves and Fernando Giraldez

P-93 *Netrin-4 promotes and modulates inner ear spiral ganglion neurite outgrowth in vitro*
Yves Brand, Michael Sung, Eduardo Chavez, Kwang Pak, Eric Wei, Soledad Levano, Vesna Radojevic, Daniel Bodmer and Allen F. Ryan

P-94 *Spiral ganglion-derived fibroblast cell culture*
Annett Anacker, Karl-Heinz Esser, Thomas Lenarz and Gerrit Paasche

P-95 *Rolipram improves survival of spiral ganglion cells in vitro*
Katharina Kranz, Athanasia Warnecke, Kirsten Wissel, Thomas Lenarz and Verena Scheper

P-96 *Extracellular matrix from human mesenchymal stem cells for cell-based drug delivery into the inner ear*
Kirsten Wissel, Athanasia Warnecke, Thomas Lenarz, Marina Prewitz and Carsten Werner

P-97 *Differentiation of Boettcher's cells during postnatal development of rat cochlea*
Marie Cloes, *Thomas Renson, Nicolas Johnen, Nicolas Thelen and Marc Thiry*

16:00 **Session 7:** ***Novel therapies and diagnostic tools***
Chair: Allen Ryan

16:00—16:30 Target Lecture:
Exploring disrupted cochlear homeostasis: a second youth for "old" diagnostic tools?
Paul Avan (UdA, Clermont-Ferrand, FR)

16:30—18:00 Oral presentations:

O-30 *Auditory threshold estimation using short-pulse DPOAE input-output functions*
Dennis Zelle, Anthony W. Gummer and Ernst Dalhoff

O-31 *Video head impulse test may represent a method for high frequency vestibular function and compensation phenomena assessment*
Hayo A. Breinbauer, Karina Aracena, José L. Anabalón, Diego Nazal and Jorge Caro

O-32 *Visualization of internal structures in the vestibule and the semicircular canals using optical coherence tomography (OCT)*
Tatsunori Sakamoto, Yosuke Tona, Akiko Taura, Takayuki Nakagawa and Juichi Ito

O-33 *Probing auditory nerve fiber loss using round-window neural noise*
Charlène Batrel, Jing Wang, Marc Lenoir, Jean-Luc Puel and Jérôme Bourien

O-34 *Intratympanic administration of triamcinolone-acetonide: In-vivo release and proof of oto-compatibility*
Elisabeth Engleder, Clemens Honeder, Gottfried Reznicek, Michael Wirth, Christoph Arnoldner and Franz Gabor

O-35 *Targeting the somatostatin receptors as a therapeutic approach for the preservation and protection of the mammalian cochlea from excitotoxicity*
Daniel Bodmer, Yves Brand, Soledad Levano, Cristian Setz and Vesna Radojevic

18:00—18:45 Plenary Conference: ***50 years of IEB Science***
Chair: Ignacio del Castillo

Inner ear biology at fifty—a science timeline
Jonathan Ashmore (UCL, London UK)

18:45—19:15 Spoendlin Award Lecture
Chair: Marcelo N. Rivolta

19:15—19:30 Spoendlin Award

21:00 Workshop dinner

Venue: Parador de Alcalá de Henares
Colegios Street, 8
Alcalá de Henares

Friday, September 13th

08:00 Registration

09:00 **Session 8:** ***Molecular bases of inner ear development and regeneration***

Chairs: Brigitte Malgrange and Takayuki Nakagawa

09:00—09:30 Target Lecture:
Translating development into regeneration for the ear
Marcelo N. Rivolta (UoS, Sheffield, UK)

09:30—11:00 Oral presentations:

O-36 *Function and regulation of Hey1 and Hey2 in cochlea development*
Ana Benito González and Angelika Doetzlhofer

O-37 *Effect of IGF-1 for spiral ganglion afferent dendrite and synapse regeneration*
Nakarin Asaka, Takayuki Nakagawa, Norio Yamamoto, Tomoko Kita, Akiko Taura, Masaaki Ishikawa, Yushi Hayashi and Juichi Ito

O-38 *Transcriptional regulation of the Pou4f3 gene*
Allen F. Ryan, Yan Li, Kwang Pak, Eduardo Chavez, Masatsugu Masuda and Ryoukichi Ikeda

O-39 *Translational research of cell-based therapy for restoration of spiral ganglion neurons*
Takayuki Nakagawa, Hiroe Ohnishi, Masaaki Ishikawa, Yosuke Tona, Nakarin Asaka, Yoshitaka Kawai and Juichi Ito

O-40 *Electrophysiological properties of stem-cell derived sensory neurons*
Bryony A. Nayagam, Tomoko Hyakumura, Mirella Dottori and Karina Needham

O-41 *Derivation of otic progenitor and inner ear cells from normal or pathological human induced pluripotent stem cells for modeling hereditary hearing loss*
Grobarczyk Benjamin, Laurence Borgs, Audrey Purnelle, Philippe Lefebvre, Laurent Nguyen and Brigitte Malgrange

11:00—11:30 Coffee break
Poster Session II

11:30—12:15 Closing conference:

Chair: Miguel Ángel Moreno

Insights into deafness from new mouse mutants
Karen P. Steel (King´s College, London, UK)

12:30—3:30 Business Meeting
Closing Ceremony
End of Poster Session II
End of Workshop

SYMPOSIUM
PRESENTATIONS

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Learning to preserve the inner ear function during cochlear implantation

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Introduction: Some clinical experiences show it is possible to invade intracochlear spaces when inserting a cochlear implant electrode array while preserving, to a greater or lesser extent, existing residual hearing. The objective of this presentation is to establish the degree of hearing preservation in implanted human patients and in normal-hearing experimental animals after the insertion of an electrode array and, in case hearing deteriorates, establish the cause.

Human CI patients: Tonal audiometries before and after the implantation were registered for 209 ears. Perimodiolar Countour Advance Soft tip cochlear implants were used in 92 cases and Straight electrodes Nucleus 22 and 24 M-K were implanted in 163 ears. Atraumatic surgery techniques for cochlear implantation were performed: the scala tympany was the target for the cochleostomy, endosteal layer of the cochlea was preserved, an interface of Healon® was used at the level of the cochleostomy, the insertion of the electrodes was carefully done following the "Advance Off-Stylet" (AOS) technique for the perimodiolar electrodes and reduction of the inflammatory response after surgery was promote with the use of Metilprednisolona. Results: Statistically significant loss of residual hearing for all the frequencies, pre and post-surgery was found with the use of straight electrode arrays Nucleus 22 and 24 M-K. We found almost complete residual hearing preservation (0-10 dB pre-post) in 80% percent of ears implanted with Contour Advance CI, "Atraumatic surgery" and a learning period.

Experimental animal trial: A 10-mm long (Hybrid S Nucleus) electrode array was placed in a group of 10 Macaca fascicularis specimens, following the same surgical steps as those performed in cochlear implant surgery in humans. Audiometric tests (BAEP and dpOA) were performed before and after implantation (during 8 months). Once the survival period had elapsed, the animals were put to sleep and the temporal bones were processed for its histopathological study. Results: The average difference in hearing thresholds pre- and post-implantation, as determined by the BAEPs, was 20 dB SPL (Range: 0 to 60 dB SPL). Hearing preservation levels were higher in the last specimens that were operated on, which suggests there is a learning curve in the development of greater atraumacity during surgeries. There was a correlation between audiometric and histological results, so the group of animals that preserved their hearing did not present intracochlear lesions, while it showed minimal reactions to foreign bodies around the electrode array.Although the first of these publications were pro-duced by researchers in Madrid and Barcelona, a growing number of groups (many in Spain's National Health System hospitals) in other cities would begin to publish. Researchers from all over the country are now represented.

Keywords: Atraumatic cochlear implant surgery

Acknowledgements: This work has been sponsored by Cochlear AG.

Drug delivery through cochlear implants

Claude Jolly

Drug delivery directly into the inner ear is a promising field for the treatment of sensory neural hearing loss (SNHL). Pharmacological intervention into the cochlea would bypass the barrier between blood and cochlear fluids and be able to reach sensory epithelium and neural tissues targeted for treatment, regeneration and neural growth much more effectively as compared to systemic application. The inner ear is easiest to access during cochlear implantation. Cochlear implant surgery is the principal mode of treatment for severe to profound SNHL today. However, it is likely that the sensory epithelium of the young and very young patients will be treated pharmacologically in situ at some point during their lifetime expectancy of over 80 years. Preservation of neural tissue is essential for best results with electrical stimulation today as well as for future alternative interventions which could eliminate the need for the implant. Cochlear implant electrode insertion and preservation of residual hearing demonstrate that in many cases the inner ear can be opened without major injuries to the sensory epithelium. Furthermore the electrode can be advanced into the scala tympani over a certain length and left in place for years without significant foreign body insult to the tissues. The reproducibility of such intervention and preservation mechanism are topics of intense studies among surgeons and clinicians. Results of residual hearing preservation are becoming routinely reported. The use of corticosteroids to prevent inflammation and improve hearing preservation at all frequencies after cochlear implantation is the first in situ therapy ever applied to the inner ear. Corticosteroid therapy has been mainly intra operative through the injection of a small bolus at the electrode entry point or in the middle ear for some time. More daring approaches have used crystalline forms of drugs with depot function which can treat the tissue for several weeks. The latest research reveals that it is possible to incorporate drug crystals within the silicone of the electrode carrier and release for months and up to several years at minimal doses. Another approach uses a catheter and demonstrates that it is feasible to insert the catheter up to 20 mm in the scala tympani of the cochlea and slowly inject a bolus for treatment in a defined region and at a defined dose. A more demanding system uses a subcutaneous implantable reservoir with port and septum. The reservoir is connected to a channel within the electrode. Diffusion mechanisms from the reservoir to the scala tympani are under study. The reservoir is refillable and could facilitate the use of biomimetic peptides for a long-term treatment. The techniques and results reported here will also apply to the non-cochlear implant patients who require an otological intervention to arrest or reverse a condition leading to SNHL or other conditions.

PET scan, an alternative to discover the effects of auditory deprivation

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The development of modern noninvasive brain imaging techniques, such as Positron Emision Tomography (PET), the increasing prevalence of cochlear implantation, and new, effective rehabilitation methods for profound or total hearing loss have led to increasing interest in human functional reorganization in auditory-related cortex in individuals with deafness.

Evidence of Neuroplasticity

In brain activation studies of prelingual deafness using PET the auditory cortex was found to be activated by sign language presented visually. These findings demonstrate the capacity of the auditory cortex for cross-modal reorganization after auditory deprivation of the human brain.

Other functional evidence of neuroplasticity is the resting cerebral glucose metabolism in the primary auditory and related cortices that increase or even exceeded normal level of activation in individuals with prelingual deafness. This finding was explained by plastic changes in the auditory neuronal circuitry due to the expansion of the afferent neural network by other sensory systems. That expansion was possible because of the lack of functional specialization of the auditory cortex in younger individuals and its resultant vulnerability to other forms of sensory stimulation. That plasticity was demonstrated to prevent the recovery of the designated hearing function of auditory neural substrates after cochlear implantation and rehabilitation in prelingually deaf patients. Furthermore, patients with postlingual deafness also show a positive correlation between rsting metabolism of anterior cingulate gyri and superior temporal cortices and the duration of deafness. In adults, auditory deprivation decreased neuronal activity transiently in primary auditory and auditory-related cortices, and functional reorganization likely takes place in the auditory cortex over time.

Prediction of Cochlear Implant outcome

The duration of auditory deprivation plays an important role, and currently is the main predictor of implantation success in children. However, among patients with identical duration of deafness, performance remains highly variable, suggesting there are other more fundamental factors that determine clinical outcome.

When speech perception scores obtained three years after cochlear implantation were correlated with resting PET brain metabolism before implantation (deafness duration was factored out to leave only time-independent effects), a positive correlation with speech outcome in dorsal brain regions encompassing the left prefrontal and parietal cortices has been observed. Children with a high metabolism in dorsal cortical regions were those most likely to successfully learn speech with a cochlear implant. Using the same experimental paradigm, similar dorso-ventral segregation was replicated in post-lingually deaf adults. Therefore, resting metabolism in the deaf brain can be a fairly good indicator of speech performance after auditory rehabilitation. In addition, resting metabolism proved to be a very useful exploratory tool, as it not only reveals the well-known time-dependent cross-modal re-organisation of auditory temporal cortices, but also adaptive higher cognitive mechanisms.

Selection of adult complex cases for cochlear implantation

Difficult patients to counsel are typically those young-to-middle age adults suffering from a long-term profound hearing loss on one side and generally unaided, and the other side affected with a long-term severe to profound hearing loss and generally aided. These patients demand an improvement in their communication abilities via cochlear implant. We have performed PET studies using H2O15 or 18FDG radiotracers to asses resting and activation of the auditory pathway in an attempt to elucidate the optimum set for an effective evaluation for selecting the better ear to carry out a cochlear implant. PET scan suggested the better ear to implant was the one still functional, as a result of significant increments in metabolic activity in the auditory cortices, and was confirmed by speech perception scores achieved after CI rehabilitation.

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Awaking the central auditory system with cochlear implants

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The developing brain is shaped by the input it receives. Without sound, association areas of the auditory cortex are vulnerable to reorganization while primary pathways remain immature. Early implantation promotes development in the auditory brainstem and cortex and allows children to develop spoken language. At the same time, however, unilateral implant use restricts excitatory input to the auditory pathways from the deprived ear and removes inhibitory input involved in binaural processing. In developing animal models, this results in overexpression of neural connections from the hearing ear and compromises binaural responses. In the present study, we asked whether such reorganization occurs when children with bilateral deafness hear through unilateral cochlear implants and, if so, whether providing bilateral electrical input in early development protects them from such changes. Responses from the auditory brainstem and cortex in children using unilateral and bilateral cochlear implants revealed that unilateral implant use allows abnormal strengthening of pathways from the stimulated ear after > 1.5 years. During this period, the auditory brainstem approaches maturity. Implantation of the opposite ear thereafter does not reverse the asymmetric auditory function created by unilaterally driven development despite 3-4 years of bilateral cochlear implant use. The data suggest a benefit of providing bilateral input within a sensitive period to the developing human auditory system.

From hearing aids to ABI. A world of options

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The management of hearing loss has suffered an impressive evolution in the last three decades. Whereas in 1980 the only available options for the hearing impaired patient were conventional analogical hearing aids or surgical management of otosclerosis and chronic ear diseases, thirty years later we have a wide range of different options. These include semi-implantable hearing aids, osseointegrated hearing aids, middle ear implants, cochlear implants, and auditory brainstem implants. The evolution in this field seems to be limited only by technological factors.

This conference deals with the indications, basic surgical technique, and results of each one of the aforementioned options, emphasizing the evolution of the management of hearing loss over the last years, based on the personal experience of the authors. Clinical cases will be used to illustrate the usefulness of every option. Upon completion of this conference, attendants should have a general idea of the options for the treatment of hearing loss in 2013, be able to suggest the best treatment option for a hearing impaired patient, and increase their knowledge about the current available implants and hearing aids. Between hearing aids and ABI there is a real world of options. Taking the right choice is in our hands.

Keywords: cochlear implants, hearing loss, hearing aids, middle ear implants

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Inner Ear Biology at fifty

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Fifty years is a remarkable age for a conference that never was associated with a scientific society. From the very beginning, the glue that held this group together was the conviction that the advancement of our science is based on communication, constructive criticism and collegiality. A mailing list that was passed from one organizer to the next was the only written document. This reminiscence on our history will pay tribute to the handful of clinicians that created the “Inner Ear Biology Workshop” as a discussion group without agenda and abstracts; trace its growth and expansion; follow its transformations, trials and tribulations; and append some personal reflections.

Keywords: Inner Ear Biology, history

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Inner Ear Biology at fifty - A science timeline

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We can hardly expect science to stand still. The scientific concerns and obsessions of fifty years ago are very different from today and yet some of themes that run through the Inner Ear Biology Workshops are still with us today. How do we best measure hearing function? What do the cells in the inner ear do? How do we protect hearing? What does the inner ear tell the brain? Of course the technology to address such questions has moved on and the molecular genetics revolution starting a little over 20 years ago now permeates the business of workshop. But when the IEB workshop began we knew little, for example, of how the inner ear transduced sound or whether the basilar membrane was adequately tuned. Who but a few might have imagined then that the inner ear emits sound?

Keywords: Inner Ear Biology, history.

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Insights into deafness from new mouse mutants

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Mutations causing hearing impairment can provide valuable insights into the molecules that are essential for auditory function and the pathological processes that can underlie dysfunction. The mouse has proven a rich source of new gene associations with deafness, informing our understanding of the biological basis of hearing and providing models for the many different forms of deafness in humans. In this talk, I will outline the findings from two large-scale mutagenesis programmes that have led to the discovery of many new genes underlying deafness.

These mutations lead to a wide range of defects including ossicle malformations, middle ear inflammation, gross inner ear malformation, organ of Corti patterning defects, multiple types of hair bundle abnormality, retarded development of hair cells, reduced endocochlear potentials, and synaptic defects, among others. The range of genes involved is equally wide. However, our ongoing screen of newly-generated mouse mutants screened by Auditory Brainstem Response measurements has been particularly useful in discovering several mutant lines with progressive increase in thresholds, and these may be valuable as models for understanding the molecular basis of age-related hearing loss in humans. Furthermore, some of these lines affect pathways that can be manipulated by small molecules and we plan to investigate whether we can slow down or stop progression of hearing loss in the corresponding mouse mutants.

Keywords: Mouse mutants; mechanisms of deafness; progressive hearing loss; genetics

Acknowledgements: This work was supported by the Wellcome Trust, MRC, EC, Action on Hearing Loss and Deafness Research UK.

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Behavioral responses in a complex auditory discrimination task depends on the sequence of cues

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The order of introduction of auditory cues such as ‘pitch’ and ‘place’ in a complex auditory discrimination task matters for the final performance. Pitch discrimination and sound location constitute two channels of auditory information processing that are not independent, neither parallel nor sequential. Complex interactions between these channels are likely to depend on the plasticity of neuronal networks activated by the sensory and memory information processing enabled during the priming phase. In the present study, behavioral responses of freely-moving rats are characterized in a GO/NOGO reaction time task aimed to assess the difference between pitch priming followed by place discrimination vs. location priming followed by pitch discrimination. Performances are compared in a complex task that involved a mixing of both pitch ad place cues in the same experimental condition. The results suggest that in both tasks a competition between two processes is enabled: one process involving stimulus evaluation, response preparation and execution, and the other process involving recognition of the stimulus features associated with inhibition of the GO response. The model is extended to analyze performances in a vowel discrimination task.

Myosins as system integrators for the regulation of mechanotransduction in the inner ear

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The apical cytoplasm of inner ear sensory hair cells contains three populations of filamentous actin that are distinct in position, organization and function: the rigid cross-linked parallel actin filaments within the stereocilia core, the dense network of actin forming the cuticular plate, and the perijunctional actin belt. The specific roles of each of these prominent actin-based structures are reliant upon their characteristic architectural features and dynamic properties. At the same time, their formation and function must be tightly coordinated to achieve the exquisite mechanosensitivity of hair cells. Several myosins, mechanochemical actin-based motors driving cargo transport and contractility, have recently been identified as essential for the development and maintenance of hair cell-specific actin networks. MyoXVa and MyoIII [1] localize to the tips of stereocilia, and are required for transport of essential protein cargo and for actin regulation. MyoVI concentrates at, and is thought to shape or stabilize, the tapered stereocilia base. MyoVIIa localizes at the upper tip-link insertion site where it is hypothesized to be involved in tip-link tensing [2]. Non-muscle MyoII interlaces with actin at the perijunctional actin belt and regulates epithelial geometry and tensional homeostasis [3]. MyoIc has long been assumed to have a role in adaptation, however a recent study showed that it is localized along the entire stereocilia. To work in concert these myosins and their corresponding isoforms, in particular those within the crowded molecular environment of the stereocilia apical pole must be intricately regulated, and likely exhibit a degree of functional redundancy. In this presentation we will review the current state of knowledge regarding myosins in the inner ear and highlight important future directions to identify ways that their regulation and compensatory potential may be exploited for repair and recovery from damage or disruption.

Keywords: Myosins; hair cells; stereocilia; deafness, cuticular plate, actin regulation

Acknowledgements: This work has been sponsored by NIDCD, IRP, NIH.

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Genetics of monogenic deafness unraveled at fast pace

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With the introduction of newborn hearing tests, early diagnosis of congenital hearing impairment is warranted in many countries worldwide. However, the underlying causes often remain elusive and therefore questions on prognosis and risks for further children in the family remain unanswered. Based on family structure and pattern of inheritance, we have combined different strategies to identify genetic defects underlying autosomal recessive nonsyndromic hearing impairment (arNSHI) in about 180 Dutch individuals with presumed arNSHI and about 20 subjects with presumed autosomal dominant nonsyndromic hearing impairment (adNSHI). High-density SNP arrays for homozygosity mapping and genotype sharing combined with candidate gene selection revealed several novel genes for arNSHI. Whole exome sequencing (WES) only was performed in 40 probands with presumed arNSHI and in 20 probands with presumed adNSHI. In the arNSHI cases, defects in GJB2 and deletions in GJB6 were excluded prior to WES. As a first step in the analysis, variants in known deafness genes are evaluated. In 5 arNSHI cases underlying mutations were detected LOXHD1, TMC1, MYO15A and STRC and for adNSHI mutations in 5 probands in MYO6, EYA4, POU4F3, MYH14, MYH9. X-linked deafness with an underlying mutation in SMPX was found in one boy. Analyses of the remaining part of the exomes are ongoing. Also, mutations in known genes for syndromic HI were identified in 3 probands with presumed arNSHI. Reevaluation of patients is being performed to confirm that HI is nonsyndromic in these cases.

In conclusion, the presented results demonstrate that employing a combination of strategies facilitates the identification of novel deafness genes in populations with small families. Genotype-phenotype correlations are emerging which are of utmost importance for prognosis, rehabilitation and genetic counseling in families.

Keywords: hearing impairment, deafness, genetics, genes

Acknowledgements: NWO/ZonMW, Action on Hearing Loss, The Oticon Foundation, The Heinsius Houbolt Foundation

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Mouse models for human hereditary deafness DFNA8/12 and DFNB21

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Mutations in TECTA, the gene encoding alpha tectorin (one of the major non-collagenous proteins of the tectorial membrane), cause recessive (DFNB21) and dominant (DFNA8/12) forms of human hereditary deafness, and a recent study (Hildebrand et al., 2011) has suggested that missense mutations in TECTA are one of the more common causes of autosomal dominant non-syndromic hearing loss. Mouse models for five DFNA8/12 missense mutations in Tecta indicate the mutations are all semi-dominant and that there is a distinct genotype-phenotype correlation in heterozygous animals, with the observed structural defects varying according to whether the mutation lies in the zona pellucida or the zonadhesin-like domains of alpha-tectorin. Null or functional null mutations in Tecta are recessive, and lead to a detachment of the residual tectorial membrane from the organ of Corti, a phenotype that is similar to that observed in mice that are homozygous for the semi-dominant missense mutations. These data from mouse models therefore provide a basis for the variations in the severity of the hearing loss that is observed in DFNA8/12 and DFNB21 patients. Missense mutations in TECTA that involve the loss of cysteine residues and are reported to cause progressive forms of hearing loss in DFNA8/12 families do not, however, lead to structural or functional changes that become worse with time, at least over the periods that have been studied thus far (up to 1 year). Biolistic transfection of fluorophore-tagged tectorin constructs into explant cultures of the early mouse cochlea provides a method for studying how missense mutations in TECTA affect matrix production. Preliminary data indicate the missense mutations in the zona pellucida domain of TECTA that cause deafness in an Austrian and a Spanish family respectively, both prevent tectorin secretion.

Keywords: tectorial membrane; Tecta; DFNA8/12; DFNB21; deafness gene

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Deep phenotyping of vestibular disorders for next generation sequencing

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Vestibular disorders (VD) have a major impact on healthcare systems. Benign paroxysmal positional vertigo (BPPV), vestibular migraine (VM) and Ménière's disease (MD) are the most common phenotypes of the episodic vertigo syndrome in adults and their joint prevalence is around 3-4% in European descendent population. Genetic factors contributing to the pathogenesis of VD have seldom been investigated and the knowledge of the biological basis of VD is still poor compared to pathophysiology of hearing loss. Several factors can explain the slow progression of biomedical research in VD, including the lack of standard definitions for diagnosis of the major VD, the absence of models of disease, the few research groups dedicated to the vestibular system and VD and the scarce of funding resources for VD.

The Barany Society, an international organization committed to vestibular research from epidemiology, neurology, neurophysiology and otolaryngology has set up a Committee for an International Classification of VD. Current ongoing work includes definition for the major vestibular syndromes and a deep phenotyping proposal to characterize complete and partial phenotypes of the episodic vertigo syndrome.

High throughput sequencing represents a paradigm shift for clinical medicine and has the potential to dramatically change health care systems in the world. Whole-exome sequencing and whole-genome sequencing (NGS) technologies have started to solve the primary molecular etiology of Mendelian diseases, including sensorineural hearing loss. So, a deep phenotyping of vestibular diseases (medical and family history) will split patients into precise categories, refining and debating differential diagnoses, facilitating NGS research and leading to the discovery of mutations causing VD.

A large collaborative effort has been coordinated in Europe to promote a COST Action on European Network for the Study of Vestibular Disorder (EuroVED). The network brings together clinical and molecular genetics researchers to improve diagnosis of VD and to identify genetic factors contributing to VD. This Action includes physicians, epidemiologists and basic researchers and the main objectives include the dissemination of standardized criteria for definition of VD, the setting of an European registry of families with VD, coordinated biobanking activities for NGS and the attraction and involvement of new early-stage researchers to the field of vestibular research.

ACEMg in the prevention and treatment of hearing impairment

J. Miller [1], *D. Prieskorn* [1], *G. Green* [1], *R. Altschuler* [1], *Y. Raphael* [1] *and C. LePrell* [2]

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A growing body of literature indicates a key role for excess free radical formation in stress induced pathology in the auditory system. Empirical data and theoretical considerations indicates that excess noise, ototoxic drugs, age, and surgical trauma share the common pathology-inducing mechanism of excess free radical formation, and an ensuing common pathway to cell death. Because free radical-induced lipid peroxidation can lead to cell death, we developed a dietary supplement of antioxidants (Vitamins A, C, & E) plus the vasodilator magnesium (ACEMg) which we have shown reduces noise- and drug-induced free radical formation, hearing loss and inner ear pathology in animals. We next tested whether ACEMg can influence the progression of pathology and hearing loss in 2 mouse models for human genetic deafness, Gjb2 and Auna1. Connexin 26 conditional knock-out and Diap3 transgenic mice were fed an ACEMg or control diet from 4 to 16 weeks. ABR thresholds at 12, 16 and 24 kHz were significantly (3 – 4 fold) better in diet fed Cx26 mice at 16 weeks compared to their 4 week values. Diap3 transgenic mice treated with ACEMg showed worse hearing at 24kHz compared with controls. These findings are consistent with a human case study showing cessation and some reversal of progressive deafness in a child with a Connexin defect (Green et al, 2011, ARO). Targeting free radical formation for intervention to prevent and treat stress-induced hearing loss has the appeal of attenuating the 'up-front' initiator of pathology. Given the safety of this micronutrient formulation and efficacy in reduction of noise-, drug-, trauma-induced hearing impairment and now specific hereditary hearing loss, ACEMg and similar formulations would seem appropriate for human trial study.

Keywords: free radicals, antioxidants, cell death, stress- & hereditary-induced hearing loss, clinical trials

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Drs Miller, Green & LePrell are co-inventors of a University of Michigan patent for the use of ACEMg to treat hearing loss & Miller is a minority stock holder in a company licensed to commercialize this treatment. Conflict Management Plans are in place.

TARGET LECTURERS

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Exploring disrupted cochlear homeostasis: a second youth for 'old' diagnostic tools?

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The responses of cochlear hair cells to sound stimuli are highly sensitive to the resting environment and operating point of their stereocilia bundles, which need to be adjusted for optimal operation. Among the conditions whereby stereocilia operation is likely disrupted, cochlear hydrops, assumed to be a hallmark of Menière's disease (MD), has the best acknowledged clinical consequences. For several decades, objective diagnosis of endocochlear hydrops has rested upon electrocochleography, and the increased size of the summating potential SP relative to the compound action potential of the cochlear nerve AP has been thought to reflect an exaggerated depolarization of hair cells in relation to a deformed, inflated scala media. More recent attempts, which will be reviewed in this presentation, suggest that otoacoustic-emission changes when symptoms of MD are present signal a biased cochlear outer-hair-cell operation, likely in relation to hydrops. We performed a comparison of the two tests in a cohort of 80 MD patients at different stages of their condition, SP/AP ratio and otoacoustic emissions being measured almost simultaneously. A gerbil model was also used in which short pressure steps were applied in scala media so as to simulate transient hydrops. Discrepancies between the outcomes of the SP/AP and otoacoustic tests, observed in both models, suggest that these tests do not react to the same aspects of disrupted cochlear homeostasis; notably, otoacoustic emissions seem much more immediately sensitive to any acute change in cochlear fluid hydrostatic pressure. The use of the two tests might thus enrich the study of the cochlear environment in patients with hydropic ears, with the long-term goal of better tracking the correlates of the symptoms, and ultimately, the efficiency of a therapy.

Keywords: endocochlear hydrops; hydrostatic pressure; otoacoustic emissions, electrocochleography; hair cell physiology.

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Translating development into regeneration for the ear

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Understanding the fundamental processes that control the development of an organ has an important consequence; it provides us the tools to devise strategies that should allow its regeneration when damaged. Pursuing the active induction of regeneration is even more necessary in organs like the mammalian inner ear; given the absence, in the adult stage, of a natural ability to replace neurons and sensory cells.

Key signaling pathways like Wnt and Fgf play an important early role in placode induction and the restriction of the otic field, but also late in development, during the specification of the sensory lineages. In the postnatal cochlea, Lgr5+,Wnt-responsive cells proliferate and have the capacity to differentiate into hair cell-like cells (1-2). More recently, a different population of Axin2+ cells with similar properties has been identified (3), raising hopes that cells with dormant stem cell potential could reside in the mature cochlea.

Furthermore, these pathways are not only relevant as potential targets for endogenous regeneration, but they are fundamental for a complementary regenerative strategy based on cell replacement. In our lab, we are generating otic progenitors from human pluripotent stem cells by manipulating the Fgf signaling pathway. These progenitors have the capacity to restore auditory thresholds when transplanted into an animal model of deafness (4). Moreover, combined modulation of Wnt signaling, together Fgf seems to enhance the efficiency of progenitor production.

In summary, the presentation will revise how our most recent understanding of the way the ear develops is paving the way for strategies that should help us regenerate the damaged inner ear.

Keywords: development, regeneration, stem cells, Fgfs, Wnts.

Acknowledgements: Work in my laboratory is supported by the MRC, AoHL, DRUK and collaborations with Cochlear and Pfizer-Neusentis.Management Plans are in place.

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ORAL PRESENTATIONS

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Sensorineural hearing loss and ischemic injury: Development of animal models to assess vascular and oxidative effects

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Objective: Hearing loss (HL) can have genetic causes, can be associated with aging or exposure to noise or ototoxic substances, and the aetiology can be attributed to vascular injury, trauma, tumours, infections or autoimmune response. All of these factors could be ascribed to alterations in cochlear microcirculation resulting in hypoxia. This condition can damage cochlear hair cells and neurons possibly leading to HL. Hypoxia and ischemia can then be identified as possible factors contributing to the aetiology of deafness, but they have not been experimentally studied yet.

The purpose of this work is to develop animal models of ischemia and infarction suitable for the study of cochlear vascular damage, and to characterize them with electrophysiology and gene/protein expression analyses.

Methods: It was decided to monitor the effects of ischemia in thrombosis mimicked by complete temporary carotid occlusion, and in stroke mimicked by incomplete permanent left coronary artery. In particular this study focused on electrophysiology (ABR), analysis of organ of Corti and spiral ganglion structures and coagulation, oxidative stress and apoptosis pathways (Real-time PCR and immunohistochemistry).

Results: In our models, both infarction and ischemia cause a slight but significant hearing loss, localized at the cochlear apex. Furthermore, there is a slight induction of the coagulation cascade, both in procoagulant and anticoagulant part, and an activation of c-Jun N-terminal Kinase (JNK) that may lead to cell survival. In addition, only in the carotid ischemia the cuticular plate of outer hair cells is damaged.

Conclusions: The two models of ischemia developed are suitable for the study of cochlear vascular damage, as they produce hearing loss and give modifications in coagulative, oxidative and apoptotic factors gene expression.

We can therefore say that the damages taken into consideration act on the inner ear with vascular damage and oxidative mechanisms.

Keywords: infarction, ischemia, vascular damage, oxidative damage.

Acknowledgements: This work has been sponsored by Cochlear LTD.

ORAL PRESENTATIONS O-01

Dexamethasone prevents electrode insertion trauma-initiated inflammatory response and fibrosis

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Immediately post-trauma, an inflammatory response is activated to restore the area of damage. The insertion of a cochlear implant (CI) electrode array can be associated with an inflammatory response that is known to induce loss of hair cells and growth of fibrotic tissue around the electrode array. An undesirable consequence of fibrosis is the requirement of an increase in the level of the stimulating current and therefore current spread. We present a study of the inflammatory response upon electrode insertion trauma (EIT), and the co-operation between TGF-ß and Wnt/ß-catenin pathway to elicit fibrosis. We demonstrate how dexamethasone (DXM) is an excellent drug to prevent these events and preserve residual hearing.

Cochleae from 3 days rat pups were or were not subjected to EIT and then treated with either TGF-ß1 (0.4 nM); TGF-ß3 (0.4 nM); LY294002 (2.5 uM) or DXM (50 uM). Studies of the mRNA levels of IL-1ß, TNF-α, COX-2, iNOS, TGF-ß1, TGF-ß3, CTGF, Axin2; Wnt4 and Wnt5b were performed on the organ of Corti (OC) and lateral wall tissue (LW) explants after 24 and/or 96h of incubation.

Confocal studies for oxidative stress, apoptosis, activated ß-catenin, epithelial to mesenchymal differentiation, presence of stress fibers, and the expression of Collagen-1A1 as an indicator of fibrosis were carried out in OC and LW explants at 96h.

The results from this EIT in vitro model show an initiation of a wound healing process in which an inflammatory response characterized by an increase of pro-inflammatory cytokines (IL-1ß, TNF-α) and inducible enzymes (COX-2, iNOS). This is followed by a proliferative-fibrosis phase where an increase of TGF-ß and CTGF was observed. Our results suggest the activation of the Wnt/ß-catenin pathway following EIT in OC, which is exacerbated by TGF-ß1 treatment and leads to cell growth and differentiation. Wnt pathways were not shown to participate in the fibrotic response initiated in the LW explants. DXM treatment abolished the inflammatory response and fibrogenesis, making this drug a first choice in, for example, CI eluting electrodes.

Keywords: Cochlear implant; residual hearing preservation; electrode insertion trauma; fibrosis; dexamethasone.

Acknowledgements: This work has been supported by grants from MED-EL to TRV and AHRF to EB

The inferior colliculus *is not* an obligatory relay in the ascending auditory pathway

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Despite the general assumption that the main source of ascending projections to the auditory thalamus is the inferior colliculus (IC), several isolated reports suggest that the medial geniculate body (MGB) receives direct projections from auditory subcollicular nuclei [1-3].

To investigate the sources of ascending projections to the auditory thalamus, we made iontophoretic injections of the retrograde tracer FluoroGold into different regions of the auditory thalamus (the ventral, dorsal and medial divisions of the MGB, the posterior intralaminar nucleus, the marginal zone, the posterior limitans nucleus, and the subparafascicular nucleus) and analyzed the distribution of the neurons labeled in all subcollicular auditory nuclei.

Our results show that the auditory thalamus receives direct convergent projections from a surprisingly high number of neurons in many subcollicular nuclei, including the contralateral dorsal cochlear nucleus and lateral superior olive; the ipsilateral medial superior olive (MSO), superior paraolivary nucleus (SPON), and ventral nucleus of the lateral lemniscus (VNLL); and the dorsal nucleus of the lateral lemniscus (DNLL) and the nucleus sagulum/horizontal cell group (Sag/HCG) of both sides. We also found numerous labeled neurons ipsilaterally in an ill-defined territory wedged between the medial nucleus of the trapezoid body and the SPON, which we termed the dorsomedial wedge (DMW). In the cases with the most retrograde labeling, virtually all neurons in the DNLL and Sag/HCG were seen to innervate the ipsilateral or the contralateral thalamus, and the percentage of labeled neurons in the ipsilateral MSO, SPON, and DMW approached 50%. The single subcollicular nucleus that contributes the most to the innervation of the auditory thalamus is the VNLL. These data suggest that many of the axons from lower centers that innervate the auditory thalamus must be collaterals of axons that also innervate the IC. Moreover, our quantitative, multivariate analysis indicates that each nucleus or region of the auditory thalamus receives a different combination of inputs from subcollicular centers.

These findings lend strong support to Cajal's "central acoustic tract", improve our understanding of the organization of the auditory pathway, shed light on the complex parcellation of the auditory thalamus, and provide novel morphological frameworks for future functional studies.

Keywords: cochlear nuclei; superior olivary complex; lateral lemniscus, inferior colliculus; medial geniculate body

Acknowledgements: This work has been supported by grants FIS PI10/01803 (to ES) and NIH DC-02266 (de ASB)

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ORAL PRESENTATIONS O-03

On the controversy about the sharpness of human cochlear tuning

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In signal processing terms, the operation of the mammalian cochlea in the inner ear may be likened to a bank of filters. Based on otoacoustic emission evidence, it has been recently claimed that cochlear tuning is sharper for human than for other mammals [1]. The claim was corroborated with a behavioral method that involves the masking of pure tones with forward notched noises (NN). Using this method, it has been further claimed that human cochlear tuning is sharper than suggested by earlier behavioral studies [2]. These claims are controversial [3-5]. Here, we contribute to the controversy by theoretically assessing the accuracy of the NN method at inferring the bandwidth (BW) of nonlinear cochlear filters. Behavioral forward masking was mimicked using a computer model of the squared basilar membrane response followed by a temporal integrator. Isoresponse and isolevel versions of the forward masking NN method were applied to infer the already known BW of the cochlear filter used in the model. We show that isolevel methods were overall more accurate than isoresponse methods. We also show that BWs for NNs and sinusoids equate only for isolevel methods and when the levels of the two stimuli are appropriately scaled. Lastly, we show that the inferred BW depends on the method version (isolevel BW was twice as broad as isoresponse BW at 40 dB SPL) and on the stimulus level (isoresponse and isolevel BW decreased and increased, respectively, with increasing level over the level range where cochlear responses went from linear to compressive). We suggest that the latter may contribute to explaining the reported differences in cochlear tuning across behavioral studies and species. We further suggest that given the well-established nonlinear nature of cochlear responses, even greater care must be exercised when using a single BW value to describe and compare cochlear tuning. A printed version of this work may be found elsewhere [6].

Keywords: nonlinear frequency selectivity, comparative hearing, inner ear, cochlear nonlinearity, cochlear mechanics

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Voltage under fire: fast IR laser-induced shifts in prestin's Boltzmann distribution along the voltage axis

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Outer hair cells (OHCs) are mechanically active components of the inner ear that underlie cochlear amplification. Prestin motor units (SLC26a5), localized to the OHC lateral membrane, drive rapid mechanical changes in OHCs and are associated with a nonlinear capacitance (NLC). NLC arises as the first derivative of a two-state Boltzmann function relating prestin voltage-sensor charge as a function of transmembrane voltage; it reflects the movement charged residues within these motor units and peaks at a voltage (Vh) where OHC mechanical response is maximally sensitive to voltage. NLC is vulnerable to many biophysical forces, including temperature [1-3].

Recently, infrared (IR) laser-induced temperature jumps have been found to induce changes in linear membrane capacitance [4]. Consequently, previous observations attributed to fast temperature dependent voltage-sensor charge movements in OHCs require re-inspection [3]. Here, we examined the effects of fast temperature jumps induced by an IR laser in control and prestin (SLC26a5)-transfected HEK cells under whole cell voltage clamp. Prestin's voltage sensor imparts a characteristic bell-shaped, voltage-dependent nonlinear capacitance (NLC). Temperature jumps in control HEK cells cause a monophasic increase in membrane capacitance (Cm) regardless of holding voltage, as previously described. Prestin-transfected HEK cells, however, additionally show a biphasic increase/decrease in Cm with a reversal potential corresponding to the voltage at peak NLC (Vh), attributable to a rapid temperature-following shift in Vh, with shift rates up to 14 V/s over the course of a 5 ms IR pulse. Treatment with salicylate, a known inhibitor of NLC, re-establishes control behavior. A simple kinetic model recapitulates our biophysical observations. These results verify a voltage-dependent protein's ability to respond to fast temperature perturbations on par with double layer susceptibility, likely arising from prestin's unique ability to move sensor charge at kilohertz rates, a requirement for the OHC's role as cochlear amplifier.

Keywords: outer hair cell; SLC26a5; prestin.

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Intravital calcium imaging of cochlear hair cells upon sound stimulation

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Objectives: Intravital confocal microscopy provides instant histopathology at the cellular and subcellular level and is ideal for investigating dynamic events involved. Here, we describe the application of Intravital Real-Time Confocal Laser Scanning Microscopy (IVRTCLSM) [1] for calcium imaging of cochlear hair cells upon sound stimulation.

Methods: Adult guinea pigs were anesthetized. Apical turn of the cochlea was surgically exposed via ventral approach, leaving tympanic membrane and auditory ossicles intact. A membrane-permeant green-fluorescent calcium indicator, Fluo-4 AM, was applied. All picture/movie acquisitions were performed using a Nikon A1R confocal laser scanning microscope system. The A1R incorporates a high-speed resonant scanner, which allows an acquisition speed of 30 frames per second. Fluo-4 AM was excited with a 488 nm argon laser, and emission was detected through a 525/50 nm band pass filter. Fluorescent images were obtained before, during, and after sound stimulation.

Results: Inner hair cells (IHCs), outer hair cells (OHCs), and supporting cells could be clearly identified, indicating successful loading of Fluo-4 AM dye. OHC region of the epithelium was selected, and the mean fluorescent intensity was calculated for each image frame. Increased fluorescence intensity of the OHCs was observed upon sound stimulation.

Conclusions: We demonstrate a technique to image dynamic fluorescent changes within sound-stimulated cochlear hair cells in live guinea pigs. Although apical opening was necessary for visualization, the preparation well preserved physiological hearing functions and conditions such as sound conduction, innervation, blood supply, and body temperature. Our technique offers opportunities to investigate hair cell transduction of living animals in both spatial and temporal resolution.

Keywords: cochlear hail cells; confocal microscope; intravital imaging

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Identification of tympanic border cells as slow-cycling cells in the cochlea

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Mammalian cochlear sensory epithelial cells are believed to possess minimal regenerative potential because they halt proliferation during late stage of embryogenesis [1] and never regenerate after birth. This means that sensorineural hearing loss caused by the death of cochlear sensory epithelial cells is a permanent condition. However, stem cells were recently identified in neonatal mice following dissociation of their inner ear organs [2]. This suggests that regenerative therapy for sensorineural hearing loss may be possible. Unfortunately, dissociation distorts the microanatomy of the inner ear, making it difficult to determine the precise location of stem cells in unaltered specimens. To develop new therapeutic approaches based on sensory epithelial cell regeneration, the location of these stem cells must be elucidated.

Stem cells normally proliferate at a slow rate in adult organs [3]. In fact, so-called label-retaining cells, or slow-cycling cells, of the brain and skin are recognized as stem cells [4]. In this study, using the exogenous proliferation marker, 5’-bromo-2’-deoxyuridine (BrdU) in combination with the endogenous proliferation marker Ki-67, we identified tympanic border cells. These cells, which are located beneath the basilar membrane in vivo, represent slow-cycling cells of the murine cochlea. Immunohistochemically, these cells stained positive for the immature cell marker Nestin. But it will be difficult to achieve regeneration of the cochlear function because these slow-cycling cells disappear in the mature murine cochlea.

Keywords: cochlea, slow-cycling cell, tympanic border cell

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A voltage sensitive dye assessment of electrical coupling in cell networks of the inner ear

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Gap junction channels formed primarily by connexin26 (Cx26) and connexin30 (Cx30) protein subunits couple non-sensory cells (supporting and epithelial cells) of the mammalian cochlea, forming vast functional syncytia. Mouse models confirmed that hearing acquisition relies not only on the functional maturation of hair cells, but also on differentiation and proper organization of gap junction-coupled non–sensory cell networks [1-3].

Objectives: To estimate quantitatively the degree of electrical coupling in non-sensory cell networks of the mouse cochlea.

Methods: We used a newly synthesized photo-induced electron transfer-based voltage sensitive dye, named Vf2.1.Cl [4], in combination with the patch clamp technique and a lock-in method to map the extent and the degree of electrical coupling in cell syncytia using fluorescence imaging.

Results: We examined electrical coupling in cochlear organotypic cultures from transgenic mice with hearing defects due to absence or mutation of Cx30. Despite the mild hearing loss phenotype of adult Cx30T5M/T5M mice [1], the high sensitivity of the technique permitted us to detect a small but statistically significant reduction of electrical coupling in postnantal organotypic cultures of the cochlea compared to wild type (WT) cultures. In addition, we examined cultures from Cx30−/− mice that, in the adult stage, present with profound deafness, lack of endocochlear potential and significantly decreased endolymphatic K+ levels [5]. These cultures showed a severe reduction of electrical coupling compared to WT mice. By comparing our experimental results with numerical simulations, we obtained an estimate of the average intercellular junctional conductance in cochlear non–sensory cells.

Conclusions: Due to its high sensitivity, our technique could potentially be applied in the study of other types of electrically-coupled cell networks, such as astrocytic and neuronal networks.

Keywords: Connexins; electrical coupling; non-sensory cells; voltage sensitive dye.

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Circadian rhythms in the auditory system

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More than 15% of the population has noise-induced hearing loss (NIHL) related to the exposure to loud noise at work or in leisure time and these numbers are predicted to increase within the two next decades. At present there is no effective treatment for this sensory deficit. How the hearing system responds to noise overexposure during different times of the day is unexplored area. Here we show that the murine cochlea possess self-sustained oscillations of the core circadian clock genes and proteins. In the cochlea, Period 2 (per2) is the most prominent, with clock gene expression showing high circadian amplitudes such as those found in the liver. Mice displayed permanent hearing loss when exposed to noise during the awake phase (night) whereas exposure during sleep phase (day) showed complete recovery. Noise overexposure during the sleep phase caused a phase shift in the expression of cochlear Period genes. It is likely that the mouse audio-clock described here will have similar properties in humans since the auditory and circadian systems in mammalian species are highly homologous. For this reason, our findings could have important implications with reference to the circadian variation in the potential for recovery from noise trauma that may play a role for people working in noisy environments, shift workers, flight crew that frequently travel across time zones and for those who recurrently visit night clubs.

The role of transmembrane channel-like proteins in hair cell mechanotransduction

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Transmembrane channel-like (Tmc) proteins comprise a family of eight isoforms, two of which, Tmc1 and Tmc2, occur in the inner ear and are central to hair cell transduction (Kawashima et al 2011). Mutations in Tmc1 cause deafness in humans, DFNA37 and DFNB7/11, and in mice, deafness (dn) and Beethoven (Steel & Bock, 1980; Kurima et al. 2002; Vreugde et al. 2002) but the functional defect is not well understood. We characterized mechanotransducer (MT) currents in response to hair bundle deflections in isolated cochleas of neonatal mice with null mutations in Tmc1 and Tmc2. Mice with the Tmc1 mutation 'dn' possessed MT currents in the first few neonatal days, but the current in outer hair cells (OHCs) declined after postnatal day (P) 6 (Kim & Fettiplace, 2013). The MT channels in 'dn' mutants up to P6 had an increased permeability to calcium and a reduced single-channel conductance, these effects being most prominent at the high frequency basal region. Recordings from Tmc1/Tmc2 double knockouts in P2 to P6 mice showed OHCs often exhibited MT currents but with anomalous properties. The current occurred on the opposite phase of hair bundle displacement and, although being blocked by FM1-43 and external calcium like wild type, was unaffected by exposure to sub-micromolar calcium buffered with BAPTA. The underlying channel also had significantly smaller permeability to calcium than wild type. OHC hair bundles of P6 double knockouts examined with scanning electron microscopy were abnormal in shape but still retained both tip links and horizontal lateral links. Exposure to BAPTA before fixation destroyed most of the tip links but left many of the side links intact. The results do not unequivocally establish Tmc proteins as pore-forming subunits of the MT channel. If they were, the anomalous reversed current may flow through a second type of mechano-sensitive channel present in the hair bundle, but it is possible that, in the absence of Tmc1 and Tmc2, the MT channel is not targeted to the stereociliary tips, which alters its properties. Acknowledgements: supported by RO1 DC01362 from NIDCD to RF and DRUK grant to DNF, CMH and RF.

Keywords: hair bundle, mechanotransducer channel, scanning electron microscopy, tip-links, Tmc1.

ORAL PRESENTATIONS O-10

Inner hair cell membranes in three dimensions: links between membranes, mitochondria and synaptic vesicles

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The ribbon synapse is a specialised structure that allows for rapid and sustained release of neurotransmitter from inner hair cells (IHCs). The ribbon body is anchored to the membrane near the synapse, and vesicles are tethered to it. Those vesicles on the side of the ribbon facing the synapse form the readily releasable pool, which can be quickly released at the onset of stimulus. This pool is then replenished from other vesicles tethered to the ribbon. However, how the vesicles tethered to the ribbon are themselves replenished is not understood. Previous work has suggested that the generation of synaptic vesicles may involve a membrane system linked to mitochondria, observed in the basal cytosol of the IHC. The aim of this work was to examine the three dimensional structure of this membrane system, to establish the potential roles it may play in neurotransmitter synthesis and transport. Serial block face scanning electron microscopy (SBF-SEM) was used to examine the membrane system in IHCs, and electron tomography was used for high-resolution characterisation of the membrane and its association with organelles. The membrane system was shown to be an asymmetric structure that traversed the cell from the apical region to the base. Density of the membrane varied laterally across the cell. Nearly every mitochondrion present in the infra-nuclear portion of the IHC was associated with these membranes. Electron tomography revealed links tethering the mitochondria to strands of membrane, and membrane-like links between vesicles at the synaptic ribbon. Links between mitochondria and synaptic vesicles and between vesicles and decorated membrane were also observed. These results show that an organised system of membrane and mitochondria traverses the IHC from the synthetic machinery at the apical end of the cell to its base. Parts of this structure have links to synaptic vesicles in the region of the ribbon synapse. Such a system is a strong candidate structure for the transport of neurotransmitter to these synapses.

Keywords: Inner hair cell, Membrane, Neurotransmitter, Ribbon synapse

ORAL PRESENTATIONS O-11

A nonsense mutation in *CLIC5* causes autosomal recessive sensorineural hearing impairment with vestibular dysfunction

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In a Turkish consanguineous family (W05-009) with autosomal recessive non-syndromic hearing impairment (arNSHI), homozygosity mapping revealed a 47 Mb homozygous region in chromosome 6p21.1-q15. This region includes the known arNSHI gene MYO6, however, no pathogenic variants were found in this gene neither with sequence analysis of the coding region and splice site boundaries nor with MYO6 mRNA analysis. The homozygous region contained another excellent candidate gene, *CLIC5*, and the orthologous mouse gene was described to be mutated in the jitterbug (jbg) mouse exhibiting congenital progressive hearing loss and vestibular dysfunction [1]. Sequence analysis of the *CLIC5* gene in family W05-009 revealed a homozygous nonsense mutation (c.96C>T; p.Cys32X; NM_016929_3), which segregated with the hearing loss in the family. This nonsense mutation may result either in the degradation of the mRNA by nonsense mediated decay or in a truncated protein. The c.96C>T variant was not present in 222 Turkish control alleles, the Exome Variant Server [2] and the Nijmegen in-house Mutation Database (1302 exomes). Screening of *CLIC5* in 55 Dutch arNSHI index patients with a comparable ski-slope audiogram configuration as seen in the affected subjects of family W05-009, did not reveal any putative causative variants. The hearing loss in the patients of family W05-009 is likely to be congenital and shows progression. Although motor development was normal, vestibular testing revealed vestibular areflexia at the ages of 16 and 11 years in the two affected sibs. Therefore, the phenotype of the patients closely resembles that seen in the jbg mice.

The *CLIC5* protein is a member of the chloride intracellular channel protein family. It localizes to stereocilia of both the cochlear and vestibular hair cells and also on the surface of Kolliker's organ during cochlea development in mice. The jbg mice have dysmorphic stereocilia and progressive hair cell degeneration. The *CLIC5* protein colocalizes with radixin in hair cell stereocilia and may help form or stabilize connections between the plasma membrane and the filamentous actin core [1].

Keywords: arNSHI, chloride intracellular channel, stereocilia

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Novel genetic tools to screen for deafness-causing mutations in the tandemly-duplicated region that contains the *STRC* (stereocilin) gene and its pseudogene

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Devising successful molecular diagnostic strategies for hereditary, nonsyndromic hearing impairment (NSHI) meets two major hurdles: the extraordinary genetic heterogeneity of this condition and the lack in most cases of distinctive clinical signs that provide clues about which genes may underlie NSHI in a given patient. Thus, current molecular diagnosis of NSHI is usually restricted to screening those genes or mutations most prevalent in each population. Usually, over half of patients with recessive NSHI remain undiagnosed after this screening, although this situation is bound to change when the use of next-generation sequencing techniques for diagnostic purposes becomes widespread.

We report here a novel protocol for the molecular screening of the *STRC* gene, encoding the hair-bundle protein stereocilin, at the DFNB16 locus. The 29-exon *STRC* gene lies on chromosome 15q15 within an 83-kb region, harbouring 4 genes, which is duplicated in tandem. The distal duplication contains a non-processed *STRC* pseudogene (ψ*STRC*) with 99.97% sequence identity to *STRC*, which has hampered molecular diagnosis so far. Critically, the existence of this highly similar non-processed *STRC* pseudogene prevents screening the region by current next-generation sequencing techniques, since any sequence variants detected cannot be assigned to either *STRC* or ψ*STRC* with certainty. Data gleaned from human structural variation databases suggest that deletions in this tandem-duplicated region are common.

We have developed and validated quantitative real-time PCR (qRT-PCR), amplification fragment length polymorphism (AFLP) and fluorescent restriction fragment length polymorphism (RFLP) assays to specifically detect *STRC* deletions. We have also developed a long-range PCR protocol to specifically amplify and sequence the *STRC* gene without interference of ψ*STRC*. By applying our novel protocol, we have detected biallelic *STRC* mutations in 5 familial and 3 simplex cases of NSHI. All affected individuals present with moderate hearing loss across all frequencies, with down-sloping or flat audiometric profiles. Our results indicate that the *STRC* gene should be screened in all cases of recessive NSHI who present with moderate hearing loss.

Keywords: *STRC*, DFNB16, moderate hearing impairment, molecular diagnostic methods

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Identification of rare variants in Mt-ND1 and LARS human genes in Meniere's disease suggest a two-hit model

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Familial Meniere's disease (FMD) is found in 8-10% of cases in European population (1,2). Although genetic heterogeneity is observed, most of the families have an autosomal dominant (AD) pattern of inheritance with incomplete penetrance. We have performed whole-exome sequencing (WES) in a family with three affected women in consecutive generations with anticipation.

DNA was isolated from peripheral blood samples of patients with MD and a healthy brother of the second generation. The libraries were prepared with the Agilent's All Exon 50MB capture kit (Agilent Tech) and WES was carried out in a SOLiD 5500xl platform (Life Technologies). Bioinformatics analyses were performed by using the Bioscope software and SAM tools, obtaining ~50.000 single nucleotide variants (SNV) per exome. Functional annotation software (ANNOVAR) and minor frequency allele (MAF) <0.05 were used to prioritize SNV according to the effect in protein structure and phylogenetic conservation. Criteria used for priorization were SIFT (Sort Intolerant from Tolerant), PolyPhen2 (Polymorphism Phenotyping v2), Graham's Matrix, GERP+ (Genomic Evolutionary Rate Profiling), Mutation taster, PhastCons y PhyloP.

We have identified and validated by Sanger sequencing SNV in Mt-ND1, FAM136A and LARS genes in all patients in this family. The variant in FAM136A leads to a stop codon not previously described which was not found in 1000 sporadic patients. The other two SNV are rare missense variants located in the Mt-ND1 gene (rs201212638, M31T) and LARS gene, encoding a Leucyl-tRNA Synthetase, (rs151245897, R457W), causing a misfolding of LARS protein. The variant in LARS gene was also found in another 2 unrelated patients with MD.

However, a single missense mutation in LARS gene probably cannot explain the differences in phenotype among offspring in the family. Thus, a deletereous mutation in mt-ND1 gene may be required to produce a complex I deficiency, increased mitophagy and neurodegeneration in MD. We suggest that a two-hit model in LARS and mt-ND1 with heteroplasmy can explain the incomplete penetrance observed in FMD. The functional impact of these variants in MD should be studied.

Keywords: Familial Meniere's disease, whole-exome sequencing andsingle nucleotide variants.

Acknowledgements: This study was funded by a PI10/00920 Grant from ISCIII and PI 242-2012 from CSBS in Andalucía.

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ORAL PRESENTATIONS O-14

Disturbance of axonal microtubules in the auditory nerve and outer hair cell degeneration accompanied by progressive hearing loss in the *Pmn/Pmn* mouse

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A detailed and profound knowledge about cochlear organization and function can be the basis for future therapeutic strategies for auditory dysfunctions. One approach to investigate the auditory system is the use of animal models with hearing defects like the progressive motor neuronopathy (*pmn/pmn*) mouse. It has been originally described as a model for a fast developing motoneuron disorder. A homozygous mutation in the TBCE gen leads to a dysfunctional co-factor of the tubulin-dimerization. This results in a disturbance of the microtubule assembly, which negatively influences the axonal function. Affected animals are characterized by a progressive axonal degeneration of peripheral nerves, beginning in postnatal week 3. Simultaneously, the animals develop a progressive hearing loss, which has been presented previously by ABR measurements [1].

In order to reveal the origin of this hearing loss, the animal was investigated by electrophysiological experiments including DPOAE and frequency-specific ABR. Additionally histological examinations of the cochlear structures and the auditory nerve including confocal microscopy and transmission electron microscopy were performed.

Electrophysiological measurements displayed elevated thresholds in ABR measurements from postnatal week 3 in *pmn/pmn* mice, accompanied by elevated DPOAE thresholds. Histological investigations revealed an age dependent loss of outer hair cells, but additional a massive degeneration of microtubules in normally myelinated auditory nerve fibres. The TBCE protein displayed a specific stain in cochlear outer hair cells and inner pillar cells.

These results imply that *pmn/pmn* mice represent a cochlear and neuronal type of hearing loss due to tubulin dysfunction. It may be interesting to further investigate the underlying interactions of tubulin for the correct function of the cochlea and the auditory pathway in the pathophysiology of hearing loss.

Keywords: Progressive motor neuronopathy, hearing loss, cochlea, auditory nerve, apoptosis, TBCE

Acknowledgements: This work has been sponsored by IZKF Würzburg, Grant N129

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Correction of hearing in the mouse model of hereditary deafness by *Gjb2* gene transfer using adeno-associated viral vectors

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A severe to profound genetic hearing loss affects approximately one in 1,600 children. Gene therapy may be a powerful technology, which fundamentally correct the disease phenotype of genetic deafness. However, gene replacement approaches for animal models of inherited deafness is extremely limited. A successful restoration of hearing mediated by gene replacement in the genetically created deaf mouse model of *Gjb2* contributes a tremendous and universal benefit on clinical application to fundamental therapy of human hereditary deafness. The ABR thresholds in $Cx26^{fl/fl}$P0-Cre mice reached around 100 dB sound pressure level (SPL) for click stimuli and ranged between 70 and 80 dB SPL for tone bursts, whereas control mice showed normal values for thresholds corresponding to those of wild type. The shift of the threshold in $Cx26^{fl/fl}$P0-Cre mice tended to be augmented from the low frequency area towards the high frequency area. A remarkable change of the collapse was recognized in the organ of Corti at P27 in the light microscope. An analysis of transmission electron microscopy showed the collapse of the tunnel of Corti, deformity of the cell shape in the supporting cells, and degeneration of hair cells in the organ of Corti. Developmental course of ABR thresholds was compared between $Cx26^{fl/fl}$P0-Cre and control mice. The onset of hearing in controls was recognized at P11to P12 and ABR thresholds almost reached the adult level at P18 to P20. In contrast, $Cx26^{fl/fl}$P0-Cre mice have never showed detectable ABR waveforms elicited by click stimuli throughout postnatal development, indicating the disturbance of auditory organ development. In order to restore deafness, an AAV vector incorporated a wild *Gjb2* gene was introduced to the cochlear perilymph through the round window membrane at P0. We measured ABR and examined the cochlear sections 65 to 80 days later. A significant improvement of ABR thresholds was observed together with a successful expression of Cx26 mediated by AAV in the supporting cells of the organ of Corti, spiral ligament fibrocytes and spiral limbus. Thus, our study will build the path to open a new era in the fundamental treatment for hereditary deafness.

Keywords: *Gjb2*, Cx26, knock-out mouse, gene therapy

ORAL PRESENTATIONS O-16

Developmental changes of epithelial Na^+ channel expression and function in the inner ear of pendrin knock-out mouse as a perspective of development of endolymphatic hydrops

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Objective: Mutation or null of pendrin (PDS) causes endolymphatic hydrops and sensorineural hearing loss. Endolymphatic hydrops develop at E14.5 in $PDS^{-/-}$ mouse; however, the mechanism of the development of hydrops is not clear. The change of Na^+ transport in the inner ear can be thought to be a candidate for the cause of endolymphatic hydrops in $PDS^{-/-}$ mouse, because Na^+ transport is a major driving force for fluid absorption and Na^+ is a main ion in the endolymph from embryonic days to early postnatal days. Furthermore, [Na^+] was reported to be increased in the endolymph of $PDS^{-/-}$ mice than $PDS^{+/+}$ mice. We tried to investigate changes of ENaC transcript expression and its function according to development of $PDS^{-/-}$ mouse.

Method: Real-time RT-PCR was performed to identify the changes of ENaC transcript expression level according to the development (P0, P6, P15, P56) of $PDS^{-/-}$ mouse. For evaluating the epithelial Na^+ transport in the inner ear epithelial cells, transepithelial current under short-circuit condition in the Reissner's membrane and saccular extramacular membrane which were reported to show ENaC-dependent current was measured with a vibrating probe.

Results: Transcript expressions of the β- and γ-ENaC in the cochlear and vestibular compartment of pendrin knock-out mice were increased from P6 and significantly up-regulated at P60, when compared to wild type. Amiloride-sensitive transepithelial current in the Reissner's membrane and saccular extramacular membrane was not different between knock-out and wild type mice at P0, but the current increased more than 3 folds in pendrin knock-out mice than wild type.

Conclusion: These findings indicate that failure of Na^+ absorption through ENaC during embryonic day to early postnatal day can be the cause of endolymphatic hydrops. In addition, transcript expression and function of ENaC were increased in pendrin knock-out mice after the development of endolymphatic hydrops as a compensatory mechanism for endolymphatic hydrops. This result provides insight into the role of Na^+ transport in the development and regulating endolymphatic hydrops in pendrin knock-out mouse.

Keywords: Pendrin, endolymphatic hydrops, ENaC

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Modulation of copper transporters in protection against cisplatin-induced cochlear hair cell damage

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Platinum agents are widely used in cancer chemotherapy. Ototoxicity is one of the limiting side-effects of platinum. For toxicity to occur platinum must first be transported into cochlear cells. Three copper transporters are considered pathways for regulating the uptake and translocation of cisplatin: Ctr1, ATP7A and ATP7B. Our recent study shows that cochlear hair cells can be destroyed by cisplatin at low concentrations from 10µM to 100µM. However, high doses of cisplatin cannot damage hair cells, maybe due to intrinsic feedback reactions that increase export of platinum by ATP7B. Cimitidine is a specific copper transporter inhibitor can block the entrance of copper and platinum. We treated cochlear cultures with cisplatin (10 µM or 50 µM), or cisplatin combined with cimitidine at concentrations ranging from 10-2000 µM for 48 hours. 10 µM cisplatin damaged about 20% hair cells, while additional cimitidine (10 µM, 100 µM and 2000 µM) protected near 100% cochlear hair cells. Higher concentration of cisplatin (50 µM) destroyed about 80% cochlear hair cells. However, 100 µM cimitidine rescued about 50% hair cells, and 2000µM cimitidine protected about 80% hair cells. Western blot data showed that CTR1 and ATP7B expressions were increased by cisplatin treatment, but cimitidine significantly depressed CTR1, ATP7B, and ATP7A. Considering that Ctr1 is involved in platinum influx, but the ATP7A and ATP7B are exporters, the results suggest that cimitidine can effectively block the entrance of copper transporters and stop the influx of cisplatin. Since the uptake of cisplatin through Ctr1 can be competitively inhibited by extracellular copper, copper sulfate (10, 50 or 100 µM) was also applied in cisplatin ototoxic model (10 µM) in culture system. Cisplatin induced hair cell loss was significantly protected by additional copper sulfate. Western blots showed that the expression of Ctr1 was greatly reduced while ATP7B was significantly increased by copper sulfate treatment. Local application of copper sulfate on round window membrane also efficiently provide significant protection against carboplatin-induced inner hair cell loss in chinchillas presumably by arising from decreased influx and increased efflux of platinum.

Keywords: cisplatin, copper trasporters, ototoxicity

ORAL PRESENTATIONS O-18

Effect in vivo of dimethyl sulphoxide (DMSO) in the ototoxicity by cisplatin, an animal model

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Objective: Dimethyl sulfoxide (DMSO) is a solvent often used to dissolve hydrophobic substances applied to the inner ear, however there is little information about its potencial ototoxic effects. Studies of DMSO effects on cochlear organotypic cultures showed a potenciated otoxicity when used together with cisplatin [1,2]. Our aim is to determine the effect of intratympanic DMSO on hearing function in vivo in the Wistar rat, alone and in combination with cisplatin, for comparison with in vitro models.

Methods: 11 Wistar rats were randomly assigned to 2 groups. One group received intratympanic DMSO 1% and a second group received intratympanic DMSO 1% in combination with intraperitoneal cisplatin (10mg/kg). Right ears were injected and left ears served as controls. ABR were performed before intratympanic injection and before sacrifice at day 5. Study cochleae were removed and processed for confocal laser scanning microscopy after phalloidin immunostaining.

Results: The results revealed that DMSO was not ototoxic by itself or in combination with cisplatin. Morphological studies showed a preservation of outer and inner hair cells in DMSO alone group, and structural changes and disappearance of some outer cells in animals treated with cisplatin and DMSO.

Conclusions: These findings show that intratympanic application of DMSO is not ototoxic, and the DMSO does not potentiate the ototoxic effect of Cisplatin when applied concomitantly in an *in* vivo model.

Keywords: Cisplatin; dimethyl sulphoxide; ototoxicity; confocal laser scanning microscopy

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Co-treatment with ototoxic nitriles and CYP2E1 inhibitors: a new mouse model for vestibular hair cell loss with limited systemic toxicity

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The ototoxic nitriles allylnitrile (ALN) and cis-crotononitrile (CCN) cause hair cell degeneration in the auditory and vestibular sensory epithelia of mice. Nevertheless, cyanide release from these nitriles through microsomal metabolism cause significant lethality. Since this metabolism is mostly dependent on activity of the 2E1 isoform of the cytochrome P450 (CYP2E1), in this study we co-administered mice with nitriles and selective CYP2E1 inhibitors, diallylsulfide (DAS) and trans-1,2-dichloroethylene (TDCE), to reduce the lethal effects. In both female 129S1/SvImJ (129S1) mice co-treated with DAS and CCN and male RjOrl:Swiss/CD-1 (Swiss) mice co-treated with TDCE and ALN, the nitrile caused a dose-dependent loss of vestibular function, as assessed by a specific behavioral test battery, and loss of hair cells, as assessed by hair bundle counts under scanning electron microscopy. In both experiments, CYP2E1 inhibitors provided significant protection against the lethal effect of the nitriles without diminishing the vestibular toxicity as assessed by behavioral effects in comparison to animals receiving no inhibitor. Furthermore, additional experiments using one single dose of ALN demonstrated that TDCE does not cause HC loss by its own and does not modify the vestibular toxicity of the nitrile in either male or female 129S1 mice. In all experiments, high vestibular rating scores in the behavioral test battery predicted extensive to complete loss of hair cells in the utricles. These results allow concluding that co-administration of nitriles with CYP2E1inhibitors is a suitable model for subsequent studies of vestibular hair cell regeneration or replacement.

Keywords: allylnitrile, cis-crotononitrile, hair cell ablation, mouse, ototoxicity, vestibular toxicity

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ORAL PRESENTATIONS O-20

Ubiquinone rescues auditory hair cells from the cisplatin-induced toxicity

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Objective: Previously, we have demonstrated that cytokines of the IL6-family protect against cisplatin ototoxicity. WP1066 (nonpeptide small-molecule inhibitor of STAT3-phophorylation on Tyr705) inhibited this protection, suggesting involvement of JAK2/STAT3 (see the poster of Elisabeth Gerschner). Here, prompted by its neuroregenerative potential described elswheree, we describe and elucidate the anti-ototoxic effect of ubiquinone (coenzyme Q10).

Methods: We have used in vitro explant cultures of the membranous cochleas dissected from the p3-p5 Wistar rats. The explants containing the organ of Corti, spiral limbus and spiral ganglion neurons were cultured for 24h with either medium plus mock control or with ubiquinone in nanodispersion (Sanomit ®) [50µg/ml]. After this, cisplatin [15µM] was added to the cultures for another 24h. In some experiments, WP1066 inhibitor [5.6µM] was also added. As a read-out served phalloidin staining and epi- or confocal microscopy combined with the hair cells scoring. In the additional experiments, cellular subfractionation and immunoblotting of PC12 cell line with STAT3 and STAT3-phospho-specific antibodies were performed. The data were statistically analyzed using ANOVA.

Results: Preincubation of explants with ubiquinone [50µg/ml] for 24h resulted in significant protection of auditory hair cells from the cisplatin-induced ototoxicity ($p<0,05$), as compared to cisplatin-only treated explants. Analysis of the PC12 lysates revealed that the addition of ubiquinone induces rapid STAT3 phosphorylation on serine 727 domain and its translocation into the mitochondria but not into the nucleus. In contrast to WP1066-abolished otoprotection induced by oncostatin M or IL6, WP1066 has not interfered with the ubiquinone-induced otoprotection, suggesting that ubiquinone-induced phosphorylation event is downstream of JAK2/STAT3 interactions.

Conclusions: Preincubation of cochlear explant tissues with ubiquinone (Sanomit ®) induces significant protection from the cisplatin ototoxicity. This novel protection seems to be related to STAT3 serine phosphorylation and translocation into the mitochondria. Ubiquinone may present a feasible option for otoprotection during chemotherapy with platinum products.

Keywords: Ototoxicity; otoprotection, ubiquinone; STAT3, WP1066

Acknowledgements: This work has been sponsored by Charité University Hospital funds

ORAL PRESENTATIONS O-21

Local delivery of brain-derived-neurotrophic factor on the perforated round window membrane: a possible clinical application

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Objective: Local delivery of neurotrophic factors on the intact round window of hair-cell deprived cochleas reduces degeneration of the cochlear nerve1. We investigated whether this otoprotective effect could be enhanced by perforation of the round-window membrane.

Materiasl and methods: Guinea pigs were deafened by co-administration of kanamycin and furosemide. Two weeks after deafening Gelfoam cubes infiltrated with a brain-derived neurotrophic factor (BDNF) solution were deposited onto the round window of the right cochlea. In the experimental condition the round-window membrane was perforated. Electrically evoked brainstem responses (eABRs) were recorded weekly. Two or four weeks after Gelfoam placement, both left (untreated) and right (BDNF-treated) cochleas were processed for histology.

Results: In BDNF-treated cochleas, both with and without perforation, neural survival in the basal turn of the cochlea was significantly larger than in untreated cochleas. Amplitudes of eABRs were larger in BDNF-treated cochleas with a round window perforation than in those without a perforation, and comparable to normal-hearing controls. Perforation did not lead to additional cochlear damage.

Conclusion: When considering application of neuroprotective agents as BDNF, delivery on a perforated round window membrane seems to be a safe and effective option.

Keywords: Brain derived neurotrophic factor, round window, gelfoam, guinea pig, neurodegeneration, auditory brainstem response

Acknowledgements: This work has been sponsored by MedEl corporation, Austria

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Aminoglycoside modulation of the acid sensing ion channels in the cochlear afferent neurons

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The ASICs (Acid Sensing Ion Channel) are members of the ENaC/DEG (*epitelial sodium channel/degenerin*) channel superfamily. There are four genes (*asic*1-4) that encodes for six subunits (ASIC1a/1b, ASIC2a/2b, ASIC3 and ASIC4). ASICs are activated by the increase in extracellular proton concentration. They have been involved in diverse neuronal functions, such as mechanotransduction, learning and memory, nociception and retinal function modulation (Chen and Wong, 2013). The ASIC2 knockout mice show a normal hearing threshold (Peng et al., 2004). However, the asic2 gene absence produces a hypersensibility to high level sounds damage. Moreover, ASIC3 knockout mice have a normal hearing at birth but eventually develop hearing loss (Hildebrand et al., 2004). Therefore, a better knowledge of the spiral ganglion neurons ASIC subunits is the first step to find the physiological role of these channels in the auditory system because they could be implicated in the mechanotransduction, mechanociception and a variety of processes involved in hearing.

In this work we studied the ASIC current of the spiral ganglion neurons (SGN) from mice cochlea. The pH dependency of the proton gated current was found to have a pH_{50} of 6.17. For that reason, in the subsequent experiments we used a pH 6.1 solution to activate the ASIC current. The pH dependence of steady state desensitization gave a pH_{50} 7.3 ± 0.03.

The aminoglycosides 100 µM streptomycin (St) (n = 10) and 100 µM neomycin (Neo) (n = 6) decreased the peak of the ASIC current in 38 ± 5 % and 26 ± 5 %, respectively. St slowed the desensitization in 840 ± 170 % and Neo in 137 ± 44 %.The integral of the current was increased by St in 46 ± 11% and by Neo in 42 ± 6 %. Neither changed the sustained current and all effects were reversible. The slowing of the desensitization and the increase in the sustained current lead to an increase of the integral of the inward current produced by extracellular acidification, as a consequence, these effects will increase the Na^+ entry probably causing a major depolarization therefore an over-excitation of the cochlear spiral ganglion neurons, which may contribute to the ototoxic effect of the aminoglycosides.

Keywords: ototoxicity, ionic current, afferent neuron, cochlea.

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Acute antioxidant response and apoptosis regulation in rats after noise exposure

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Continuous noise exposure is a common cause of sensorineural hearing loss. During the last decades, a considerable number of genetic and molecular studies have evidenced the central role of oxidative stress in the pathogenesis of noise-induced hearing loss (NIHL) [1]. In particular, reactive oxygen species (ROS) molecules are generated during normal metabolism and eliminated by cellular antioxidant defense mechanisms. However, intense noise causes an excessive production of ROS leading to oxidative stress on hair cells and subsequent cochlear damage as seen in NIHL [1]. In this regard, it is essential to elucidate the molecular mechanisms that underlie cochlear damage, among other things for its impact in the development of new therapeutic strategies for NIHL prevention and treatment. Our aim was to characterize the involvement of oxidative stress and apoptotic pathways in NIHL by evaluating the transcriptional levels of key genes using Real Time-PCR. Wistar rats were exposed to a continuous white noise at 115dB SPL for 4h during 4days to induce a permanent threshold shift (PTS). Prior to and 24h after noise overexposure, auditory brainstem responses (ABR) were evaluated [2]. Cochleas from each animal were micro-dissected and immediately frozen on dry ice. Total RNA extraction and cDNA synthesis from both control and noise-exposed cochlear tissues were performed in order to examine the expression level of genes related to oxidative stress and apoptosis. Overexposure to continuous noise caused hearing loss on animals as confirmed by auditory threshold shifts on ABR recordings. Real time-PCR analysis revealed an acute (24h) transcriptional response in the cochlear tissue characterized by an up-regulation of antioxidant enzyme genes (*Sod1*, *Cat* and *GPx1*) and disregulation of the apoptotic machinery, as shown by increased expression of anti-apoptotic (*Bcl-2*) and by diminished expression of pro-apoptotic (*Bax*) genes, respectively. This resulted in normal expression of *Casp3*. In summary, our findings suggest that the acute transcriptional response to intense noise trauma of cochlear tissue is characterized by changes in the expression of important antioxidant genes, all of which have been implicated in the regulation of oxidative stress-produced apoptosis [3, 4]. These results provide fundamental clues for the understanding of initial molecular steps in NIHL allowing novel therapeutic strategies.

Keywords: Apoptosis; noise-induced hearing loss; oxidative stress.

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Adenosine amine congener as a cochlear rescue agent: an update

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Introduction: Our previous studies have shown that Adenosine Amine Congener (ADAC), a selective A1 adenosine receptor agonist, can reduce noise- and cisplatin-induced cochlear injury and ameliorate hearing loss1,2. Here, we report a dose-dependent rescue effect of ADAC on noise-induced hearing loss (NIHL). We also demonstrate the time window for treatment after noise exposure and the pharmacokinetics of ADAC after systemic administration.

Methods: Increasing doses of ADAC (25-300 µg/kg) were administered intraperitoneally to Wistar rats (8-10 weeks old) after exposure to traumatic noise (8-16 kHz, 110dBSPL for 2 hours). ADAC was administered daily for five consecutive days, beginning six hours after the cessation of noise exposure. Hearing sensitivity was assessed using auditory brainstem responses (ABR; frequency range 4-28 kHz) measured before and 12 days after noise exposure. The optimal safe concentration of ADAC established in the dose-response study was administered 12, 24, 48 or 72 hours after traumatic noise exposure. The pharmacokinetic studies utilized reverse-phase HPLC to detect ADAC in plasma after systemic (intravenous) injection.

Results: In the control non-treated group, the average threshold shift across the frequencies tested was 34dB, with the largest shift of 40dB at 12 kHz. ADAC mitigated hearing loss at all doses tested. The most effective doses were 100 and 200 µg/kg providing 19dB and 17dB protection averaged across the frequencies (11-26dB range). ADAC (200 µg/kg) significantly reduced ABR threshold shifts at 12 and 24 hours after noise exposure (17dB and 16dB protection respectively, averaged across the measured frequencies). At 48 hours after noise exposure, the average protection was 8dB (not significant) and at 72 hours the threshold shifts were similar to non-treated animals. Pharmacokinetic studies demonstrated a short half-life of ADAC in plasma after intravenous administration (4.5 min) without detection of degradation products, suggesting that the compound was quickly taken up by tissues or protein bound. Fast tissue uptake was supported by a large Vd (the total volume of distribution) of ADAC in plasma.

Conclusion: Our data provide further support for the otoprotective effect of ADAC across a spectrum of conditions, but further studies are required to establish its translation as a clinical otological treatment.

Keywords: Adenosine amine congener; adenosine receptors; noise-induced hearing loss, otoprotection; pharmacokinetics

Acknowledgements: This work was sponsored by the Faculty Research Development Fund and UniServices (University of Auckland).

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ORAL PRESENTATIONS O-25

Autophagy is a defense mechanism against noise-induced hearing loss

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This study addresses the relationship between autophagy, reactive oxygen species (ROS) and noise-induced temporary or permanent auditory threshold shifts (TTS or PTS, respectively). Using pharmacological manipulations as well as siRNA silencing in CBA/J mice at the age of 3-months, we analyzed the autophagy marker microtubule-associated protein light chain 3 B (LC3B) and ROS marker 3-nitrotyrosine (3-NT) in outer hair cells (OHCs). LC3B increased 1 hour after either TTS- or PTS-noise exposure, with TTS exerting a significantly stronger effect than PTS. 3-NT was also elevated after either TTS- or PTS-noise, however, with PTS imposing a significantly greater elevation than TTS. Treatment with rapamycin significantly increased expression of LC3B, diminished 3-NT and prevented noise-induced PTS. In contrast, treatment with 3-methyladenine (3MA) resulted in down-regulation of LC3B expression and an increase in 3-NT in OHCs, intensifying the magnitude of both TTS and PTS. Silencing LC3B corroborated the effects of 3MA treatment, blocked rapamycin-induced elevation of LC3B, and impeded its protection against noise-induced hearing loss. In conclusion, our results suggest that autophagy plays a protective role in OHCs after noise exposure, partially through inhibition of ROS formation. Furthermore, rapamycin might be useful as a selective pharmacological agent against noise-induced hearing loss through, at least in part, the activation of autophagy.

Keywords: Autophagy; Oxidative stress; Noise-induced hearing loss.

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The early onset of oxidative stress processes in the organ of Corti after intense noise exposure: the role of membrane fluidity

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Introduction: It is well known that noise-induced hearing loss (NIHL) is due to damage of outer hair cells (OHCs) which are the source of active amplification in the cochlea. OHCs are more vulnerable to insult than inner hair cells (IHCs) to the acoustic trauma. The common basis for OHC loss by acoustic over-stimulation is triggered by the unbalance of cellular redox status due to reactive oxygen species (ROS) overload. As membrane structural organization is strongly affected by ROS, OHC electromotility may be altered by increased metabolic activity. In this issue, we investigated in OHC different functional zones the mechanisms linking metabolic functional state: NAD(P)H (its spatial distribution and time evolution), lipidic peroxidation (4HNE staining) and the membrane fluidity examination (Laurdan staining). These analyses were carried out in the middle/basal turn inner, middle and outer OHC different rows

Material and methods: The guinea pigs were used as model of acute acoustic trauma (6kHz, 1h, 120 dB). Functional, morphological and immunohistochemical changes in OHC during the first 48 hours after noise exposure were studied. Two-photon microscopy was applied to study the NAD(P)H fluorescence as a measure of superoxide production and membrane stiffness in OHCs in a living explanted organ of Corti preparations.

Results: One hour after noise exposure the threshold shift was elevated to about 40-50 dB and the hearing loss level and its recovery was paralleled by a rapid ROS production in OHCs. NADPH oxidation showed a different distribution related to the different vulnerability of noise induced damage of OHCs and activation of endogenous mechanisms of protection. Furthermore, the acoustic trauma induced both a loss of fluidity polarization of the plasma membrane.

Conclusion: In control animals a functional relation is established between membrane fluidity and NAD(P)H distribution. After noise exposure, the time course of NAD(P)H oxidation, lipid peroxidation and membrane fluidity indicate that a perturbation of OHC metabolism triggers lipid peroxidation, which impairs OHC cell function through membrane fluidity loss. On the whole, membrane fluidity is regulated by NAD(P)H redox state and lipid peroxidation which represent therefore key targets for a therapeutic rescuing plan from noise insults.

Hyperacusis and tinnitus studies in the aging rat

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Progressing loss of auditory sensation is a major problem of aging populations. In humans, loss of hearing function can be seen through increased thresholds, altered sound processing of temporally and spatially modulated auditory stimuli, but also through abnormal perception of above-threshold sounds or phantom perceptions, like hyperacusis and tinnitus (Rüttiger et al., 2006; Zuccotti et al., 2012; Kujawa and Liberman., 2006; Rüttiger et al., 2003).

The age related loss of synaptic structures, afferent synaptic contacts, and auditory fibers and the impact on the hearing sensation, was studied in an animal model on young and aged rats. Hyperacusis and tinnitus sensation were tested using a behavioral approach (Rüttiger et al. 2003, Hearing Res), and hearing function was studied on auditory evoked brainstem responses (ABR) and otoacoustic emissions (DPOAE). Above-threshold responses to click and frequency specific stimuli were analysed in detail for recruitment and latencies of ABR wave deflections (wave amplitudes and latencies), to gain insight into the central brainstem function. Results from behavior studies on young and aged rats, before and after auditory overstimulation, are presented in correlation with individual hearing functions and morphological specifications of hair cell molecular phenotype specified on the level of behavior studies for tinnitus and hyperacusis.

Keywords: age-related hearing loss, ribbon synapses, temporary threshold shift, waveform analysis

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ORAL PRESENTATIONS O-28

C-RAF deficiency causes cochlear abnormalities and profound sensorineural deafness in the mouse

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Insulin-like growth factor I (IGF-I) is fundamental for neurogenesis and neuronal differentiation during inner ear development. IGF- I deficiency is associated with deafness in man and mice. IGF-I binds to its high affinity receptor and activates downstream signaling as the Ras-RAF-MEK-ERK pathway. RAF kinases are essential for cell proliferation, survival and differentiation during development and in the adult tissues homeostasis. RAF proteins have redundant but also specific cellular and tissular functions. In the developing chicken inner ear the activation of C-RAF and B-RAF are critical for otic neurogenesis. To further study the role of RAF kinases in the auditory receptor, we have analyzed C-RAF mRNA and protein expression patterns in the mouse inner ear along development. Our results show that C-RAF is differentially expressed and that the Ras-RAF-MEK-ERK pathway is active. To explore its functional relevance we have studied the phenotype of the *C-RAF*$^{-/-}$ null mouse. *C-RAF*$^{-/-}$ mutants present an all-frequency profound sensorineural hearing loss with a mean auditory threshold of 90 dB SPL. The study of the general cochlear cytoarchitecture indicates that the main structures and cell types have been formed, although the expression of proteins essential for hearing is altered. Thus the levels of the Kir4.1 potassium channel in the stria vascularis are reduced in the *C-RAF*$^{-/-}$ null when compared to the wild type littermates. In addition, noise-exposure experiments shows that *C-RAF*$^{+/-}$ presented a greater susceptibility to noise damage, with higher threshold shifts and worst recovery compared to noise-exposed *C-RAF*$^{+/+}$ mice. Accordingly, *C-RAF*$^{+/-}$ noise-exposed mice showed morphological defects in the cochlea. In summary, these results show that C-RAF is expressed in the developing cochlea and that its activity is essential for correct hearing under normal conditions and for protection from exposure to noise.

Keywords: C-RAF, IGF-I, hearing loss, noise

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Auditory threshold estimation using short-pulse DPOAE input-output functions

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Distortion product otoacoustic emissions (DPOAEs)are generated by the nonlinear cochlear amplification processin response to two tones of frequencies f1 and f2. This results in so-called primary- and secondary-source components. To date, objective assessment of the state of the cochlear amplifier by means of DPOAEs is limited by interference between these two components. Recently, we showed thatpresenting a pulsed f2 tone leads to successful quantification of the primary-source component (Vetešníket al., 2009) and increases the accuracy of estimated auditory thresholds using semi-logarithmic input-output (I/O) functions (Dalhoff et al., 2013). Here, we present a refined method using short-pulse DPOAEs enabling both the reliable acquisition of I/O-functions and the quantification of both components in the time domain at low cost of measurement time and independent of interference type.

DPOAEs were acquired from four healthy subjects with distinctive fine structurein the frequency region f2 = 1.7–2kHz and for six primary tone levels with L2= 25–65 dB SPL. The ratio f2/f1 was kept constant at 1.2. For the investigated frequency range, the primary-source component attains its steady state approximately 8–10 ms after onset of the f2 tone, while the secondary-source component mainly occurs 8–10 ms later (Vetešníket al., 2009). Therefore, presenting a short-pulsed f2 tone of 8 ms lengthenables extraction of the primary-source component and the computation of I/O-functions without interference from the secondary-source component.

I/O-functions utilizing the extracted primary-source component from short-pulse measurements yield reliable auditory threshold estimation with similar standard deviation compared to pulsed DPOAE while decreasing the measurement time by approximately 80%. Application of objective evaluation criteria (Boege and Janssen, 2002) to the acquired I/O-functions leads to an exclusion rate of 15% when using continuous DPOAE while none of the I/O-functions acquired with short-pulse DPOAEs needs to be neglected.

In conclusion, short-pulse DPOAEs present a new timesaving approach to acquire I/O-functions allowing reliable and accurate auditory threshold estimation. Furthermore, additional information about the state of the cochlear amplifier may be obtained by extraction of both source components.

Keywords: DPOAE, input-output functions, auditory threshold estimation, cochlear amplifier.

ORAL PRESENTATIONS O-30

Video head impulse test may represent a method for high frequency vestibular function and compensation phenomena assessment

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Recently, the introduction of the video head impulse test (vHIT) has broadened the concept of semicircular canals' function assessment. vHIT evaluates the vestibulo-ocular reflex, which pursuits the stability of the visual field in response to head movements. By means of goggles equipped with a high velocity camera and an accelerometer, the compensatory eye movement that arises in response to a head rotation can be thoroughly observed.

While caloric testing relies on extremely low frequency/velocity stimuli (0,002 Hz) for measuring only the horizontal semicircular canal activity, vHIT is able – within few minutes, and without inducing any discomfort – to assess the function of each of the six semicircular canals individually, in response to high frequency/velocity head movements (5-7 Hz, more similar to everyday head rotations).

We present our initial experience with vHIT for the horizontal semicircular canal. 124 patients consulting for dizziness and vertigo were examined with vHIT and caloric testing. Considering calorics as gold standard, vHIT presented good correlation (rho=0,85; p<0,001), a specificity of 94% and a sensitivity of 77%.

Interestingly, every supposedly "false positive" patient reported active vestibular symptoms at the moment of examination. We hypothesize that these were actually "true positive" cases for vestibular dysfunction.

On the other hand, most of "false negative" patients reported having symptoms weeks ago, but were already recovered when tested. In light of these results, we believe that vHIT assesses a vestibular response to high frequency stimuli that may be highly related to the presence of vestibular symptoms. We hypothesize that compensation processes may focus on this "high frequency function" (in the range of everyday head movements), while an isolated low frequency dysfunction, as assessed by caloric testing, may suggest an already compensated previous vestibular damage.

Furthermore, we performed vHIT daily since symptoms' onset on a subgroup of hospitalized patients, where we registered signs (behavior and latency changes of refixation "catch-up" saccades patterns during the first week) that may represent early central compensation phenomena.

Although these ideas require further research, vHIT appears to be an important complement for vestibular function testing. Moreover, catch-up saccades behavior could represent a measurable phenomenon for central compensation study.

Keywords: Vestibulo-ocular reflex, Vestibular functional tests

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Visualization of internal structures in the vestibule and the semicircular canals using optical coherence tomography (OCT)

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The inner ear has two compartments; the membranous labyrinth and the perilymphatic space. Morphological change in these compartments deteriorates inner ear functions. To visualize these structural changes, it has been necessary to process the tissue into sections. OCT is an imaging modality that provides a series of 'optical sections' of biological tissue without sectioning. It utilizes near-infrared light, which propagates in the biological tissue. Researchers including us have shown that structures in the cochlea of rodents are well visualized using OCT. In the current study, we tried to visualize structures in the vestibule and the semicircular canals (SSCs). Inner ears of CBA/N mice at the age of 7 months are removed, fixed and decarcified using 10% ethylenediaminetetraacetic acid. Each specimen was placed on the imaging stage under the objective lens of the OCS1300SS OCT imaging system (Thorlabs, New Jersy, USA). The system was equipped with the light source with a central wavelength of 1325 nm and the axial resolution of 9 µm. Then optical sectional images were obtained. The image sets were post-processed using ImageJ and other imaging softwares. In addition to the cochlea, membranous labyrinth, nerves, maculae and cristae were clearly visualized in the saccule, the utricle and the SSCs. This suggests the use of OCT as an evaluation tool for the endolymphatic hydrops in the vestibule and the SSCs.

Keywords: Endolymphatic hydrops, Optical coherence tomography (OCT), optical section, Saccule, Semicircular canal (SSC), Utricle

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Probing auditory nerve fiber loss using round-window neural noise

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Recent studies have shown that a selective degeneration (up to 50%) of auditory nerve fibers (ANFs) may coexist with normal auditory brainstem thresholds [1, 2, 3], making the detection of auditory neuropathies difficult. Here, we described a robust and minimally invasive method to probe the degree of ANF loss in gerbil. Spontaneous and sound-evoked neural noise were recorded through an electrode placed onto the round window niche. The difference between the power spectral density of spontaneous and sound-evoked neural noise enable to probe the sound-driven activity of the ANFs. From 2 to 32 kHz, the amplitude of the ANFs sound-driven activity matches the bimodal histogram of the ribbon synapse distribution (indicated by the double immunostaining of synaptic ribbons and glutamate receptor clusters with CtBP2 and GluR2, respectively). In animals treated with ouabain, known to induce selective ANFs loss [4], the ANFs sound-driven activity became uni-modal (i.e., reduction of the response above 8 kHz), and thus predicting a partial deletion of basal ANFs. Accordingly, confocal microscopy revealed ribbon-synapse deletion in the basal region above 8 kHz. These results suggest that the cochlear neural noise can be a faithful proxy to quantify the degree of ribbon-synapse loss and could be translated into clinic to phenotype human neuropathies.

Keywords: Neuropathy, cochlear neural noise, auditory nerve fibers, gerbil

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Intratympanic administration of triamcinolone-acetonide: in-vivo release and proof of oto-compatibility

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To reach therapeutically relevant drug levels in the perilymph of the inner ear intratympanic drug-administration onto the round window membrane is an interesting route for drug delivery. For that purpose thermoreversible hydrogels being a fluid at room temperature and semi solid at 37°C [1] seem to be appropriate formulations. In addition, the semi-solid delivery system might prolong the contact time with the membrane leading to improved drug release into the inner ear.

To approach this aim, rheological studies were carried out to simulate the shear forces occurring during intratympanic injection and their effect on subsequent formation of the hydrogel structure at 37°C. For in-vivo drug monitoring, Poloxamer407 (POX) based hydrogels containing Triamcinolone-acetonide (TAAc) were intratympanally administered in guinea pigs. After incubation for 24, 72, and 240 hours samples of the perilymph, the liquor and the plasma were collected and quantified by HPLC-MS [2]. Additionally, to estimate ototoxicity of TAAc, incubated pullas were resected and fixed for 48 hours. Subsequently, the organ of corti was isolated and the hair cells were stained for microscopic visualization [3]. A defined section was counted and the results were compared with the control group without exposition to TAAc.

The rheological-studies confirmed that shear forces affected before sol/gel transition exert no influence on the gelation properties of the hydrogel at 37°C. TAAc-screening of the perilymph-samples revealed highest levels after 24 hours. Nevertheless, therapeutically relevant concentrations of TAAc were observed even after 240 hours. Liquor and plasma samples contained lower drug amounts than those of the perilymph. Upon counting the hair cells after TAAc-exposition, significant differences ($p<0.5$) to the control group were not detected excluding any ototoxic effects of TAAc at the applied drug levels.

All in all, intratympanic injection of TAAc-loaded thermoreversible hydrogels facilitates drug-administration onto the round window membrane. In-vivo, obviously the prolonged release of TAAc from the POX-hydrogel led to therapeutically relevant drug levels in the perilymph. Considering the otoprotective effect of the applied amounts of TAAc as well, intratympanal administration of corticoid-loaded thermoreversible hydrogels is a promising approach for local therapy of inner ear diseases and after cochlea implantation.

Keywords: drug delivery, in-vivo release, ototoxicity, rheological characteristics

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Targeting the somatostatin receptors as a therapeutic approach for the preservation and protection of the mammalian cochlea from excitotoxicity

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Somatostatin is a modulator of neurotransmission in the CNS, it acts by binding to G-protein coupled receptors (SSTR1-5) on target cells. We analyzed the expression of SSTR1-SSTR5 in the mammalian cochlea. The peak in the expression of SSTR1 and SSTR2 at mRNA and protein level is around the onset of hearing at postnatal day 14. We demonstrated that all five receptors are expressed in inner hair cells and outer hair cells as well as in defined supporting cells in the organ of Corti of the adult mouse cochlea. A similar expression of SSTRs was found in cultivated mouse organ of Corti explants as well as in neuroepithelial cell culture.

To investigate the regulation of SSTRs, we used mice with either a deletion of SSTR1 or SSTR1/SSTR2. In SSTR1 knockout (KO) mice we found an upregulation of SSTR2. In double KO mice, SSTR5 was up-regulated and SSTR3 and SSTR4 were down regulated. These findings provide evidence of a compensatory regulation as a consequence of a receptor deletion. Also, we observed reduced levels of phospho-Akt and total-Akt in SSTR1 KO and DKO mice as compared to wild type mice. Akt is likely to be involved in hair cell survival. Most importantly, we found improved hair cell survival in somatostatin and octreotide (somatostatin analogue) treated organ of Corti explants that had been exposed to gentamicin compared to explants exposed to gentamicin alone. Finally we demonstrated that organ of Corti explants from SSTR1 mice displayed less hair cell loss after gentamicin exposure compared to wild type mice or SSTR1/SSTR2 double KO mice. These findings propose that the somatostatinergic system has neuroprotective properties in the cochlea.

Keywords: gentamicin, hair cells, octreotide, organ of Corti, somatostatin, somatostatin receptors

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Function and regulation of Hey1 and Hey2 in cochlea development

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Auditory mechano-sensory hair cells (HCs) are critical for our ability to detect sound. In mammals, auditory HCs are arranged in rows, with one row of inner HCs (IHCs) and three rows of outer HCs (OHCs) spanning the length of the inner ear cochlea. In mice, HC differentiation initiates at E14.0 at the cochlea base and progresses over a 4-day period towards the cochlea apex. Despite the importance for proper HC patterning, little is known about the underlying mechanisms controlling the onset of HC differentiation and its basal to apical progression. In this study, we investigate the involvement of Hey1 and Hey2 in both of these processes.

The transcriptional repressors, Hey1 and Hey2 are highly expressed in undifferentiated cochlea sensory progenitors, and are rapidly down regulated as these sensory progenitors differentiate into HCs [1];. Based on their known function as HC fate repressors [2]; we hypothesized that Hey1 and Hey2 block premature HC differentiation. Our analysis of the Hey1/Hey2 double knockout mice reveals that in the Hey1/Hey2 cochlea HC differentiation occurs prematurely. Further, HCs in the Hey1/Hey2 mutant cochlea are miss-patterned along the basal to apical axis. There is a significant overproduction of OHCs in the basal regions of the cochlea duct, while the apical region shows a significant deficit in OHC production, with only two rows of OHCs.

Hey1 and Hey2 have been commonly described as Notch effectors. However, recent studies have shown that Hey2 expression can be maintained by Notch independent mechanisms [3];. We hypothesized that Shh signaling, which has been recently shown to oppose HC differentiation [4];, maintains Hey1/Hey2 expression in prosensory progenitor cells, preventing premature onset of HC differentiation. To test if Shh positively regulates Hey2 and/or Hey1, we analyzed changes in gene-expression in response to Shh pathway activation or inhibition. Our results indicate that Shh positively regulates Hey1 and Hey2 expression at the prosensory stage. Moreover, cochlea explants treated with ectopic Shh showed a delay in HC differentiation, while in those treated with the Shh antagonist, HC differentiation occurred earlier. Based on these findings we hypothesize that Shh pathway negatively controls HC differentiation through a Hey1/Hey2 dependent mechanism.

Keywords: Hair cells, development, differentiation, Hey1/Hey2, Sonic hedgehog (Shh).

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Effect of IGF-1 for spiral ganglion afferent dendrite and synapse regeneration

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Recenly, roles of IGF-1 on the development and maintenance of the auditory systems have been gained considerable attention. We have recently reported a protective effect of IGF-1 on cochlear hair cells and its intracellular signaling. The present study aimed to examine therapeutic effects of IGF-1 on spiral ganglion neurons, focusing on regeneration of synaptic contacts with inner hair cells.

Cochlear explants obtained from P2-3 mice were used in this study. We cut down the second turn of cochleae including the modiolus, and maintained as a slice in the culture media. Degeneration of afferent dendrites between spiral ganglion neurons and inner hair cells was induced by application NMDA and kainite to the media. Firstly, we evaluated toxic effects of NMDA and kainite, and determined the doses at which remarkable degeneration of afferent dendrites was induced, while spiral ganglion neurons were maintained. We then assessed therapeutic effects of IGF-1 for afferent dendrites of spiral ganglion neurons. After intoxication by NMDA and kainite, IGF-1 was applied to the culture media at different concentrations. We then quantified the numbers of afferent dendrites attaching to inner hair cells, those of pre- and post- synaptic vesicles using immnostaining for neurofilament, myosin VIIa, CTBP2 and PSD95. As results, IGF-1 exhibited dose-dependent effects on an increase of numbers of afferent dendrites attaching to inner hair cells, pre-and post-synaptic vesicles. Such therapeutic effects of IGF-1 on spiral ganglion neurons were eliminated by application of an IGF signaling pathway inhibitors, MEK/ERK inhibitor or PI3K/Akt inhibitor.

In conclusions, present results indicate that IGF-1 has an effect for restoration of afferent dendrites of spiral ganglion neurons and synaptic contacts between spiral ganglion neurons and inner hair cells.

Keywords: Afferent dendrites; IGF-1; regeneration, spiral ganglion neuron; synapse.

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Transcriptional regulation of the *Pou4f3* gene

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The transcription factor POU4F3 plays an essential role in the development and survival of the hair cells (HCs) of the inner ear. While HCs are present prenatally in POU4F3-null mice, they do not develop the apical specializations required for transduction, lack other features characteristic of this cell type, and die soon after birth. We evaluated the regulation of the *Pou4f3* gene by 8.5 kb of genomic DNA 5' to the ATG, which is sufficient to direct GFP expression to HCs in transgenic mice. Homology analysis identified three regions that are highly conserved across four widely-spaced mammalian species: 0 to -400 bp, -1630 to -1700, and –8150 to -8450 5' from the *Pou4f3* ATG of the mouse.

Successive deletion analysis in transgenic mice revealed that sequence from 7.0 to 8.5 kB 5' to the *Pou4f3* ATG is required to target gene expression to HCs. Moreover, removal of the sequence from -400 to -6000 kB 5' to the ATG demonstrated that the remaining DNA was sufficient to drive HC gene expression. Finally, we substituted proximal promoter sequence from the *Ela1* gene for the proximal sequence 0 to -400 prior to the *Pou4f3* ATG. In these transgenic mice, GFP expression was restricted to HCs, but the level was dramatically reduced from that seen with the native *Pou4f3* gene proximal sequence. Taken together, our results suggest that HC targeting is mediated by *Pou4f3* gene distal enhancers located several thousand bp 5' to the transcription start site, while the degree of gene expression is regulated by proximal promoter sequences.

The highly conserved regions of the *Pou4f3* gene contain motifs to which Atoh1 can bind, and chromatin immunoprecipitation confirmed this binding. Additional transcription factors with conserved binding sites were evaluated for their ability to enhance Atoh1-induced expression of the reporter gene and/or myosin-VIIA by nonsensory cells of the neonatal cochlear sensory epithelium. Four transcription factors that enhanced the number of cells co-expressing both the reporter and myosin-VIIA were identified, while two factors proved to be inhibitory. This is consistent with combinatorial regulation of both *Pou4f3* gene expression and adoption of the HC phenotype by a common transcriptional control mechanism.

Keywords: Atoh1, bioinformatics, hair cells, *Pou4f3* gene, promoter, transcription factors

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Translational research of cell-based therapy for restoration of spiral ganglion neurons

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Objectives: Previous studies have demonstrated that a transplantation approach using pluripotent stem cells has the potential of functional restoration of spiral ganglion neurons. To realize clinical application of cell-based therapy for degeneration of spiral ganglion neurons, we should overcome several problems associated with a source of transplants, methods for preparation, surgical procedures. The safety and stable outcomes are included in critical issues for clinical application. In the current study, we examined the feasibility of transplantation of human iPS cell-derived neurons into the guinea pig cochlea.

Methods: We used terminally differentiated neurons derived from human iPS cells as transplants, because use of terminally differentiated neurons could reduce a risk for tumorigenesis. Instead of an injection of transplants into the cochlear modiolus, placement of a collagen matrix on which human iPS-derived neurons were cultured in the scala tympani of the basal turn of cochleae was used as a transplantation method. The fate of transplanted cells was assessed by immunohistochemistry.

Results: Human iPS cells cultured on a collagen matrix were differentiated neurons expressing beta III-tubulin, and elongated neurites on collagen fibers. One week after transplantation, the survival of transplanted cells were found in guinea pig cochleae, when appropriate treatment for immune suppression was applied. Transplanted cells were localized on collagen fibers and positive for betaIII-tubulin. Some of transplanted cells projected neurites to host spiral ganglion neurons. When immune suppression treatment was insufficient, a number of inflammatory cells infiltrated into the cochlea, and virtually no survival of transplanted cells was observed.

Conclusions: The present results demonstrated the feasibility of transplantation of terminally differentiated neurons derived from human iPS cells into guinea pig cochleae, and that appropriate immune-suppression treatment is necessary for xenograft.

Keywords: Cell therapy; translational research; iPS cells; xenograft; auditory nerve.

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Electrophysiological properties of stem-cell derived sensory neurons

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Objectives: In severe cases of sensorineural hearing loss where the numbers of auditory neurons are significantly depleted, stem cell-derived neurons may provide a potential source of replacement cells. The success of such a therapy relies, among other things, upon producing an appropriate population of physiologically functional neurons from stem cells, in order to relay the precise sound information to the brainstem. The primary auditory neurons of the cochlea are glutamatergic and capable of firing at high rates [1,2], with much higher rates observed in response to electrical stimulation [2,3]. This raises important considerations if stem cell-derived neurons are to be used in a cell replacement therapy, including: are stem cell-derived neurons electrically active; do they possess an appropriate compliment of ion channels; can they faithfully transmit electrical signals to the target neurons in the brain; and will they be able to respond to the high stimulus rate provided by the cochlear implant?

Methods: Using our published differentiation assay to produce sensory neurons from human stem cells [4], whole-cell patch-clamp recordings were made from cells displaying a bipolar neuronal morphology (n=78).

Results: Recorded cells did not fire spontaneously, but were all capable of generating an action potential in response to membrane depolarisation. Stem cell-derived neurons were observed to entrain reliably to stimuli up to 20 pulses per second (pps), with 50% entrainment (0.5 probability of firing) at 50 pps. General firing properties were considered relative to the period of time in culture. Recordings were made between 31 and 58 days *in vitro* (DIV), and grouped into the following clusters on the basis of mean time in culture; 31 DIV (n=10), 35 DIV (n=29), 42 DIV (n=18), 48 DIV (n=15), and 55 DIV (n=6). There was no significant difference in resting membrane potential, threshold or firing latency between groups.

Conclusion: Stem cell-derived neurons did not entrain to high stimulation rates as effectively as cultured mammalian auditory neurons [5], however their electrical phenotype is stable in culture and consistent with that reported for embryonic (day 15) auditory neurons *in situ* [6].

Keywords: Stem cells, deafness, patch-clamp recording

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Derivation of otic progenitor and inner ear cells from normal or pathological human induced pluripotent stem cells for modeling hereditary hearing loss

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Alström Syndrome (AS) is a human, autosomal recessive, genetic disorder characterized by numerous clinical symptoms including deafness. AS is caused by mutations in the ALMS1 gene. ALMS1 protein is located in the basal body and implicated in cell polarity, microtubules arrangement and intracellular. Knowing that ALMS1 is express in the inner ear [1], we are interested in understanding the cellular mechanisms involving this protein in the genetic deafness in AS patients.

To develop a model closer to the human pathology, in our project we will use human induced pluripotent stem cells (hiPSCs). As the embryonic stem cells, these hiPSCs have two essential properties: pluripotence and self-renewal. In 2006, Yamanaka's team discovered that somatic cells, like fibroblasts, can be reprogrammed into an undifferentiated state [2]. These fibroblasts can be infected with lentiviral particles containing the genes OCT4, SOX2, KLF4 and cMYC. Once expressed, the fibroblast will progressively pass from a differentiated to an undifferentiated state. This reprogramming technique is currently used in our lab to generate hiPSCs from healthy patients. hiPSCs can then be differentiated in vitro into any cell type of the body.

Recently it was demonstrated that mouse embryonic stem cells can be directed to an otic fate [3], therefore we hypothesize that hiPSCs can also be directed to an otic fate. Using a stepwise protocol, we demonstrate that healthy hiPSCs can generate a population of cells with a gene and protein expression profile consistent to the ones of otic progenitor cells. Ongoing studies aim to adapt the growth factors and signaling molecules added to the cell culture medium in order to optimize the differentiation of pluripotent stem cell types into inner ear cells.

The application of the differentiation protocol to hiPSCs generated from AS patients will furthermore create an avenue to other studies that envisage the understanding of the AS pathological symptoms. For example, it will be interesting to study ALMS1 function in cell polarity, microtubules arrangement and intracellular trafficking in the in vitro generated inner ear cells.

Keywords: inner ear, otic progenitors, human induced pluripotent stem cells, genetic deafness, in vitro differentiation, ALMS1.

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POSTER PRESENTATIONS

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IGF-I deficiency causes upregulation of glutamatergic neurotranmission in the mouse cochlear nuclei

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Insulin-like growth factor 1 (IGF-1) is an activity-dependent peptide that plays an important role in the development and maturation of the nervous system, and modulates synaptic plasticity and neuronal function in the adult brain. Mice lacking the *Igf1* gene suffer from hearing loss, increases in their auditory thresholds and latencies, abnormalities in stria vascularis and the cochlear innervation as well as significant decreases in the number and size of spiral ganglion neurons. An issue that remains unknown however, is whether these cochlear abnormalities in IGF-1 deficient mice may result in an imbalance between excitation and inhibition in the cochlear nuclei, the first central relay station in the central auditory system. Accordingly, the expression and distribution of the vesicular glutamate transporter 1 (VGLUT1) and the vesicular inhibitory transporter (VGAT), specific markers for labeling excitatory and inhibitory terminals, were examined in the cochlear nuclei of a 4-month-old mouse model of IGF-I deficiency and neurosensorial deafness (*Igf1*$^{-/-}$ homozygous null mice) in comparison with *Igf1*$^{+/-}$ heterozygous and *Igf1*$^{+/+}$ wild type animals. The results demonstrate significant increases in the overall mean gray levels and the immunostained area of VGLUT1 but not VGAT immunostaining in the cochlear nuclei of *Igf1*$^{-/-}$ when compared to *Igf1*$^{+/-}$ and *Igf1*$^{+/+}$ animals. In conclusion, these findings provide evidence of an upregulation of the glutamatergic neurotransmitter system in the cochlear nuclei of IGF-1 null mice that may reflect a compensatory synaptic mechanism due to an IGF-1 deficient cochlea.

Keywords: infarction, ischemia, vascular damage, oxidative damage.

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Polyphenols effect on oxidative stress and apoptosis in cochleas of experimental animal associated with age

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Introduction: Under normal conditions, the consumption of oxygen by cells under aerobic metabolism is capable of generating different reactive oxygen species (ROS, Reactive Oxygen Species) and/or nitrogen (RNS, Reactive Nitrogen Species), which normally shall be eliminated by various enzymes and physiological antioxidants. A possible decoupling of the production and disposal of these ROS / RNS leads to a condition known as oxidative/nitrosative stress, which involves the consequent cellular damage. Oxidative/nitrosative stress also seems to increase with age, suggesting as one of the main factors causing cell damage and increased apoptosis in many aged tissues.

Objective: determinate the antioxidant effects of polyphenols on increased oxidative stress / nitrosative and apoptosis in the cochlea of experimental animals associated with aging.

Methods: Male Sprague-Dawley rats (n = 100), grouped in different ages: 3, 6, 12,18 and 24 months, each group in turn were divided into control and treated with a mixture of polyphenols, 100 mg / kg bw / day dissolved in water for 4 months. The cochleas were extracted to measure the activity of superoxide dismutase enzyme (SOD), the presence of free oxygen and nitrogen radicals by dihidroetidium probe (DHE) and anti-nitrotyrosine in histological sections of cochlea. Was quantified by Western blot protein 3-nitrotyrosine and Caspase-3, also was measured by caspase-3 activity luminescent techniques.

Results: The apoptosis increases with the age of the rats and significantly prevented treatment. Moreover, polyphenols increase the total SOD activity in the rat cochlea, reducing ROS and RNS and nitrated proteins in rat cochlea.

Conclusion: Our data support the hypothesis of the involvement of oxidative/ nitrosative stress as an inducer of apoptosis causes of presbycusis observed in the rat cochlea. Also suggest that preventive treatment with antioxidants has a significantly beneficial action on the integrity of the inner ear even at higher ages.

Keywords: polyphenols, oxidative/nitrosative stress, apoptosis, cochlea

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Sources of input to the lateral superior olive

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The lateral superior olive (LSO) is one of the so-called principal nuclei of the superior olivary complex (SOC), the first level of the auditory pathway where the information from the two ears converges. LSO neurons encode interaural intensity differences, one of the essential cues to locate sounds in space. Although it is well established that the LSO sends chemically asymmetric projections to the ipsilateral and contralateral inferior colliculus, there is conflicting information as to the sources of input to the LSO (reviewed by Thompson and Schofield, 2000 [1]).

To analyze systematically the neural connections of the LSO, we made tiny injections of the bidirectional tracer biotinylated dextran amine (BDA; 3,000 Da or 10,000 Da) into different tonotopic regions of the LSO of albino rats (one injection per animal). The animals were treated according to current European regulations. BDA labels very efficiently the cell bodies, dendrites and axons of the neurons that innervate the injection, as well as the axons of the neurons at the injection site. In this study we paid attention exclusively to the morphology and distribution of retrogradely labeled neurons.

In all cases with BDA injections restricted to the LSO, we found three discrete and conspicuous populations of labeled neurons on the side ipsilateral to the injection site: spherical bushy cells in the anteroventral cochlear nucleus (AVCoN), planar multipolar neurons in the posteroventral cochlear nucleus (PVCoN), and principal neurons in the medial nucleus of the trapezoid body (MNTB). On the contralateral side, the only labeled cells were small multipolar neurons of the ventral nucleus of the trapezoid body (VNTB). In all four locations, the position of the labeled neurons shifted systematically with the position injection site along the tonotopic axis of the LSO, thus confirming the tonotopic order of connections between auditory nuclei. Neuron counts revealed the relative contribution of each one of the sources of input to the LSO: AVCoN ($\approx$ 45 %) > MNTB ($\approx$ 37%) > PVCoN ($\approx$ 12%) > VNTB ($\approx$ 6%).

Our data extend previous descriptions of the connections of the LSO and provide a morphological framework for future physiological and computational studies.

Keywords: biotinylated dextran amine (BDA); cochlear nuclei; lateral superior olive; medial nucleus of the trapezoid body; planar multipolar neurons; spherical bushy cells; three-dimensional reconstruction; ventral nucleus of the trapezoid body.

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The posterior limitans nucleus is the main thalamic target of the external cortex and the nucleus of the brachium of the inferior colliculus

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The posterior limitans nucleus of the thalamus (PLi) is an enigmatic structure interposed between the diencephalon and the pretectum. Because it receives projections from photosensitive retinal ganglion cells [1] and from the intergeniculate leaflet [2], the PLi is usually considered part of the visual system.

As part of a systematic investigation of the projections from the auditory midbrain to the thalamus, we made injections of the sensitive retrograde tracer FluoroGold (FG) into the PLi of albino rats. In the auditory midbrain, two conspicuous populations of neurons are labeled: small neurons grouped in clusters or modules in layer 2 of the ipsilateral external cortex of the inferior colliculus (ECIC) [3], and multipolar neurons of the ipsilateral nucleus of the brachium of the inferior colliculus (NBIC).

To verify these projections, we performed additional experiments with anterograde tracers. The injection of Phaseolus vulgaris-leucoagglutinin (PHA-L) o biotinylated dextran amine (BDA) into the ECIC shows a very dense projection to the ipsilateral PLi; ECIC terminal fibers fill the nucleus, which extends for considerable rostrocaudal and dorsoventral distances as a narrow and vertical, rostrocaudally oriented sheet along the lateral border of the anterior pretectal nucleus and medial to, but separated from, the medial geniculate body (MGB). Injections of BDA into the NBIC reveal an even denser projection to the ipsilateral PLi whose distribution matches that of the ECIC projection. The neurons of the ECIC modules that innervate the PLi are immunopositive for parvalbumin, whereas NBIC neurons are not; this suggests that the PLi receives inhibitory projections form the GABAergic modules of ECIC [4, 5] and excitatory projections from the NBIC. Other minor thalamic targets of the ECIC and the NBIC revealed by our experiments include the posterior intralaminar nucleus, the medial and dorsal divisions of the MGB, and the marginal zone.

These findings improve our understanding of the organization of the initial stages of the extralemniscal, belt auditory pathway, shed light on the complex parcelation of the auditory thalamus, and provide novel morphological frameworks for future functional studies.

Keywords: biotinylated dextran amine (BDA); external cortex of the inferior colliculus, FluoroGold (FG); GABAergic neurons; medial geniculate body; nucleus of the brachium of the inferior colliculus; Phaseolus vulgaris-leucoagglutinin (PHA-L); posterior limitans nucleus of the thalamus.

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Olivary Complex and inner ear plastic reorganization after auditory cortex ablation

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Unilateral auditory cortex (AC) ablations were performed in adult wistar rats by stereotactically guided fine-needle aspiration of the auditory brain surface to analyze the impact of the lost of descending control on both the superior olivary complex (SOC) and the cochlea. The effect of cortical ablation on the olivary complex was studied through the immunocytochemical analysis of the c-Fos expression. Three groups of rats (n = 4) were acoustically stimulated with a pure tone of 5 kHz at 80 dB during 1 hour in open field system at 1, 7 and 15 post surgery days (PSD) respectively. After sound stimulation, animals were allowed to a rest in a quiet environment for 100 min, and then perfused under deep anesthesia. Subsequently, serial sections of the brains were labeled for c-Fos and the number of c-Fos immunoreactive neurons was quantified using a standard densitometric procedure developed by our laboratory. A different ipsi- versus contralateral effect of cortical deprivation may be expected on c-Fos olivary expresion because the descending projection to this nuclei ends bilaterally, albeit with a weak contralateral component.

Using RT-qPCR we analyzed changes in gene expression in the inner ear comparing the ipsi- and the contralateral side. The cochleas from three groups at 1, 7 and 15 PSD were removed and homogenized in order to study the expression of the subunits of the postsynaptic receptors of glutamate *GluA 2/3, GluA 4*, dopamine *Drd1a and Drd2*, and acetylcholine alpha 7 and 10, and the genes for Prestin and Parvalbumin.

There were significant variations over time and side analyzed, of the values of both the qPCR values and the densitometric analysis of the activity of c-Fos immunoreactive olivo-cochlear neurons. Our data suggest a correlation between the activity of neurons in the COS and the organ of Corti, consistent with a progressive homeostatic compensation in the olivo-cochlear feed back loop after the lost of the top down control.

Keywords: Cortical top down control, olivo-cochlear system, inner ear receptor plasticity, c-Fos activation.

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Changes of the olivocochlear efferent system in the hamster GASH:Sal

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Objectives: The golden hamster (Mesocricetus auratus) is often used in auditory research, but little is known about the anatomical organization of its olivocochlear (OC) neurons, the source of the efferent innervation of the organ of Corti (1). A hamster strain from the University of Salamanca, the Genetic Audiogenic Seizure Hamster (GASH:Sal), is being validated as a new model of epilepsy. As a part of this process, we are studying its auditory nuclei to determine the anatomical substrate involved in the mechanism of inducing audiogenic seizures.

Material and Methods: Four control hamsters and six adult GASH:Sal were used in this study. Accordingly, we labeled the OC neurons projecting to one cochlea by means of retrograde axonal transport of FluoroGold. In four animals, all labeled OC neurons were counted and digital images of the labeling were captured and analyzed morphometrically. In one case, a 3D computer reconstruction of the bilateral distribution of OC neurons was made and also of the LSO. In addition, we study the activity of the efferent system by the measuring of distortion-product of otoacoustic emissions (DPOAEs).

Results: The GASH:Sal shows morphological changes in the nuclei origin of the olivocochlear system, more pronounced in the LSO, with a 30% of volumen reduction comparing with controls. The analysis of the DPOAEs reveals functional alterations in the medial efferent system of this strain, with a functional asymmetry between both ears. Further studies are needed to test if the changes in the efferent system could be involved in the genesis or the maintenance of the audiogenic epilepsy that exhibit these animals.

Keywords: audiogenic seizures, efferent system, Genetic Audiogenic Seizure Hamster (GASH:Sal)

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Voltage-dependent inhibition of N-type calcium channels by activation of ORL-1 receptor in the vestibular afferent neurons of the rat

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Opioid peptides have been found to participate in the postransductional processing of vestibular information and to modulate afferent input to vestibular nuclei (Vega and Soto, 2003). To further contribute to determine the opioid participation in the integration of vestibular information we evaluated the potential role of the neuropeptide orphanin FQ nociceptin (N/OFQ), and its receptor the opioid receptor-like (ORL-1) in the voltage-gate calcium current (I_{Ca}) in isolated vestibular afferent neurons. For the experiments we used Long Evans rats of 7-10 posnatal days for evaluate the I_{Ca} of isolated vestibular afferent neurons and of 14-18 postnatal days for evaluate the vestibular nerve activity.

The I_{Ca} was recorded using the perforated patch technique. Both low voltage activated (LVA) and high voltage activated (HVA) currents were found, similar to previously described (Limón et al., 2005). The perfusion of 1 μM N/OFQ decreased the HVA current without significantly changing of the LVA current. The use of the ORL-1 receptor antagonist UFP101 occluded the N/OFQ effect showing that the inhibitory action is consequence of the ORL-1 receptor activation. The inhibitory effect of the N/OFQ in the HVA current was reverted by a pre-pulse of 80 mV, indicating that the voltage-dependent signaling mechanism is taken place in the inhibition. The application of 3 μM ω-ctx-MVIIA decreased the peak of the I_{Ca}, in this condition the application of 1 μM N/OFQ did not significantly affected the remnant current. These results showed that in the isolated vestibular afferent neurons the ORL-1 receptor activation inhibits the N-type calcium current in a voltage-dependent manner.

We hypothesize that the ORL-1 receptor could act as a presynaptic modulator at the synapse between the vestibular-afferent neurons and the vestibular-nucleus neurons.

Keywords: isolated vestibular afferent neurons, ORL-1 receptor, N/OFQ, pre-synaptical inhibition, voltage-dependent inhibition and calcium current.

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Folate deficiency alters homocysteine cycle in the cochlea and causes premature hearing loss in mice

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The methionine/homocysteine cycle is modulated by nutritional factors and its alterations have been associated to several pathologies including deafness [1].

We have studied the impact of a dietary-induced folic acid deficiency on cochlear methionine metabolism and in hearing.

C57BL/6J mice were fed with normal diet or folate deficient (FD) for 8 weeks. Hearing was evaluated by ABR threshold analyses and cochlear morphology was evaluated by hematoxylin-eosin staining and immunohistochemistry. RT-qPCR and Western blotting were used to determine cochlear levels of the methionine cycle enzymes, peptide mass fingerprint was carried out for protein identification, and plasma Hcy (pHcy) levels were determined by HPLC.

The control group showed normal ABR thresholds (8 to 28 kHz, 27-48 dB SPL) whereas the FD group presented moderate to profound hearing loss (8 to 28 kHz, 52-85 dB SPL). Folic acid deficiency caused a reduction in plasma folate levels whilst pHcy levels were increased. All Hcy cycle enzymes studied were expressed in the cochlea. But some of them showed altered mobility in Western blot as compared to the reference liver mobility patterns, suggesting post-translational modifications in the cochlea.

In summary our data indicate that: i) the main enzymes of Hcy metabolism are expressed in the cochlea; ii) alterations in the methionine cycle secondary to folic acid deficit caused hearing loss; and iii) hearing loss correlated with alterations in the expression of Hcy cycle enzymes, and elevations of systemic pHcy.

Keywords: ABR, deafness, homocysteine, folic acid, BHMT

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Burst activity and ultrafast activation kinetics of CaV1.3 Ca^{2+} channels support presynaptic activity in adult gerbil hair cell ribbon synapses

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Auditory information transfer to afferent neurons relies on precise triggering of neurotransmitter release at the inner hair cell (IHC) ribbon synapses by Ca^{2+} entry through CaV1.3 Ca^{2+} channels. Despite the crucial role of CaV1.3 Ca^{2+} channels in governing synaptic vesicle fusion, their elementary properties in adult mammals remain unknown. Using near-physiological recording conditions we investigated Ca^{2+} channel activity in adult gerbil IHCs. We found that Ca^{2+} channels are partially active at the IHC resting membrane potential (–60 mV). At –20 mV, the large majority (>70%) of Ca^{2+} channel first openings occurred with an estimated delay of about 50 µs in physiological conditions, with a mean open time of 0.5 ms. Similar to other ribbon synapses, Ca^{2+} channels in IHCs showed a low mean open probability (0.21 at –20 mV), but this increased significantly (up to 0.91) when Ca^{2+} channel activity switched to a bursting modality. We propose that IHC Ca^{2+} channels are sufficiently rapid to transmit fast signals of sound onset and support phase-locking. Short-latency Ca^{2+} channel opening coupled to multivesicular release would ensure precise and reliable signal transmission at the IHC ribbon synapse.

Keywords: Inner Hair cell; Ca^{2+} channel; Ribbon synapse

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Subnuclear endocytic activity of the outer hair cell

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Mechanical impedance of the organ of Corti can be influenced by efferent feedback through outer hair cells (OHCs). This mechanism requires endocytic activity at the subnuclear pole of OHCs. It has already been shown that plasma membrane is endocytosed at the apical pole of OHCs and trancytosed to different locations; namely, the basolateral membrane and the subnuclear region. The significance of endocytic activity in the subnuclear region is still not clear. Therefore, in this study we investigated endocytosis at the synaptic pole of OHCs.

Confocal laser scanning microscopy was used to detect FM1-43 staining in isolated OHCs from the functionally mature guinea-pig cochlea. The dye was applied extracellular to the cell through a local perfusion system. The time course of intracellular signal intensity changes were recorded in the apical and basal poles and normalized to the signal intensity change of the plasma membrane.

Signal intensity changes at depths of 2 and 6 µm beneath the membrane were 0.15 ± 0.03 and 0.06 ± 0.04 in the basal pole and 0.16 ± 0.03 and 0.04 ± 0.03 in the subcuticular area after 5 s onset of dye application, respectively (N=5). These data show no significant difference in the staining between the opposite poles of the OHCs – the subnuclear and the subcuticular areas –, indicating similar endocytic activity mechanisms at the opposite poles of OHCs.

Excluding the apical pole of OHCs from the FM1-43 staining by drawing that pole of the cell into a glass capillary had no significant effect on the relative fluorescence intensity change in the subnuclear area. In this configuration, the measured signal intensity changes at depths of 2 and 6 µm were 0.17 ± 0.03 and 0.07 ± 0.03, respectively (N=3). These data imply that vesicles accumulating in the subnuclear pole of OHCs in the first 5 seconds cannot have originated from the apical pole of the cell, but are locally endocytosed.

In conclusion, these data indicate that endocytic activity in the subnuclear region of OHCs is significant and contributes equally to the total endocytic activity of the cell.

Keywords: Endocytic activity, outer hair cell, FM-43, confocal microscopy

3D imaging of the guinea pig cochlea and spiral ganglion neuron quantification

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Various methods have been developed for imaging of the cochlear structures, cochlear implants (CI) and for the time-consuming neuron counting. We tested a semi-automatic method based on Confocal Laser Scanning Microscopy (CLSM) [1] to visualize the cochlea, implanted devices and Rosenthal's Canal three-dimensionally and to quantify the spiral ganglion neurons (SGN) within.

Cochleae of 8 normal hearing guinea pigs and 5 implanted with a silicone filament were fixed in paraformaldehyde (PFA), decalcified, dehydrated and cleared in Spalteholz solution. Using the tissue's autofluorescence, CLSM was performed generating z-series stacks of the modioli and the basal turns with implants (labelled additionally with vimentin and Cy5 for connective tissue), respectively. The perimeters of the Rosenthal's Canal were surveyed, representative neuron diameters were measured and the neurons first counted manually and then software-assisted. For comparison, 8 normal hearing guinea pig cochleae were embedded in paraffin and examined similarly.

The CLSM method has the advantage that the cochleae remain intact as an organ and keep their geometrical structure. Z-stack creation is nearly fully-automatic and frequently repeatable with various objectives and step sizes and without visible bleaching. The tissue shows minimal or no shrinking artefacts and damages typical for embedding and sectioning. As a result, the cells in the cleared cochleae reach an average diameter of 21µm and a density of about 18 cells/10.000µm2 with no significant difference between the manual and the automatical counts. Subsequently we compared the CLSM data with those generated using the established method of paraffin slides, where the SGN reached a mean density of 9.5 cells/10.000µm2 and a mean soma diameter of 13.6µm.

We were able to prove that the CLSM method provides a high grade of tissue preservation in comparison to paraffin or epoxy resin embedding. The automatic stack-generation and the counter software reduce the effort considerably. In addition this visualization technique offers the potential to detect the position and orientation of CI within the cochlea and tissue growing in the scala tympani around the CI due to the fact, that the implant does not have to be removed to perform histology as in case of the paraffin method.

Keywords: CLSM, cochlea, histological technique, spiral ganglion neuron.

Acknowledgements: This work has been sponsored by DFG Transregio 37 / A5.

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Formation and properties of the acoustic field in the cochlear duct
I. The diapason of perceived sound frequencies

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The sound field in the human ear cochlear duct forms the Reissner's membrane. Under the influence of stapes oscillations in the vestibular scale and onto the membrane are committed processes, which represents the acoustic-wave hearing model [1]. Reissner's membrane creates standing waves and directs the sound energy flow to receptor cells.

Mathematically the hearing model is expressed the equations system that represent the distribution of maxima coordinate of standing waves on the vestibular membrane (axial $x_{max}(f)$ or $\ell_{max}(f)$, dimensionless $\delta_{max}(f)$, and time $t_{max}(f)$ in accordance with experimental data [2]) and dispersion relation of the velocity distribution of the sound waves in frequencies v(f). These equations contain parameters ideal cochlear duct: the length L_o=32 mm, the minimal frequency of perceiving sound f_o=20 Hz, the maximal – f_{mo}=20 kHz, with a maximum speed of spreading v_{mo}=1600 m/s.

The ratio $\ell(f)=L_o\delta(f)$ for a frequency fm (set by audiometric as maxima perceiving in real-ear) determines the maximal coordinate of the cochlear duct, and identifies its length $L_d=L_o.\delta(f_m)=L_o.2^{2\log(fm/fmo)}$ Feedback $f_m=f_{mo}(L_d/L_o)^{1/(2\log2)}$ claims that the observed decline in the perceived of the upper frequency fm structurally due to the reduction of cochlear duct length.

A similar ratio with the determining minima audiometric perceived frequency f_a for real-ear as $L_a=L_o.2^{2\,\lg(fa/fmo)}$ determines the width of the apical ligament duct L_a. Feedback $f_a=f_o(L_a/L_o)^{1/(2\log2)}$ describes the observed raise in the lower sound frequency, f_a, which is created by the broadening of the apical ligament of duct membranes L_a.

These relationships make it clear that the observed decline in actual top f_m and the growth of the lower limits of the frequency f_a perceived sound linked to changes in the biological characteristics of the cochlea. The cochlea selects from the overall sound diapason that range, which morph- and physiologically perceives.

Keywords: acoustic-wave hearing model; Reissner's membrane functions; perceived sound frequencies.

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Formation and properties of the acoustic field in the cochlear duct
II. The dispersion of acoustic waves and relaxation of the sound field

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Statement [1] that in the cochlea is not possible the sound dispersion, is not correct: the sound propagation in a real environment, has a tendency to disperse. [2] The distribution relation of sound velocities to frequencies in the human cochlea v(f) describes acoustic-wave hearing model [3]: $v(f)=\varepsilon(f).v_{mo}$, where dispersion $\varepsilon(f)=(1+\delta(f))/2$ with dimensionless coordinate $\delta(f)=2^{2\log(f/fmo)}$.

To calculate the maximum time of order zero $t_{max,0}(f)$: will use the data obtained previously (see Part 1): it sets the duration of the relaxation sound field $\tau(f)$ in the cochlear duct.

Since the $\tau(f)=t_{max,0}(f)$, it is easily seen that the relaxation time

$$\tau(f)=(L_d+\ell_m(f))/v_m(f)=2.Ld/v(f_m)=(2.Lo/v_{mo}).\delta(f_m)/\varepsilon(f_m)$$

is determined only by maximal perceived frequency f m and is independent of any other. These results leads to the explanation of the formation process of the sound field in the cochlea and to the calculation of the start time for auditory sensations of sound (time relaxation) – time during which is created a set of standing waves (a maxima time of zero order) on the vestibular membrane and, therefore, is formed a sound field in the cochlear duct.

Thus, in the cochlea creates a sound field, in which the dispersion of the sound leveled dispersion effects by design of auditory organ.

Keywords: acoustic-wave hearing model; dispersion of acoustic waves; relaxation of the sound field.

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Morphological changes of cochlear gap junction plaques in Brn4 deficient mouse, a mouse model of DFN3 non-syndromic deafness

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Objective: Brn4, which encodes a POU transcription factor, is the gene responsible for DFN3, an X chromosomelinked nonsyndromic hearing loss. Brn4 deficient (KO) mice show low endocochlear potential (EP), hearing loss and severe ultrastructural alterations in spiral ligament fibrocytes [1]. Mutations in the connexin26 (Cx26) and connexin 30 (Cx30) genes, which encode gap junction proteins and are expressed in cochlear fibrocytes and nonsensory epithelial cells (cochlear supporting cells) to maintain proper EP, are thought to be responsible for hereditary sensorineural deafness. The molecular pathologie of Brn4 deficiency causing low EP are still unclear.

Methods: It has been hypothesized that gap junction in the cochlea provide an intercellular passage by which K+ are transported to maintain high levels of the endocochlear potential essential for sensory hair cell excitation. In this study, we analyzed the formation of gap junction plaques in cochlear supporting cells of Brn4 KO mice in different stages by confocal microscopy and three dimensional graphic constructions.

Result: Gap junction composed of mainly of Cx26 and Cx30 in wild type mice showed horizontal linear gap junction plaques along the cell-cell junction site with the adjacent cells and these formed pentagonal or hexagonal outlines of normal inner sulcus cells and border cells. However, the gap-junction plaques in Brn4 KO mice did not show normal linear structure, but the round small spots were observed around the cell-cell junction site. The size of gap junction units of Brn4 KO mice was significantly shorter than control mice.

Conclusions: Our results demonstrated that Brn4 gene mutation affects on the accumulation and localization of gap junction proteins at the cell border among cochlear supporting cells. It may suggest that Brn4 significantly associates with cochlear gap junction properties to maintain proper EP in cochlea as well as the mutations of Cx26 or Cx30.

Keywords: Brn4, POU3F4, Connexin, Gap Junction, Endocochlear Potential

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POSTER PRESENTATIONS P-14

sTSLIM imaging of rodent and human inner ears

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Using a scanning thin-sheet laser imaging microscope (sTSLIM) that we have developed, we have digitized the inner ear of a mouse, rat, guinea pig and human. Light-sheet illumination and optical plane detection uses air-mounted objectives to accommodate different size temporal bones. sTSLIM imaging produces a well-registered z-stack of high resolution, mid-modiolar images of the soft and bony tissues of the inner ear that are ideal for 3D reconstruction of structures. Direct volume rendering allows for visualization of present and missing cells (e.g., hair cells and spiral ganglion neurons). Segmentation of individual structures allows determination of volumes, cell counts, and measurements of the spiral length of structures along the length of the cochlear duct and these morphometric data will be presented. sTSLIM detects fluorophores within the tissues after whole inner ear labeling using a general stain (i.e., rhodamine B isothiocyanate) or using immunofluorescent antibodies for specific structure labeling. Whole cochlea antibody labeling can be accomplished if structures are accessible to the antibodies such as through the basilar membrane. Labeling of hair cells has been successful using anti-prestin, myosin VIIa and synapsin antibodies. Hair cell, SGN and fibrocyte loss after kanamycin/furosemide treatment was mapped along the complete length of the cochlea from a complete serial reconstruction and segmentation of structures. Matrix glycoproteins like laminin and type IV collagen, that are deeply embedded in tissues were not accessible to whole cochlea antibody labeling; however, they were accessible after removal of the cells using fresh tissue decellularization. The 3D geometry of extracellular matrix scaffolds has been revealed using type IV collagen labeling of acellular tissues. Such decellularized cochlear sections are being used to engraft and differentiate stem cells toward an auditory phenotype. sTSLIM imaging, antibody labeling of specific structures, and 3D reconstruction of structures using computer assisted algorithms allow for an accurate morphometric assment of normal and pathological cochlear structures.

Keywords: 3D reconstruction; ototoxicity; regeneration; stem cells

Acknowledgements: This work has been sponsored by NIDCD grant #U24DC011968 and the Lions Hearing Foundation.

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Cell therapy for hereditary deafness with bone marrow mesenchymal stem cell and the activation of stem cell homing

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Congenital deafness affects about 1 in 1000 children and more than half of them have genetic background such as Connexin26 gene mutation. The strategy to rescue such heredity deafness has not been developed yet. Recently, a number of clinical studies for cell therapy have been reported and clinically used for several intractable diseases. Inner ear cell therapy for sensorineural hearing loss also has been studied using some laboratory animals, although the successful reports for the hearing recovery accompanied with supplementation of the normal functional cells followed by tissue repair, recovery of the cellular/molecular functions were still few. Previously, we developed a novel animal model for acute sensorineural hearing loss due to fibrocyte dysfunction and performed cell therapy with bone marrow mesenchymal stem cells (MSC) as supplementation of cochlear fibrocytes functioning for cochlear ion transport [1]. We injected MSC into the lateral semicircular canal and a number of these stem cells were then detected in the injured area in the lateral wall. The transplanted animals showed a significantly higher hearing recovery ratio than controls. We analyzed the machinery of this stem cell induction to the targeted site in cochlea and found that monocyte chemotactic protein 1 (MCP1:CCL2) and stromal cell-derived factor-1(SDF-1:CXCL12) played important roles for this cell induction as stem cell homing factors. To enhance MSC invasion to cochlea tissue, we developed a novel transplant strategy by induction of MCP1 /SDF1 expression in host cochlear tissue and enhanced expression of their receptors, chemokine (C-C motif) receptor 2 (CCR2) and C-X-C chemokine receptor type 4 (CXCR4) in MSC. With this strategy, we induced efficient invasion of MSC to inner ear tissue and differentiation to form gap junctions with Cx26 among transplanted MSCs in Cx26-deficiente mouse inner ear.

Keywords: Bone marrow mesenchymal stem cell, Connexin26, Hereditary deafness, Inner ear cell therapy

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Deafening guinea pigs using locally applied kanamycin and furosemide

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The systemic administration of aminoglycosides combined with diuretics to induce selective hair cell loss has been used since the 1970s [1]. However, in guinea pigs, drug injection into the *Vena jugularis* externa is laborious, resulting in substantial mortality and variable hair cell loss. We therefore examined the effect of local application of a mixture of kanamycin and furosemide to the middle ear. The tympanic bulla of guinea pigs (300-600 g, strain BFA bunt) was exposed with a retroauricular approach, and the middle ear was opened to visualize the basal part of the cochlea. A gold wire electrode was chronically implanted near the round window to measure hearing threshold. The middle ear was completely filled with 150 µl drug mixture under visual control. After 1 or 2 h of exposure, the liquid was removed and the bulla was rinsed with Ringer's solution. The following drug concentrations were used: kanamycin (200 mg/ml) and furosemide (50 mg/ml) for 1 and 2 h, respectively, kanamycin (100 mg/ml) and furosemide (25 mg/ml) for 2 h, and Ringer's solution for 2 h as control. Hearing tests were carried out for several months after drug exposure. The cochleae were histologically processed, and the remaining hair cells and spiral ganglion cells were examined in serial sections. One week after drug exposure and beyond, profound hearing loss or complete deafness was observed in 80% of the ears treated for 2 h with kanamycin (200 mg/ml) and furosemide (50 mg/ml). After 1 h exposure, 50% showed at least profound hearing loss, while the other half was severely affected in the high-frequency range. Variable results (no to complete deafness) were observed in the ears exposed to 100 mg/ml kanamycin and 25 mg/ml furosemide for 2 h. No animal showed drug-related side effects. The middle ear application of kanamycin and furosemide proved less invasive than systemic administration, while reliably producing profound hearing losses.

Keywords: local drug application, hair cell loss, aminoglycoside, diuretic, guinea pig

Acknowledgements: Supported by a Erasmus scholarship to Peter Bako and BMBF (BioTransporter, FKZ 13N11301).

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BDNF mediated neurite outgrowth in a mouse spiral ganglion cell model

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Cochlea implant (CI) technology is a milestone in the treatment of deafness. Yet electrical stimulation by a CI does not reach the performance of normal hearing. In part, this is due to the limited number of electrode contacts that stimulate the auditory nerve. One way to improve the nerve-electrode interface is to stimulate the outgrowth of neurites towards and onto the electrode. This may be achieved by neurotrophins such as brain derived neurotrophic factor (BDNF). Via TrkB receptors neurite outgrowth is stimulated, however as a paradox, this effect is inhibited by simultaneous BDNF binding to the low-affinity p75NTR receptor. We thus set out to investigate the TrkB and p75NTR mediated pathways in more detail. An organotypic culture model of the postnatal (P4-6) mouse spiral ganglion was used. Neurite outgrowth was analyzed and quantified by customized Sholl analysis using ImageJ. Stimulation of neurite outgrowth was quantified after application of BDNF, synthetic, selective TrkB ligands and Rho-associated kinase (ROCK) inhibitors. Inhibition of neurite outgrowth was assessed for application of MAG-Fc (myelin-associated glycoprotein), TrkB-inhibitors, PI3K (phosphoinositide-3-kinase) inhibitors and PKA (protein kinase A) inhibitors. BDNF application resulted in a dose- and a time-dependent response of neurite outgrowth. Outgrowth was also stimulated by a synthetic TrkB ligand. Inhibitors were capable to inhibit these effects in a dose dependent manner. Using MAG-Fc a p75NTR associated inhibitory environment was created which suppressed BDNF activated outgrowth. The effect of the inhibitory environment circumvented by the application of a selective TrkB ligand and the interruption of p75NTR signaling by a Rho-associated kinase inhibitor. The organotypic culture model of the spiral ganglion is suitable for the evaluation of compounds stimulating or inhibiting neurite outgrowth. A synthetic, selective TrkB ligand proves to be a potential candidate for selective stimulation of neurite outgrowth. To overcome inhibitory environment, more potent TrkB-agonists are required. Selective inhibition of the p75NTR pathway improves neurite outgrowth in an inhibitory environment.

Keywords: spiral ganglion; brain derived neurotrophic factor; inhibitory environment; neurite outgrowth.

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POSTER PRESENTATIONS P-18

Aquaporins constitute a molecular water shunt in the cochlear apex – Implications for longitudinal endolymph flow and Menière's disease

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In the cochlea, the perilymph-endolymph-barrier (PEB) separates the perilymphatic and endolymphatic fluids. However, the PEB exhibits water permeability higher than for ions such as potassium (K+) and allows rapid water exchange between the two fluid compartments. The physiological significance of the high water permeability and the molecular pathways of water flow across the PEB still need to be unraveled.

Immunohistochemical analysis of aquaporin (AQP) 4 and AQP5 expression in the cochlear duct of different rodent species (rat, mouse, gerbil, guinea pig) and humans revealed their localization in the baso-lateral (AQP4) and apical (AQP5) membranes of outer sulcus cells (OSCs) in the cochlear apex. Ontogenetically, this localization of both AQPs in OSCs first occurs at the onset of hearing function, as determined in the rat and mouse cochlea. Based on previous *in vivo* experimental data [1; 2] on diffusional and osmotic water exchange and *in silico* simulation data on diffusional water exchange, we computed the trans-epithelial diffusion (PD) and osmotic (Pf) water permeability coefficients between endolymph and perilymph in the guinea pig cochlea. We calculated the P_D and/or P_f for the whole cochlear duct epithelium (CDE) and the AQP4/5-expressing OSCs in the lateral wall of the cochlear apex. Compared to the relatively low P_f of the CDE, the OSCs exhibit a very high P_f as found in highly water permeable renal tubule epithelia.

The membranous localization of AQP4 and AQP5 in OSCs in the cochlear apex constitutes a high capacity molecular water shunt between endolymph and perilymph that presumably drives longitudinal endolymph flow in response to transepithelial osmotic gradients. Impaired transcellular flow through this water shunt is potentially involved in the formation of endolymphatic hydrops in Ménière's disease.

Keywords: Aquaporin; Cochlea; Endolymph; Water permeability; Ménière's disease

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Expression and distribution of Connexin 26 and 30 in the Human Cochlea – A confocal laser immunohistochemistry study

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The copious expression of connexin-26/30 proteins (Cx26, Cx30) was assessed in the normal human cochlea using confocal laser immunohistochemistry. Their expression in the membrane-associated gap junctions was always separate in the organ of Corti suggestive of a homomeric arrangement in man. Cx30 dominated while Cx26 was mostly expressed diffusely in the cytoplasm of the outer pillar and Deiter cells. A small number of Cx26-associated gap junctions appeared in Hensen´ cells separated from Cx30. Outer pillar cells expressed Cx26 and Cx30 in the cytoplasm but in different domains but there was also Cx30-associated gap junctions present between individual pillars. The findings suggest that human pillar cells are coupled. In the lateral wall and spiral limbus both connexins were found abundantly distributed and mostly co-expressed. The diverse chemical arrangement of the gap junction proteins Cx26/30 in the OC and lateral wall suggest that they may serve different functions in the human cochlea.

Keywords: Human Cochlea; Organ of Corti; Stria vascularis; Connexin30; Connexin 26; Gap Junction; Cochlear implant

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Morphologic change in the inner ear of the Aquaporin (AQP)-11 knockout mice

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Objectives: To maintain water homeostasis in the inner ear, both of the absorption and production of inner ear fluid is well balanced. Generally the stria vascularis (SV) is thought to be the site of endolymph production, and the endolymphatic sac (ES) of the site of endolymph absorption. For the purpose of evaluating the role of Aquaporin (AQP)-11 in the water channel system of the inner ear, we have examined the morphology of the cochlea, vestibule and endolymphatic sac using the AQP-11 knockout mice.

Methods: AQP-11 knockout mice (2 and 5 weeks later after birth) were used in this study. Temporal bones were immersed in fixative overnight, and decalcified in 0.12 M EDTA for 5 days. They were then dissected into the inner ear block under a stereomicroscope. For light microscopy some tissues were embedded in paraffin. And the others were used for immunocytochemistry, which embedded in OCT compound and prepared frozen sections by Cryostat (10µm thick). The primary reagent used to detect AQP-11 was a rabbit anti-AQP-11 polyclonal antibody.

Results: AQP-11 knockout mice expressed cell swelling in the endolymphatic duct near the sac. However any other morphologic abnormality was not seen.in the cochlea and vestibule.

Conclusions: Endolymphatic hydrops is generated when homeostasis of inner ear fluid is broken. Focused on the endolymphatic sac, AQP11 might work the important role of water flux.

Keywords: AQP-11 knockout mouse, inner ear

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Functional and morphological characterization of NOD-SCID inner ear

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Objectives: Sensorineural hearing impairment is a consequence of hair cells (HC) and/or spiral ganglion neurons loss. In mammals, HCs are unable to regenerate, so their loss cause irreversible damages. For this reason it is very important to study the mechanisms to stimulate cochlear tissues regeneration with stem cells. Moreover it is necessary to choose the correct animal model. The NOD-SCID is a mouse with hampered immune system useful for stem cells transplantation, but there are no information about its auditory capacity. The aim of the project is to characterize the functionality and the morphology of the NOD-SCID inner ear because it derives from NOD mouse which presents a progressive hearing loss.

Methods: Animal models: 4-8-12 week-old CBA/J (non hearing-impaired), NOD (hearing-impaired) and NOD-SCID. Investigations: electrophysiology (ABR), histology of cochlea, spleen and tongue. All tissues were fixed, paraffin-embedded, and the sections were Hematoxylin-Eosin stained. The cochleae were cut into ten series of slides with sequential sections (thick 5um), for each section we evaluated inner and outer hair cells number, neuronal density, stria vascularis (SV) area and spiral ligament (SL) area. The percentage of fungiform and filiform papillae was counted along the superficial side of the tongue sections [1]. The morphological changes of red and white pulp of the spleen were studied. All data were analyzed with ANOVA Bonferroni's test.

Results: NOD-SCID mice show a progressive hearing impairment at high frequencies from 4 to 12 weeks. The deafness appears to be associated with hair cells loss in the medium/basal region of the cochlea, likely as a consequence of SV and SL morphological and functional alterations. The histological study of NOD-SCID tongue demonstrates abnormalities in the organ development and an erythroid cells accumulation is observed in the spleen red pulp.

Conclusions: Deafness of NOD-SCID mice could be due to premature aging, or could be a consequence of physiological/metabolic alterations that interfere with Organ of Corti functionality causing hair cells death.

Keywords: NOD-SCID, hair cells, hearing impairment

Acknowledgements: This work has been sponsored by Antonveneta Foundation (Italy)

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Hearing impairments in diet-induced obesity in mice

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Obesity-related cardiometabolic disorders are known to be associated with hearing impairment. We hypothesized that a high-fat diet (HFD) could exacerbate hearing degeneration in C57B6 mice.

Forty C57BL/6J mice were randomly assigned to control or HFD groups. They were then divided into the following subgroups: 1-month group, 3-month group, 5-month group, and 12-month group (HFD, n = 5; control, n = 5; each). Ten CBA/N-slc mice were also assigned to 12-month control or 12-month HFD groups (n = 5, each). Obesity was induced by feeding HFD (High Fat Diet 32). Mice in the control group were fed a standard diet.

HFD group mice showed significantly higher body weight than the controls. There was no significant difference in blood glucose levels between the HFD group and controls. At 3 and 5 months, compared to the baseline, auditory brain stem response (ABR) threshold shifts were significantly decreased in the HFD group (at 32 kHz) compared with controls, in the C57B6 mice. After 12 months, ABR thresholds were significantly decreased in the HFD group at all frequencies, in the C57B6 mice. On the other hand, CBA mice showed the opposite outcome in that, ABR thresholds were significantly increased in the HFD group compared with controls at all frequencies, at 12 months. These results in CBA mice showed similar findings to some previous reports.

C57B6 mice used in the current study are well known to develop sensorineural hearing loss much earlier in life than other mice. The results in this study suggest that HFD may suppress the progression of age-related hearing loss in C57B6 mice.

Keywords: hearing loss, ABR, high fat diet

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Morphological and electrophysiological cochlear changes in two models of epilepsy, the WAR and GASH:Sal strains, after audiogenic kindling

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Introduction: Animal models of audiogenic seizures are important for the study of neural substrates and mechanisms involved in epileptic seizures phenomena. Few studies report on the morphology of the inner ear of these animals, on audiological thresholds and on electrophysiological responses of brainstem (ABR) in audiogenic animals after the development of epileptic audiogenic seizures. For this reason we study auditory electrophysiological changes and characterize the microanatomy of the Corti's organ after successive stimulations in WAR and GASH:Sal strains.

Material and Methods: Six animals of the WAR and GASH:Sal strains underwent repetitive noise stimulations that trigger audiogenic seizures. The animals were evaluated by ABR before and after the audiogenic kindling and the morphology of the Corti's organ was evaluated by scanning electron microscopy, compared with control groups of wistar rats (*Rattus norvegicus*) and hamsters (*Mesocricetus auratus*).

Results: The ABR and the scanning electron microscopy allowed us to characterize the functional and morphological changes in the inner and outer hair cells of the GASH:Sal and WAR audiogenic strains, after audiogenic kindling. Compared to controls, both strains showed high threshold in the ABRs. We also found morphological damage, mainly in the spatial organization of the stereocilia of the outer hair cells. The inner hair cells also exhibit changes in the stereocilia number and disorganization in the stereocilia links. The findings suggest that the audiogenic kindling in these strains models of epilepsy causes functional and morphological impact in the inner ear.

Keywords: audiogenic seizures, audiogenic kindling, inner ear, Wistar Audiogenic Rat (WAR), Genetic Audiogenic Seizure Hamster (GASH:Sal)

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Transepithelial K^+ secretion and Na^+ absorption in human endolymphatic sac epithelium: ex vivo study for understanding net physiologic current of endolymphatic sac epithelium

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Objective: Endolymphatic sac has been thought to be an organ that involve in the regulation of inner ear fluid volume and endolymphatic ion homeostasis. Various ion channels were identified in the luminal epithelial cells of endolymphatic sac using animal models, however, there is no studies using human tissue. In addition, no study has identified net physiologic transepithelial current in the endolymphatic sac epithelium. This study was performed to investigate characteristic of net physiologic current and identify specific ion dependent current from human endolymphatic sac (ES) epithelium.

Method: Human endolymphatic sac was harvested during acoustic tumor surgery via translabyrinthine approach. The luminal side of ES was exposed and folded for current measurement. Scanning vibrating electrode technique was used to measure transepithelial current under short-circuit condition. Specific ion-dependent current was measured using electrophysiological and pharmacological method; artificial perilymph solution was perfused during current measurement and various ion channel blockers for Na^+ channels, K^+ channels, Cl^- channels, and ion pump were applied. Ion channel expression was also investigated using microarray and proteomics.

Results: Net current from ES epithelium of cystic portion was very tiny. Sometimes small cation absorption/anion secretion current was measured, but sometimes cation secretion/anion absorption current was observed. However, Net current from epithelium in the duct portion showed only cation absorption/anion secretion current. The cation secretion current was inhibited by Ba^{2+} (100µM and 1mM), 4-AP (1mM) and apamin (10nM). The cation absorption/anion secretion current was inhibited by amiloride (10µM), DIDS (10µM). Microarray and proteomics data indicated that the candidates of those channels were Kir, Kca, ENaC and various chloride channels.

Conclusion: These results suggest that transepithelial current from ES epithelium of cystic portion is nearly neutral or showed cation secretion current in physiologic condition. This means that cation secretion and absorption is balanced or has more cation secretion current. The candidate of the current is thought to be Kir and Kca. Duct portion has more cation absorption activity than cystic portion. The cation absorption current was ENaC and Cl^- channel dependent.

Keywords: Endolymphatic sac, short circuit current, potassium, sodium

Effect of reduced frequency selectivity on hearing impaired listener in perceiving nonlinearly distorted speech and music

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Problem: Most current assistive hearing devices are nonlinearly distorting speech or music while they are trying to improve audibility and compensate for loudness recruitment in hearing impaired listeners. The quality of the output from these devices may be perceived differently when their frequency selectivity reduced.

Objective: This study aims to establish the relationship between the frequency selectivity measured using Psychophysical Tuning Curves (PTC) and the perceived quality of speech and music subjected to different nonlinear distortions by hearing impaired subjects. The outcomes would be used to refine our model [1] in objectively predicting this perceived quality.

Method: 11 hearing impaired subjects participated in the study. PTCs were measured using the fast method [2] with a 10dBSL pure tone signal at frequencies 500 Hz, 1000 Hz and 2000 Hz, and a narrowband noise masker. Subject was also asked to rate the perceived quality of speech and music subjected to various forms of artificial and real nonlinear distortions in two separate sessions. The artificial distortions are inherent to most assistive hearing devices, including clippings, compressions, and full-range nonlinear distortion. The real nonlinearly distorted speech and music were recorded at output of 3 different compression hearing aids with different compression settings. The recordings were digitally filtered so that the long-term spectrum of the output matched to that of the input closely. All stimuli were bandpass-filtered between 300 and 5000 Hz and amplified by the “Cambridge formula” [3] before presentation via Sennheiser HD600 earphones.

Results: All subjects were able to rate the perceived quality of speech and music between sessions with good consistency. Their performance was inversely correlated to width of their PTCs obtained at both low and high frequencies. Broader PTCs which is associated with reduced frequency selectivity has hindered them from making consistent rating. PTCs obtained using signal frequency at 2000Hz exhibit a strong correlation to the performance of the subjects with distorted music stimuli. It seems to suggest that reduced frequency selectivity at high frequency region has a greater influence on the subjects’ perceived quality rating of nonlinearly distorted music.

Keywords: Distortion, psychophysical tuning curves, hearing impairment, sound quality, correlation.

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A multiscale computational model of guinea pig cochlea to probe neuropathy mechanisms

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Sound-evoked compound action potential (CAP) of the auditory nerve is a common proxy to probe deafness in experimental and clinical framework. It is generally accepted that this electrophysiological index reflects the progressive activation of different pools of fibers that populate the auditory nerve: the high-spontaneous rate (SR) auditory nerve fibers, which detect lowest-sound level, and the medium- and low-SR fibers, which translate higher-sound level stimulation. To examine the weight of each auditory fibers fraction on CAP threshold and amplitude, we designed a multiscale computational model of guinea pig cochlea from the seminal model of Ray Meddis [1]. This model includes all the components involved in the cochlear sound-transduction and enables to analyze the ANF firing assembly (up to 800 ANFs) as well as the CAP in response to basilar membrane velocity evoked by increasing sound level. Using such model, we found that the low-SR fibers have a small contribution into the CAP.

Keywords: Auditory neuropathy, compound action potential of the auditory nerve, computational modeling, guinea-pig.

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POSTER PRESENTATIONS P-27

Coupling between inner hair cells in the adult mouse cochlea

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The inner hair cells (IHCs) of the mammalian cochlea signal to the auditory neurites through ribbon synapses, with multiple release sites per cell and it is generally thought that the sharp neural tuning seen in single nerve fibres reflects the selectivity of individual IHCs [1]. Using a preparation which allows recording from adult mouse IHCs, rather than cells in immature systems more commonly used, we have employed a fluorescent probe to investigate signalling at the synapses by using simultaneous multiphoton confocal imaging and patch clamp recording.

Cochlear inner and outer hair cells were visualised in the intact isolated temporal bones of mice aged between P25 and 10 months, opening the bone to reveal the 10-15 kHz cochlear region with the cells placed in identifiable positions and orientation. The chamber was superfused with hepes-buffered extracellular solutions at room temperatures. Cells were recorded in the whole cell tight seal mode, the pipette including 0.2 mM OGB5N as the calcium indicator. Caesium was used in the pipettes to reduce large outward currents to less than 1.5nA at 0 mV.

These recordings showed distinct calcium entry sites, 'hotspots', when the IHC was depolarized to 0 mV from -60 mV. After OGB5N loading for 2-5 mins it was also often found that a proportion of neighbouring cells appeared to be dye-coupled. Up to 9 adjacent cells could be found filled with dye. Recording time constants in such cases exceeded the single cell values and also indicated the cells were electrically coupled. Calcium responses imaged in such coupled IHCs showed that the kinetics generally differed from that of the recorded cell, suggesting that the coupling did not permit the movement of large calcium buffers between cells, but were sufficient to allow small molecules, including dyes, to pass.

The present data thus indicate that adult IHCs can be coupled. The extent of the observed coupling would not significantly degrade the frequency selectivity of individual auditory nerve fibres, at least for low characteristic frequencies, but may enhance the signal/noise ratio in the pathways leading from the apical cochlea.

Keywords: Inner hair cells, cochlear, multiphoton imaging, coupling.

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POSTER PRESENTATIONS P-28

NMII forms a contractile transcellular sarcomeric network to regulate apical cell junctions and tissue geometry

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Nonmuscle myosin II (NMII) is thought to be the master integrator of force within the epithelial apical junctional complex (AJC), mediating both morphogenesis and tensional homeostasis [1]. It is thus not surprising that mutations in NMII are associated with a number of diseases due to failures in cell-cell adhesion [2]. However, the organization and mechanism by which NMII generates and responds to tension along the intercellular junctional line are not known. Current models depict a random distribution of NMII at the perijunctional actin belt [3], but it is unclear how the activities of individual NMII filaments are integrated and coordinated to generate and transmit force across the epithelium.

We use the organ of Corti, one of the most striking examples of mammalian epithelial patterning, to investigate the role of NMII within the AJC. Using immunofluorescence, exogenous expression of tagged NMII in explant cultures, and a transgenic NMIIC-GFP mouse, we show that periodic assemblies of NMII interlace with perijunctional actin and α-actinin to form a continuous belt of sarcomere-like units (~400 – 600 nm) around the apical perimeter of epithelial cells, at the interface between tight- and adherens junctions. Double labeling NMII head- and tail-domains showed that NMII forms bipolar filaments, oriented parallel to actin filaments along the junctional line, confirming a sarcomeric organization. Remarkably, the sarcomeres of adjacent cells are precisely paired across the junctional line forming an integrated, transcellular network. Using chemical inhibition we show that NMII sarcomeres are contractile, and demonstrate that changes in NMII sarcomere length concomitantly impact apical cell shape and tissue geometry. A differential localization of NMII isoforms across heterotypic junctions suggests isoform-specific roles in the formation and/or regulation of the apical shapes of different epithelial cell types. Importantly, the presence of the sarcomeric belt in various gastrointestinal epithelia suggests it is a universal feature of epithelial systems. Our data provide a direct link between the organization of NMII and its role in modulating epithelial dynamics and cytoarchitecture. The novel sarcomeric network within the epithelial AJC also presents a well-defined target to investigate the multiple roles of NMII in junctional homeostasis, development and disease.

Keywords: non-muscle myosin II; apical junctional complex; sarcomere; apical constriction, adherens junction.

Acknowledgements: This work has been sponsored by NIDCD, IRP, NIH.

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Molecular genetic diagnosis of Usher syndrome

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Usher syndrome (USH) is an autosomal recessive disorder characterized by sensorineural hearing loss, retinitis pigmentosa and, in some cases, vestibular areflexia. Three clinical types are distinguished (USH1-USH3) and, to date, 10 genes have been identified as responsible for the disease.

The purposes of this study were the identification of the disease-causing mutations in our cohort of patients with Usher syndrome and the establishment of a diagnostic algorithm for this disease.

Our cohort was composed of 270 families diagnosed with Usher syndrome: 70 USH1, 136 USH2, 23 USH3 and 41 families clinically non-classified. The molecular study was carried out using diverse techniques: SSCPs, linkage analysis, direct sequencing, MLPA, genotyping microarray and next generation sequencing.

In our series of patients, 182 different mutations were identified, including 175 point mutations and 7 large rearrangements. Eighty-two of these mutations (45%) were described only in Spanish population. The genetic cause of the disease was identified in 226 families (83.7%).

The genetic diagnosis of Usher syndrome is challenged by its high genetic and allelic heterogeneity. Next generation sequencing techniques will allow to reduce the turnaround time of the molecular study and increase the efficiency of the diagnosis of Usher syndrome patients.

Keywords: Usher syndrome, sensorineural hearing loss, retinitis pigmentosa, molecular epidemiology.

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Hearing impairment and human inner ear degeneration caused by missense mutation in WFS1 gene

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A Swedish family that was clinically described with symptoms of optic atrophy, hearing impairment as well as psychiatric problems was originally described in 1940 [1] and was reinvestigated with genetic analysis for mutations in the WFS1 gene causing Wolfram syndrome in 2010 [2].

Here, we present histology of the inner ear from a patient with molecular diagnosed mutation in the WFS1 gene caused by a de-novo mutation in this family. The patient had cochlear implantation at age 57 in one ear, experienced considerable improvement of hearing and died at age 81 in 2012.

Sequencing of the WFS1 gene indentified a heterozygote sequence change c2051C>T in exon 8 leading to a substitution of alanine for valine at position 684 of Wolframin (p.A684V). Plastic embedding and sectioning of the un-implanted inner ear for light- and electron microscopy revealed absence of hair cells and great loss of peripheral processes. Spiral ganglion neurons were found in Rosenthal's canal mainly as mono-polar neurons surviving even with severe hearing impairment. Lipofuscin-like granules were accumulated in neurons of the cochlea and vestibular organ.

Heterozygote missense mutation in the WFS1 gene causes hair cells loss but not severe loss of neurons. The good outcome of cochlear implantation in the contra-lateral ear emphasizes our previous results of robust survival of spiral ganglion neurons in humans suggesting a much slower degeneration of neurons following de-afferentiation [3].

Keywords: Wolfram syndrome, sensorineural hearing loss, optic atrophy, genetics, mutation

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A novel splice-site mutation in the *GJB2* gene causing mild postlingual hearing impairment

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DFNB1 deafness, caused by mutations in the *GJB2* gene (connexin-26), is the most frequent subtype of autosomal recessive non-syndromic hearing impairment. *GJB2* contains just two exons, with the coding region fully contained within the second exon. To date, more than 100 pathogenic mutations have been identified in the *GJB2* coding region, but some DFNB1 patients carry mutations in non-coding parts of the gene or in distant regulatory regions. Previously, a mutation inactivating the donor splice site of *GJB2* intron 1 (c.-23+1G>A or IVS1+1G>A) was reported in hearing-impaired subjects in compound heterozygosity with another *GJB2* mutation. Sequencing of cDNA from a lymphoblastoid cell line derived from a c.-23+1G>A mutation carrier did not detect the mutant transcript.

We have identified a novel substitution mutation that replaces one of the invariant nucleotides at the acceptor splice site of *GJB2* intron 1 (c.-22-2A>C or IVS1-2A>C). Three affected siblings from the same family harbored this mutation in compound heterozygosity with the frameshift mutation c.35delG and presented with mild postlingual deafness. Bioinformatic analyses predicted the existence of a cryptic acceptor splice site located 38 bp upstream of the standard acceptor site. Taking advantage of our recent finding that *GJB2* transcripts can be detected in total RNA extracted from saliva, we generated GJB2 cDNAs from saliva samples obtained from the three affected siblings. Transcripts generated by use of the standard acceptor splice site were only detected for the 35delG allele, indicating that the c.-22-2A>C mutation abolished the standard acceptor splice site. However, we did detect longer transcripts harboring the c.-22-2A>C mutation. Sequencing confirmed the use of the upstream cryptic acceptor splice site predicted *in silico*, although with much lower efficiency than that of the standard acceptor splice site. This residual expression may underlie the mild hearing phenotype observed in these c.-22-2A>C/c.35delG heterozygotes.

Keywords: DFNB1, *GJB2*, mild postlingual deafness, hypomorphic mutations, functional assays.

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Eps8 is necessary for the normal expression of cochlear, but not vestibular hair cell K^+ channels

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Epidermal growth factor receptor pathway substrate 8 (*Eps8*) is an evolutionarily conserved signal transducer endowed with multiple functions in the control of actin dynamics and in the integration of these events with other receptor-activated signaling pathways. We have recently shown that *Eps8* knockout (KO) mice are deaf and that the normal development of cochlear hair cells stereocilia and ion channels is prevented [1]. More specifically, $I_{K,n}$, a low voltage activated K^+ current which appears at around hearing threshold, was absent. Curiously, though *Eps8* is also expressed in vestibular hair cells [2], no gross vestibular defects have been reported in *Eps8* KO mice. However, mild vestibular defects can be hard to identify in rodents, and therefore we investigated if ion channel expression is altered in *Eps8* KO mouse vestibular hair cells. Patch-clamp whole-cell recordings were obtained from in situ vestibular Type I hair cells from control and KO mice of 7 to 19 post-natal days. At this developmental stage vestibular hair cells normally express the mature-like variety of ion channels. We were particularly interested in IK,L, a low-voltage activated K^+ current which, similar to $I_{K,n}$ in cochlear hair cells, is expressed late during development by vestibular Type I hair cells. Though $I_{K,n}$ and IK,L show different kinetics and pharmacology [3], and are presumably conducted through different ion channels, because of their negative voltage range of activation they are responsible for the cell low input resistance at rest. Here, we found that IK,L was normally present in Type I hair cells of *Eps8* KO mice (n = 12). Furthermore, no major differences in macroscopic current amplitude and time course were observed between control and KO mice. These results indicate that, at difference from cochlear hair cells, *Eps8* is not a main regulator of K^+ channels expression in vestibular Type I hair cells.

Keywords: Eps8; Hair cell; K^+ channel

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Different expression of histone modification in the spiral ganglion of Mn-SOD knock out mice

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Objetives: It is currently accepted that epigenetics plays an important role in normal genetics and differentiation and its failure triggers various diseases, such as cancer, aging, metabolic diseases, and abnormal differentiations. The typical mechanism involves the modification of histones and the methylation of DNA. We have reported that acetylated histone H3 was detected in the spiral ganglion cells and the organ of Corti of the young group but not in those of the aged group. Dimethylated histone H3 was detected in the spiral ganglion cells and the organ of Corti of the aged group but not in those of the young group[1]. In this study, we investigated the modification of histones in the aged cochlea of Mn Super Oxide Dismutase (SOD) knock out mice using immunohistochemistry.

Methods: Eight Mn-SOD knock out (+/-) mice (C57BL/6(B6)). Animals were divided to young and aged groups. Cochleas were incubated with fixative and decalcified. After removing paraffin, the sections were incubated with the primary antibody to acetyl-histone H3 Lys9 or dimethyl-histone H3 Lys9. Confocal scanning microscopy was performed for observation. Hematoxylin-eosin staining was performed for morphological study using a light microscope.

Results: Acetylated histone H3 was detected in the spiral ganglion cells of young and aged groups. However, dimethylated histone H3 was not detected in the spiral ganglion cells and the organ of Corti of the aged group but not in those of the both groups. The degeneration of the spiral ganglion cells was severe in aged group by light microscopy.

Conclusion: Acetylation was observed in young and aged groups, however, methylation was not detected. Histone modification is known to have a critical role in neuro-degeneration. Our findings suggest that epigenetic change participates in the process of presbycusis and these different histone modifications compared to wild type may be due to other mechanism of aging in the Mn-SOD knock out mice.

Keywords: epigenetics, histone modification, Mn-SOD knock out mouse

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Wbp2-deficient mice show progressive high-frequency hearing loss and abnormal cochlear innervation

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The Wellcome Trust Sanger Institute Mouse Genetics Programme generates targeted mouse mutants and screens them for a wide range of disease features, including using Auditory Brainstem Responses (ABR) at 14 weeks to detect new hearing-impaired lines. The *Wbp2* homozygous mutant showed raised thresholds at high frequencies only in this screen. *Wbp2* encodes the WW domain-binding protein 2 that acts as a transcriptional coactivator, binding to the estrogen receptor α (*Esr1*) in the nucleus (Dhananjayan et al. 2006, Mol. Endocrinol. 20:2343).

We found that ABR thresholds were raised as early as 4 weeks of age in the mutants and progressively increased and extended to lower frequencies by 28 and 44 weeks old, indicating progressive hearing loss. Immunocytochemistry revealed widespread expression of *Wbp2* in nuclei of multiple cell types in the cochlea. The gross structure of the mutant middle and inner ears appeared normal, and scanning electron microscopy of the organ of Corti showed no obvious damage or degeneration at 4 and 30 weeks old. Therefore, we examined the innervation of the cochlea using antibodies to neurofilament to label unmyelinated nerve fibres, to CtBP2 to label pre-synaptic ribbons, and to GluR2/3 to label post-synaptic densities, viewed using confocal imaging of mutant and control littermates at 4 weeks old. In the mutants, nerve endings below inner hair cells appeared swollen, synapses were more widely-spread around the basolateral hair cell membranes, synapses appeared smaller, and double labeling suggested that pre- and post-synaptic markers were not as well-aligned as in control inner hair cells. We are looking into the possibility that these features result from glutamate excitotoxicity, and also investigating the link between *Wbp2*, *Esr1* and synaptic defects.

Keywords: Mouse Genetics Programme; progressive high-frequency loss; excitotoxicity; ribbon synapse.

Is a connexin deletion associated hearing loss treatable?

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Introduction: Although numerous causative genes for hereditary hearing loss have been identified, there are no fundamental treatments for this condition. Mutations or deletions in the connexin (Cx) genes are common causes of profound congenital hearing loss in both humans and mice. We set out to examine whether gene therapy could cure hearing loss caused by deletion of the Cx gene.

Objective: We investigated whether supplementary gene therapy in Cx30 knock out (KO) mice could restore postnatal auditory functioning.

Methods: We performed Cx30 gene transfer into the otocysts of homozygous Cx30-deficient mice (C57BL/6 Cx30−/− mice; Klaus Willecke [1]). Gene transfer was achieved via Electroporation-Mediated Transuterine Gene Transfer into Otocysts (EUGO) [2] [3]. Subsequently, we evaluated the transfected area at E18.5 and at postnatal day 30 (P30). We assessed auditory function at P30.

Results: EUGO induced gene transfection in the spiral limbus, the organ of Corti, the stria vascularis, the spiral ligament, and the spiral ganglion at P30. EUGO in the wild-type Cx30 gene in C57BL/6 Cx30−/− mice rstored the lack of Cx30 expression in the cochleae and resolved postnatal auditory function.

Conclusions: These results demonstrate that supplementary therapy utilizing EUGO can restore postnatal auditory functioning of Cx30 KO mice.

Keywords: Genetic hearing loss, otocysts, gene therapy, connexins, connexin30

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Mouse models of deafness that suggest impaired calcium mobilization in inner ear cells: possible pathways that differentiate the phenotype according to the molecular nature of the mutations

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Developing new mouse deafness models will greatly increase our understanding of the mechanisms underlying hearing impairments, as well as improving the diagnosis and treatment of these disorders [1]. Here, we used an ENU-mutagenesis screening platform to identify mice with hearing impairments, with the aim of developing a novel mouse model of human deafness. We screened mice by observing the startle responses evoked by a click box and then measuring the auditory brainstem responses (ABR) [2-3]. The isolated mutants showed a phenotype that was not accompanied by other traits, which is a characteristic typical of human diagnostic-type nonsyndromic deafness. The mutations in four of the lines were mapped to the distal region of chromosome 6. Direct sequencing showed that these lines harbored missense mutations in different domains of pmca2, indicating a possible defect in hair cell calcium-ion mobilization [4]. Mutations in the other 10 lines were mapped to chromosome 10. Five of these lines harbored missense mutations in the extracellular domains of Cdh23 gene products. We observed clear phenotypic variations in the four lines harboring mutations in pmca2, including differential increases in the ABR threshold at the early stage of deafness, followed by the eventual loss of hair cells and spiral ganglion cells at different time points. This phenotypic variation appeared to be caused by differences in the enzymatic activity of the mutated gene products; however, the pathways through which these phenotypes evolve are thought to be less straightforward. A pathway that may explain these variations will be discussed in the context of our current understanding of the function of the pmca2 gene and its relevance to human hearing disorders.

Keywords: ENU mutagenesis; missense mutation; calcium ion; pmca2; Cdh23; nonsyndromic deafness

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Hearing phenotype of the conditional inducible *mapk14*-null mouse

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Human IGF-I deficiency is a rare disease associated with hearing loss, poor growth rates and mental retardation (OMIM608747). *Igf1*-null mice are dwarfs and present congenital profound sensorineural deafness. Analysis of downstream signaling in the cochlea of *Igf1*-null mice has shown an increase in the activity of the stress p38 mitogen-activated protein kinase (MAPK) and altered expression levels of MEF2, FoxM1 and FoxG1 [1]. The p38 MAPK family comprises four members (α, β, γ and δ), being the ubiquitously expressed α isoform critical for mammalian embryonic development [2].

To further explore the role of p38α MAPK (Mapk14) in the cochlea as a potential mediator of IGF-I actions, we have evaluated a conditional knockout mouse with targeted disruption of this gene using the Cre/loxP system. Comparative gene expression analysis of the four p38 MAPKs in the cochlea and brain of p38α-null and wild type mice showed that expression profiling differences of p38 MAPKs depends on the tissue rather than the genotype. Mouse embryonic fibroblasts cell lines derived from p38α-null mice showed an altered expression of MEF2, FoxM1 and FoxG1, according to the changes found in the cochlea of *Igf1*-null mice. Auditory brainstem responses (ABR) were measured in p38α-null and wild type mice before and after a 5-day i.p. treatment with tamoxifen. No changes in hearing thresholds were detected associated to treatment or genotype. Mice were then exposed to a swept sine noise at 105 dB SPL for 30 minutes in a reverberant chamber and ABR performed before and after exposure. Mice showing a total or partial deletion of Mapk14 showed a better recovery of hearing thresholds than wild type mice. ABR parameters measured in double Mapk12/13-null mice, corresponding to p38γ and p38δ isoforms respectively, were identical to those of their wild type littermates.

Keywords: hearing loss, IGF-I, noise-exposure, p38α MAPK

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Temporary dysfunction of outer hair cells after intratympanic application of *Pseudomonas aeruginosa* exotoxin A

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Objectives: To study the early effect of PaExoA on DPOAE thresholds in rat and to ascertain if a single intratympanic dose of Edaravone has an otoprotective effect against PaExoA-induced DPOAE threshold increase.

Materials and methods: Five groups of Sprague-Dawley rats were used. Group 1 (n=9) received 15 µg/20µl PaExoA as an intratympanic injection in the right middle ear. Group 2 (n=11) had 20 µg/20µl PaExoA similary injected. The left ears in these groups, used as controls, were given 20 µl NaCl solution intratympanically. DPOAE measurements were performed ½, 1, 2, 3, 4, 5 and (in group 2) 24 hours after PaExoA injection. Group A (n=4) received 20 µg/µl PaExoA + 3 mg/kg Edaravone after 1 hour; Group B (n=6) 20 µg/µl PaExoA + 3 mg/kg Edaravone after 2 hour; Group C (n=4) 20 µg/µl PaExoA + 3 mg/kg Edaravone after 4 hours. DPOAE measurements were performed 2, 4 and 24 hours after Edaravone injection.

Results: PaExoA caused an early rise in the DPOAE threshold in rats. No such increase was recorded in controls after intratympanic injection of 20 µl NaCl solution. Edaravone 3 mg/kg, given 1, 2, or 4 hours after 20 µg/µl PaExoA, had a limited effect on the rise in DPOAE threshold induced by PaExoA.

Conclusion: Pseudomonas aeruginosa Exotoxin A (PaExoA) causes an early rise in Distortion Product Otoacoustic Emission (DPOAE) thresholds, suggesting a possible dysfunction of outer hair cells (OHCs) appearing within an hour of intratympanic exotoxin injection.

Intratympanic injection of Edaravone, a free-radical scavenger at 1, 2, or 4 hours after 20 µg PaExoA dose not prevent a rise in DPOAE thresholds but has a tendency to limit the effects in the high-frequency region.

Keywords: distortion product otoacoustic emission, Pseudomonas aeruginosa exotoxin A, rat, Edaravone, hearing loss, cochlea, outer hair cells

Ototoxic effects of Mefloquine in cochlear organotypic cultures

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Mefloquine is a widely used anti-malarial drug. Some clinical reports suggest that mefloquine may be ototoxic and neurotoxic. To evaluate the ototoxic and neurotoxic potential of mefloquine, we treated cochlear organotypic cultures with spiral ganglion neurons with various concentration of mefloquine. Mefloquine caused a dose-dependent loss of cochlear hair cells at doses exceeding 0.01 mM; hair cell loss progressed from base to apex and from outer to inner hair cells with increasing dose. Spiral ganglion neurons and auditory nerve fibers were also rapidly destroyed by mefloquine in a dose-dependent manner. Mefloquine-induced hair cell and auditory neuron death is associated with apoptotic features of nuclear fragmentations and caspase activities. Quantitative RT-PCR apoptosis-focused gene arrays (96-well, 84 apoptosis related genes) was used to assess the changes of gene expression in the cochlear explants treated with 100 µM mefloquine for 3 h. Significant up-regulation or down-regulation in gene expressions was detected in 23 genes in the cochlear basilar membrane, and 32 genes in the spiral ganglion neurons with time-matched controls. One of the major causes of cell death by mefloquine is thought to be the depletion of NAD+ in the nucleus and cytoplasm. Consistent with this hypothesis, we report that NAD+ prevents hair cell and neuroaxonal degeneration caused by mefloquine treatment in cultured rat cochlea. We treated cochlear organotypic cultures with 35 µM or 50 µM of mefloquine alone or combined with 5 mM or 20 mM of NAD^+. Treatment with 50 µM of mefloquine alone resulted in nearly 100% sensory hair cell and 100% auditory axon degeneration in both basal and apical turn of the cochlea, while 50 µM of mefloquine with 20 mM of NAD treatment resulted in a significant increase in hair cell and auditory axon survival. The protective effects of NAD may be due to its antioxidation and antiapoptosis. These results suggest that novel therapeutic strategies directed at administration of NAD may be effective for the treatment of neurological side effects of hearing loss, caused by Mefloquine for malaria patients.

Keywords: mefloquine, neurotoxicity, ototoxicity, apoptosis

Otoacoustic emissions and cochlear functionality in workers exposed to styrene

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This study is aimed at testing the sensitivity of otoacoustic emissions (OAEs) as biomarkers of damage due to the exposure to styrene.

The styrene exposure of workers in a glass reinforced plastic factory in central Italy has been assessed by means of different techniques. The biological monitoring was performed by measuring the urinary concentration of Mandelic and Phenilgliossilic acids (MA + PGA), the most important metabolites of the styrene. The styrene concentration was also evaluated directly in blood and in saliva. The data relative to the styrene concentration in biological fluids were correlated to the styrene ambiental concentration. This last was performed with a passive Radiello system and with controlled flux active pumps. Transiently-evoked OAEs (TEOAEs) and distortion product OAEs (DPOAEs) were recorded and analyzed as cochlear functionality outcome variables. A new time-frequency filter based on the wavelet transform was used to suitably separate the different latency components of the TEOAE signal. The short and long latency components were individuated and separately studied as outcome variables of the styrene exposure. Levels of styrene exposure close to the BEI limit values of 400 mg/g of creatinine in urine and to the TLV TWA of 20 ppm in case of ambiental dose were found in a group of workers simultaneously exposed to moderate noise levels (around 80 dBA). A dose response curve was studied correlating the OAEs in the different frequency bands to the interaction between the end shift PGA concentration and the square root of the total life styrene exposure duration. A multivariate ANOVA model was used to study the correlation between OAEs and the exposure groups. OAEs are able to effectively discriminate exposed to noise, styrene and both, from controls. The long latency TEOAE and the filtered signal, summing up the short and the long latency components, are better outcome variables in the discrimination of the exposed from the controls with respect to the non filtered signal. This result suggests the improvement of the diagnostic power if the OAE components approximately coming from the same cochlear region and generated by the same backscattering mechanism are selected.

Keywords: otoacoustic emissions, styrene exposure, time-frequency analysis

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Afferent pathology and repair associate with vestibular dysfunction and recovery during chronic ototoxicity in the rat

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The events involved in chronic vestibular dysfunction are poorly understood. In this study, we aimed at identifying key pathological events associated with functional loss as revealed by behavioural endpoints during chronic ototoxic exposure. Adult male Long-Evans rats were exposed to 20 mM of 3,3'-iminodipropionitrile (IDPN) in the drinking water for 4, 6 or 10 weeks or to 10 mM for 4 or 10 weeks. The animals were studied at the end of the exposure, or after 4 or 10 weeks of recovery. Vestibular function was assessed at weekly intervals by a specific behavioural test battery. Vestibular epithelia were examined by scanning and transmission electron microscopy (SEM and TEM), and confocal immunostaining. Our protocol allowed for the comparison of behavioural, SEM, TEM, and confocal data on an individual basis. In the 20 mM group, vestibular dysfunction started at 2 weeks, increasing progressively afterwards. At 4 weeks, most animals showed significant vestibular impairment. SEM and TEM observations showed intact overall epithelial structure, with no evidence of hair coalescence, or HC degeneration or extrusion. Nevertheless, major changes were observed in the calyx afferents contacting type I HCs (HCI). The electron-dense material characterizing the septate junction between afferents and HCIs was no longer present in most calyx endings. In some cases, the terminals had retracted and only partially covered the HCI membrane, but the retracted afferents were not swollen. These ultrastructural changes were associated with a dramatic loss of the septate junction protein caspr. Animals exposed to 10 mM IDPN showed little or no behavioral or epithelial changes. In the more severely affected animals after 4 weeks of 20 mM, and in animals exposed for longer times, coalescence of stereocilia and HC extrusion were observed. Comparison of animals with similar behavioural dysfunction examined right after exposure or after a recovery period indicated that behavioural recovery associates with afferent repair. We conclude that loss of septate junctions, synaptic uncoupling, and afferent retraction are reversible events associated with the initial loss of vestibular function during chronic ototoxic exposure.

Keywords: Ototoxicity, septate junctions, calyx afferents, vestibular dysfunction, rat.

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Oncostatin M - induced protection against cisplatin ototoxicity is mediated *via* STAT3

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Our preliminary data has implied that oncostatin M (OSM) protects inner (IHC) and outer hair cells (OHC) in the apical and medial part of post-natal rats' organ of Corti (OC) from cisplatin-induced toxicity. Encouraged by these results, we have investigated the role of a signal transducer and activator of transcription 3 (STAT3) in the otoprotective properties of interleukin 6 (IL6)-family members.

For our experiments, we have dissected and explanted the organs of Corti together with *limbus spiralis* and spiral ganglion neurons from postnatal Wistar rats (p3-p5). Explants were preincubated with OSM [30ng/ml] for one day and then simultaneously incubated with cisplatin [15µM] and OSM [30ng/ml] for another day. A second group of explants was treated in the same way with the exception of adding STAT3 inhibitor III / WP1066 [5.6µM] simultaneously with OSM. Control explants were incubated with medium only for two days, negative controls explants were cultured with medium only for one day and with cisplatin for another day and a fifth group with STAT3 inhibitor III alone for two days. Explants were stained with phalloidin-Alexa 488 to visualize and score the hair cells under epifluorescent microscope. The data was statistically analyzed using ANOVA on ranks (Dunn's method).

Obtained data confirm our previous results by demonstrating significant damage induced by cisplatin and equally significant protection against cisplatin ototoxicity induced by OSM in both hair cells types. OSM-induced protection was abolished by using STAT3 inhibitor III. Incubation with the inhibitor alone did not alter the morphology of IHC or OHC.

Presented results corroborate our hypothesis about STAT3 being an essential piece in the OSM-mediated otoprotection. STAT3 inhibitor III does not damage hair cells but blocks the OSM-induced otoprotection. These results suggest a key role of STAT3 in the protective signaling pathway induced by OSM and other IL6 family members.

Keywords: cisplatin, oncostatin M, otoprotection, ototoxicity, STAT3

Protective role of edaravone against neomycin-induced ototoxicity in zebrafish

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Aminoglycosides are one of the most commonly prescribed types of antibiotics worldwide. However, these drugs appear to generate free radicals within the inner ear, which can result in permanent hearing loss. Although some studies were reported that edaravone has side effects such as nephrotoxicity and cytotoxicity, edaravone, a neuroprotective agent, exhibits potent free radical scavenging and antioxidant actions without causing serious side effects. We evaluated the effects of edaravone on neomycin-induced ototoxicity in transgenic zebrafish. The 5-day post fertilization (dpf) zebrafish larvae were exposed to 125 μM neomycin and various concentrations of edaravone for 1 h. Hair cells within neuromasts were analyzed under a fluorescence microscope and confocal microscope. Hair cell survival was calculated as average numbers of the hair cells in the control group, which was not exposed to neomycin. Ultrastructural changes were evaluated using a scanning electron microscope and transmission electron microscope. Edaravone protected against neomycin-induced hair cell loss in the neuromasts (1,000 μM: 11.6 ± 1.1 cells, neomycin only: 5.5 ± 0.5 cells; $n = 10$, $P < 0.05$) and decreased the TUNEL reaction. In ultrastructural analysis, structures of mitochondria and hair cells within neuromasts were preserved in zebrafish exposed to 125 μM neomycin and 1,000 μM edaravone for 1 h. In conclusion, edaravone protected against neomycin-induced hair cell loss by preventing apoptosis.

Keywords: edaravone; neomycin; ototoxicity; zebrafish

Acknowledgements: This research was supported by a Korea University Grant, by the Soo ENT Clinic and the Communication Disorders Center, Korea University.

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Systemic treatment with resveratrol and N-acetylcysteine prevents from local ototoxicity with kanamycin and furosemide in rats

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Introduction and Objective: Oxidative stress is one of the main mechanisms in the pathophysiology of aminoglycoside ototoxicity. Therefore, treatment with combination of antioxidant drugs represents a rational therapeutic approach. The objective of this study is to determine the ability of a combination of resveratrol and N-acetylcysteine in preventing hearing loss induced by local application of an ototoxic mixture of kanamycin and furosemide.

Material and Methods: Twenty-four 2 month-old male Wistar rats were used in this study. Local application of a concentrated solution of kanamycin and furosemide was performed through a bullostomy. One group (n=13) received an intraperitoneal dose of resveratrol (10 mg/kg/day) and N-acetylcysteine (NAC, 400 mg/kg/day) for 5 days starting the day before surgery. Control group (n=11) received similar volume of saline.

Hearing function was evaluated with auditory brainstem responses (ABR) before and 3, 7 and 21 days after surgery.

Results: Rats treated systemically with resveratrol and NAC showed lower ABR thresholds at the end of the treatment, with statistically significant differences compared to saline group, but not at 7 and 21 days after surgery.

Conclusions: The preliminary results of this studio suggested a protective effect of systemic antioxidant treatment with resveratrol and NAC in kanacimina and furosemide induced local ototoxicity. However the beneficial effect is temporally limited, so a prolonged treatment will be considered in the next experiments.

Keywords: antioxidants, combination, drug therapy, ototoxic damage

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Ear in a dish: development of an *in vitro* assay for ototoxic and otoprotective drugs screening

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Hearing loss is a dose-limiting severe side effect frequently observed in treatment involving aminoglycosides or platinum-containing chemotherapeutics. The mechanisms of drug induced ototoxicity are not well understood, furthermore otoprotective drugs that prevent hearing loss are lacking. Recently screening for ototoxicity was assessed using inner ear cell lines derived from the immorto mouse or a lateral line model from zebrafish larvae. Because inner ear cell lines may behave different as primary cells in response to drug treatment due to their immortalization state, and the zebrafish larvae is a non-mammalian model, we intend to develop an *in vitro* standardized assay for ototoxic/otoprotective drug screening based on murine organ of Corti progenitor cells.

Differentiation of otospheres derived from the mouse postnatal organ of Corti is known to give rise to a small population of hair- and supporting cell-like cells *in vitro* [1]. Our previous results demonstrated an improvement in the amount of hair cell- (MyosinVIIa) and supporting cell marker (SOX2) expressing cells by chemical inhibition of the Notch pathway in the apical part of the postnatal day 0 mouse organ of Corti.

To induce death of hair cell-like cells, Neomycin (1 mM, 24 h) was applied to otospheres derived from postnatal day 0 organ of Corti. Compared to the untreated spheres, inhibition of the Notch pathway increased the number of cells expressing hair cell or supporting cell markers. Furthermore, we found that newly differentiated hair cell-like cells were sensitive to aminoglycoside treatment *in vitro*, in a time window dependent manner, while a stable number of SOX2 expressing cells was maintained. Our results show that the establishment of a standardized mammalian *in vitro* assay is an outstanding tool for screening of potential ototoxic drugs. The assay may also be valuable to screen for otoprotective compounds.

Keywords: aminoglycosides; hearing loss; in vitro model; ototoxicity; progenitor cells.

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Deiters cells regulate the remodelling of aminoglycoside-injured organ of Corti, through the release of HMGB1

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To examine whether an inflammatory process occurs in the amikacin-poisoned cochlea, we investigated the presence of the cytokines TNF-alpha, IL-1beta and IL-10. No TNF-alpha, IL-1beta or IL-10 was detected in the cochlear perilymph after the loss of most auditory hair cells, indicating the absence of severe inflammation. In contrast, we observed a significant and temporary increase in extracellular HMGB1, a late mediator of inflammation which also functions as a signal of tissue damage [1]. This increase coincided with epithelial remodelling of the injured organ of Corti and occurred concomitantly with a robust and transient cytoplasmic expression of acetylated HMGB1 within the non-sensory supporting cells, Deiters cells. Here, HMGB1 was found to be enclosed within vesicles a number of which carried the secretory vesicle-associated membrane-bound protein Rab 27A. In addition, a transient up-regulation of RAGE, an HMGB1 membrane receptor [2], was found in most epithelial cells of the scarring organ of Corti when extracellular levels of HMGB1 were at their highest. Altogether these results strongly suggest that in stressful conditions, Deiters cells liberate HMGB1 to regulate the epithelial reorganisation of the injured organ of Corti through engagement of RAGE in neighbouring epithelial cells.

Keywords: cochlea, cytokines, ototoxicity, rat.

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Preliminary results with the NMDA antagonist memantine in salicylate-induced tinnitus

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Background: Short-term tinnitus develops shortly after the administration of a high dose of salicylate. Since salicylate selectively potentiates the N-methyl-D-aspartate (NMDA) currents in spiral ganglion neurons, it may play a vital role in tinnitus by amplifying NMDA-mediated neurotransmission. The aim of this study was to determine whether systemic treatment with a NMDA channel blocker, memantine, could prevent salicylate-induced tinnitus in animals. Additional experiments were performed to evaluate the effect of memantine on the auditory brainstem response (ABR) and distortion product otoacoustic emissions (DPOAE) to test for changes in hearing function.

Methods: Thirty-six rats were divided into 3 groups and were treated daily for four consecutive days. One group (n=12) was injected with salicylate (300 mg/kg/d, IP), the second (n=12) was treated with memantine (5 mg/kg/d, IP) and the third group (n=12) was injected with salicylate and memantine. All rats were tested for tinnitus and hearing loss 2, 24, 48, 72 hours after the first drug administration and 24 h post treatment; tinnitus-like behavior was assessed with gap prepulse inhibition of acoustic startle (GPIAS) and hearing function was measured with DPOAE, ABR and noise burst prepulse inhibition of acoustic startle (NBPIAS).

Results: Rats in the salicylate group showed impaired GPIAS indicative of transient tinnitus-like behavior near 16 kHz which recovered 24 h after the last salicylate treatment. Memantine did not cause a significant change in GPIAS. Combined injection of salicylate and memantine significantly attenuated GPIAS tinnitus-like behavior 48 hours after the first injection. None of the treatments induced permanent threshold shifts in the ABR and DPOAE, that recovered completely 1 day post treatment. Animals treated with salicylate plus memantine showed results comparable to animals treated with salicylate alone; confirming that there is no effect of memantine on DPOAE which reflects OHC function.

Conclusion: The present study confirms the role of cochlear NMDA receptors in the induction of salicylate-induced tinnitus.

Keywords: tinnitus, memantine, salicylate, startle reflex, NDMA receptors, rats

Effects of the calyx on the apparent properties of vestibular type I hair cells K^+ currents

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Vestibular Type I hair cells are almost entirely enveloped by a single large afferent nerve terminal, called calyx, whose functional meaning is still enigmatic. Another defining property of Type I cells is the expression of a low-voltage-activated outward rectifying K^+ current, named $I_{K,L}$. By patch-clamp whole-cell recordings from *in situ* mouse vestibular Type I cells, we have found that $I_{K,L}$ activation can result in K^+ accumulation around the cell, as inferred from the positive shift of K^+ currents reversal potential (VrevK$^+$), presumably due to the presence of a residual calyx [1]. This phenomenon accompanied to a slower $I_{K,L}$ deactivation during hyperpolarizing voltage steps. We have investigated this aspect in more detail, and found that $I_{K,L}$ deactivated with a slow complex time course, not consistent with the reported exponential decay [2]. Since most previous studies were done in isolated Type I cells, we repeated the experiments in enzimatically dissociated cells and found that both the shift of $V_{rev}K^+$ and the alteration of $I_{K,L}$ deactivation were absent or much less obvious. Our hypothesis is that most of the calyx survives *in situ*, but not after cell dissociation, which would represent a restriction to K^+ diffusion, and a resistance (R_e) to current flow between the synaptic cleft and the bath. As a consequence, large ion currents would produce a significant voltage drop (V_e) across R_e. V_e would be maximal at the peak amplitude of the instantaneous $I_{K,L}$, and then decrease with $I_{K,L}$ deactivation. Thus, $I_{K,L}$ deactivation would be distorted by voltage-clamp failure due to R_e, which we estimated in tens of MΩ, i.e. in the same magnitude order of the calyx input resistance (~90 MΩ; [1]). In conclusion, our data demonstrate for the first time that the calyx can significantly influence, via a purely electrical mechanism which adds to the effects of K^+ accumulation in the cleft, the behavior of the currents generated by the hair cell membrane.

Keywords: Type I Hair cell; $I_{K,L}$; Nerve calyx; Vestibular

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Proteomic analysis of vestibular schwannoma

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Objectives: The aims of this study were to evaluate the expressed proteins in vestibular schwannoma in comparison with vestibular nerve tissue and to identify pathways that may be altered in vestibular schwannoma.

Methods: Proteins were extracted from two vestibular schwannoma specimens and two vestibular nerve tissues and analyzed in parallel by two-dimensional electrophoresis (2-DE). We then analyzed 29 spots that were differentially expressed using matrix-assisted laser desorption and ionization time of-flight mass spectrometry (MALDI-TOF MS). Up-regulated proteins were confirmed by means of western blot.

Results: Twenty proteins were identified that show significantly changes in the expression level between vestibular schwannoma tissue and vestibular tissue. Among these proteins, 7 proteins were related to apoptosis.

Conclusion: Our findings suggested that apoptosis is involved in the pathogenesis of vestibular schwannoma. And proteomic analysis may be powerful tool for the identification and characterization of many promising candidate proteins relating vestibular schwannoma.

Keywords: Proteomic analysis; Protein; Vestibular schwannoma

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Sudden sensorineural hearing loss, inflammatory disease & TNF-alpha

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Inflammation and reductions in cochlear blood flow can induce hearing loss. Most investigations have separated the 2 etiologic factors (that is, inflammation versus vasculopathy), under the assumption that the 2 pathogenic mechanisms are distinct and mutually exclusive. Our previous experimental data demonstrate that tumor necrosis factor reduces cochlear blood flow. In this study we investigated the effect of TNF-alpha inhibitor etanercept in patients with a chronic inflammatory disease suffering from sudden sensorineural hearing loss.

In a clinical study, a total of 15 adult patients suffering from distinct chronic inflammatory diseases presenting with typical symptoms of sudden hearing loss who were not responsive or only partially responsive to prednisolone treatment were identified and selected for etanercept treatment. Etanercept (25 mg S.C.) was self-administered twice a week for 12 weeks.

9 of 15 patients treated with etanercept had significant improvement of hearing. 2 out of 15 had a recurrence after finishing the treatment.

The improvement of sudden hearing loss in patients with chronic inflammatory diseases suggests that TNF-alpha plays a major role in the pathogenesis of sudden sensorineural hearing loss in these patients.

Polymorphisms in genes involved in the free-radical process in patients with Ménière's disease and sudden sensorineural hearing loss

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Introduction: Although the etiologies of Ménière's disease (MD) and sudden sensorineural hearing loss (SSNHL) remain unclear, genetic factors could contribute, at least in part. Recently, accumulating evidence has demonstrated that free radicals are related to the pathology of inner ear disease. We investigated the association between genetic polymorphisms located in genes related to the free-radical process and susceptibility to MD and SSNHL in the present study.

Methods: Patients affected by MD or affected by SSNHL, 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: methionine synthase (*MTR*; rs1805087); methionine-synthase reductase (*MTRR*; rs1801394); nitric oxide synthase 3 (*NOS3*; rs1799983); caveolin 1 (*Cav1*; rs3840634); melatonin receptor 1B (*MTNR1B*; rs1387153); NAD(P)H oxidase p22(phox) subunit (*NADH/NADPHp22phox*; rs4673); and mitochondria 5178 (*MT5178*; rs28357984) were investigated for statistical analysis.

Results: The *Cav1* polymorphism was significantly associated with a risk of MD; moreover, the OR for the *Cav1* polymorphism and MD risk was 1.849 (CI: 1.033–3.310) with adjustment for age and sex. The *NOS3* polymorphism was significantly associated with a risk of SSNHL; in addition, the OR for the *NOS3* polymorphism and SSNHL risk was 2.108 (CI: 1.343–3.309) with adjustment for age and sex. The remaining five polymorphisms failed to show any associations with the risk of MD and SSNHL.

Conclusion: In conclusion, the *Cav1* and *NOS3* polymorphisms were significantly associated with the risk of MD and SSNHL, respectively.

Keywords: sudden sensorineural hearing loss, Ménière's disease, free radicals, case-control study, polymorphism

Acknowledgements: This study was supported by research grants (21390460, 20591979) from the Ministry of Education,Culture, Sports, Science and Technology, and research grants for Longevity Sciences (20shi-2, 21A-17) and a research grant (H20-Nanchi-021) from the Ministry of Health, Labour and Welfare of Japan.

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Reducing noise-induced hearing loss by peptides targeting transforming growth factor-β1

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Noise-induced hearing loss (NIHL) represents more than one-third of hearing impairment cases in developed countries and has become an important public health priority. Excessive exposure to noise activates inflammatory and immune responses in the inner ear that, if unregulated, could eventually contribute to cochlear damage and hearing loss. Transforming growth factor-β is a key regulator of both responses and has been associated with otic injury.

To evaluate the potential of targeting TGF-β1 as a therapeutic strategy in noise induced hearing loss, we have studied hearing, cochlear morphology and TGF-β signaling in mice treated with TGF-β1 peptidic inhibitors P17 and P144, and exposed to noise following standard methodology.

Our results indicate that systemic administration of P17 and P144 prior to noise exposure attenuated the impact of cochlear damage in mice, therefore suggesting a protective effect. Both peptides also showed a dose-related therapeutic effect when administered after noise challenge, improving significantly the evolution of hearing thresholds and ameliorating the degenerative changes, especially in lateral wall structures. This therapeutic effect was not clearly associated with the blockade of the TGF-β1 signalling pathway and the reduction in the expression of p-SMAD2, suggesting alternative molecular pathways for TGF-β1 inhibitors to control cochlear inflammation and after noise damage. In addition, local administration of P17 and P144 close to the round window by means of bullostomy also showed curative effects, achieving a significant smaller hair cell loss in the organ of Corti, compared to saline. In conclusion, TGF-β1 inhibition with these peptides represents a promising new therapeutic line for noise-induced hearing loss.

Keywords: cochlea, noise-induced hearing-loss, TGF-α1 inhibition.

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IGF-I deficit predisposes to noise-induced hearing loss in mice

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Background: Human IGF-I deficiency (ORPHA73272, OMIM608747) is a rare disease associated with poor growth rates, mental retardation and syndromic hearing loss. Equally, Igf1$^{-/-}$ mice are dwarfs with poor survival rates and congenital profound deafness [1]. IGF-I is a neuroprotective agent, and accordingly, the physiological age-related decrease in circulating IGF-I levels have been related to cognitive and brain alterations. Igf1$^{-/-}$ mice present undetectable serum levels of IGF-I throughout its life, whereas Igf1$^{+/-}$ and Igf1$^{+/+}$ littermates show an age-dependent decrease in IGF-I serum levels, especially from 6 months of age on [2], which correlates with the increase in ABR thresholds. However, there is little information on the effect of low levels of IGF-I on the susceptibility to noise induced hearing loss (NIHL) and the potential protective actions of IGF-I against this condition.

Methods: We have studied the susceptibility of Igf1$^{+/-}$ and Igf1$^{+/+}$ mice to NIHL at different ages, with functional (auditory brainstem responses, ABR), morphological (cochlear histology and stereological hair-cell quantification) and molecular (RT-qPCR, Western Blotting) studies.

Results: Noise-exposure experiments with 1 and 3 months-old mice did not reveal differences between genotypes, both genotypes were equally sensible to NIHL. However, 6 month-old Igf1$^{+/-}$ presented a greater susceptibility to noise damage, with higher threshold shifts and a poorer recovery compared to noise-exposed Igf1$^{+/+}$ mice. Igf1$^{+/-}$ mice showed several morphological changes, including the loss of hair cells, as well as alterations in the main IGF-I signalling pathways and genotype-related changes in gene expression. These changes correlated with the low IGF-I serum levels observed in heterozygous when compared with wild type mice.

Conclusion: These results support the idea that low levels of IGF-I predisposes to higher susceptibility to NIHL. Therefore, IGF-I- based therapies could contribute to prevent or ameliorate age related and noise-induced hearing loss.

Keywords: IGF-I, aging, hearing, noise-induced hearing loss.

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Functional and anatomical correlation in noise-induced hearing loss mouse model

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Introduction and Objective: The cochleogram is a graphic record that represents hair cell populations along the length of the basilar membrane of the organ of Corti (OC), and relates cell damage with threshold shifts for specific frequencies [1].

The purpose of this study is to determine the changes in hair cell density and distribution using a home-made cochleogram in a noise-induced hearing loss mice model, and to find functional and morphological correlations.

Materials and Methods: 12 two month-old male CBA/CaOlaHsd mice were evaluated with auditory brainstem responses (ABR) at different times (baseline-2-14-28 days) and divided into 2 groups: control (n=6) and noise-exposed (violet swept sine noise [2], 105 dB SPL for 30 minutes, n=6).

The cochleae were extracted, fixed, decalcified and divided into two parts (apical-middle and basal), obtaining around 80% of the whole extent of the OC. Using a fluorescence microscope and stereological software, the total length of the OC was divided into equidistant 5% sectors [3] [4]. The number of inner (IHC) and outer (OHC) hair cells in randomly distributed areas were determined, and cell density (cells/100 µm2) was estimated for each sector.

Results: Mice exposed to noise showed an evident ABR threshold shift (40-50dB) in response to click and tone burst stimuli 2 days after noise exposure and a poorer recovery. Control group maintained threshold values throughout the experimental procedure. Peak latencies decreased slightly early after noise damage and ABR peak amplitudes decayed 2 days after noise insult and did not recover one month after. Noise exposure altered cochlear morphology, with disruption of stereocilia and loss of hair cells, mainly OHC, especially in the basal turn of the cochlea. The overall mean densities were: Left IHC:4.08, OHC:8.66, ratio IHC/OHC:2.11; Right IHC:3.93, OHC:8.40, ratio IHC/OHC:2.14. In contrast, the control group presented a normal cytoarchitecture and a homogeneous distribution of hair cells along the cochlea [5]. (Left IHC:4.10, OHC:12.12, ratio IHC/OHC:2.95; Right IHC:4.20, OHC:12.22, ratio IHC/OHC:2.91; $p<0.05$).

Conclusion: This method allows us to quantify the hair cell populations throughout the cochlear length in a precise and reproducible manner and can be used to assess hair cell damage and protection in experimental mice models.

Keywords: cochleogram, hair cells damage, noise induced hearing loss

Acknowledgements: This work was supported by grants from Fondo de Investigaciones Sanitarias to TR and SAF2011-24391 to IV-N. SMC holds a postdoctoral contract of CIBERER. We appreciate very much the technical help of L. Barrios (CTI, CSIC) and Raquel Martinez Vega (IIBm, CSIC).

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Protective effect of rosmarinic acid in noise induced hearing loss: a functional and morphological study

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Introduction: Several studies have recently identified the oxidative stress, induced by acoustic trauma, as the pivotal pathway of cochlear damage. Namely, a progressive increase of ROS and of lipid peroxidation, in conjunction with an imbalance of antioxidant defenses, have been demonstrated to play a significant role in noise-induced hearing loss (NIHL) as they largely participate in cellular mechanisms that underlie hair cell death after noise exposure. It has been verified that several molecules with antioxidant and scavenging properties can reduce oxidative stress-induced hair cells death. The aim of this study was to investigate the protective effect of Rosmarinic Acid (RA) on noise-induced hearing loss. Among the molecules derived from phenolic acid, RA is a natural antioxidant found in many herbs of the Lamiaceae family with multiple biological activities such as antioxidant, anti-inflammatory and antiviral activities. Furthermore, recent studies have shown that RA is able to enter spontaneously into membranes, and it is efficiently able to prevent lipid peroxidation.

Materials and Methods: In this study Wistar rats were used as a model of acute acoustic trauma. Animals were exposed for 60 minutes to 120 dB SPL, 10 kHz. Of these, a group was treated with RA (i.p. 10mg/Kg 1h after trauma and for 2 consecutive days). We evaluated the effectiveness of RA by measuring Auditory Brainstem Responses (ABR), the extent of damage with cochleogram, the magnitude of lipid peroxidation by 4-hydroxynonenal (4HNE) expression and the superoxide amount with DHE assay.

Results: Our results demonstrate that RA can: (a) prevent hearing loss by reducing ABR threshold shifts at 1, 3 and 5 days after acoustic trauma, (b) decrease hair cell loss as shown by cochleogram, (c) decrease oxidative stress by reducing superoxide amount in the cochlea and (d) prevent lipid peroxidation as shown by decreased expression of 4HNE.

Conclusion: Based on our preliminary results, we speculate that RA treatment can reduce the cochlear damage caused by an acute exposure to noise probably by preventing lipidic peroxidation of membranes and thus RA provides a promising approach against NIHL.

Role of p66shc in noise induced hearing loss: a functional and morphological study in mice and rats

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Introduction: Oxidative stress and the increased production of reactive oxygen species (ROS) in conjunction with an imbalance of antioxidant defences have been demonstrated to play a significant role in noise-induced hearing loss (NIHL).

The p66Shc protein is a Src homologue and a collagen homologue (Shc) adaptor protein. The p66Shc isoform participates in mitochondrial ROS generation and acts as an oxidative signal regulator, which translates oxidative signals to the mitochondria. Upon oxidative stress, p66Shc is phosphorylated at a serine residue (Ser36). The phosphorylation of Ser36 is necessary for the cellular response to oxidative stress and is an important element for ROS induction. However, no study of p66Shc in NIHL has previously been reported thus, the goal of this study was to investigate the role of p66Shc in noise-induced oxidative stress and hearing impairment.

Material and Methods: Mice 126 SvEv wild type (WT) or knock-out (KO) for P66shc and Wistar rats were used in this study. Acoustic trauma was induced in animals by a continuous pure tone of 120 dB SPL, 10 kHz for 1 hour. Auditory function was measured by recording the auditory brainstem responses (ABR) at 6-32 kHz, 24h after the acoustic trauma. Immunofluorescence labelling and western blot analyses were performed for evaluate Ser36-P-p66Shc, SOD activity and superoxide amount in the cochlear tissue.

Results: The functional evaluation illustrated a greatest threshold shift in WT mice compared to KO mice. The morphological analyses revealed an increase of Ser36-P-p66Shc, SOD activity and superoxide amount in mouse WT and rat cochleae after the acoustic trauma exposure, in particular, in the stria vascularis. The later is an essential structure of the inner ear, it contains abundant mitochondria and has the highest rate of aerobic oxidation in the inner ear.

Conclusion: In conclusion, we established that the hearing impairment induced by noise exposure is associated with an oxidative stress-related increase in the expression of p66Shc and Ser36-P-p66Shc and the translocation of p66Shc to the mitochondria. Considering that oxidative stress plays a key role in NIHL, the involvement of p66Shc as a signaling agent may provide important insights into the mechanism of NIHL.

Analysis of vulnerability of hearing loss over age subsequent to the deletion of BDNF or CaV1.2 in the cochlea

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We recently showed that BDNF deletion in the cochlea is protective for IHC synapses following acoustic noise exposure (Zuccotti et al., 2012) and that this protection partly related to the L-type voltage-gated calcium channel CaV1.2 (Zuccotti et al., submitted). The present study aimed to assess if deletion of BDNF in auditory periphery and specific brainstem structures alters the vulnerability of hearing loss over age.

We compared the hearing function of young (1-3 months) and old (8-16 months) conditional knockout mice as [1] BDNF^{Pax2} KO mice (Zuccotti et al., 2012) with the deletion of BDNF in the whole cochlea, the dorsal cochlear nucleus, and inferior colliculus, and [2] the CaV1.2^{Pax2} KO mice (Zuccotti et al., submitted) with deletion CaV1.2 in the same regions as in the BDNF^{Pax2} KO mice. All mice were hosted in the animal facility for up to 16 months under the protocol reviewed and approved by the animal welfare commissioner and the regional board for scientific animal experiments in Tübingen. Animals were regularly observed by a veterinarian. The hearing function of young (1-3 months) and old (8-16 months) mice was compared by measuring the auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE). Thresholds and above-threshold ABR were analyzed for the young and old mice. We also analyzed the influence of acoustic noise exposure on the development of hearing loss over age. Our data can contribute to the better understanding of the role of BDNF in the auditory function and the loss of hearing function during aging in animals and humans.

Keywords: Age-related hearing loss, auditory brainstem response, brain-derived neurotrophic factor, CaV1.2, noise-induced hearing loss

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Analysis of acute stress on long-term vulnerability after an acoustic injury in a mature rat model

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Excessive noise is a global health hazard that leads to noise-induced hearing loss and presbycusis, currently with no successful clinical treatment. We recently found evidence for a differential influence of stress (corticosterone level) on the vulnerability of hearing function after an acoustic injury [1].

Questioning how stress might influence auditory processing we considered to study the interference of sound exposure and stress pathways. The feedback regulation of the body's stress system with the HPA axis as its core is mediated by glucocorticoids through intracellular receptor subtypes of which the mineralocorticoid (MR) and glucocorticoid (GR) receptors are best known.

To investigate the role of GR and MR for hearing function, we tested the influence of GR and MR potent drugs in the presence of acute stress on hearing processing after an acoustic injury in a mature rat animal model. Stress levels were determined via urinary corticosterone measurements using ELISA during and at the end of the testing period. The hearing function was monitored for 14 days after the acoustic injury via auditory brainstem responses (Click- and Frequency-ABR) and distortion-product otoacoustic emissions (DPOAE). We will present data that give a first insight in a presumptive role of GR and MR referring to sound processing after an acoustic injury.

The results will help to identify compounds for a suitable clinical therapeutic intervention to prevent loss of hearing after exposure to traumatizing noise.

Keywords: stress, glucocorticoid, GR, MR, threshold shift.

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Lack of BDNF in the cochlea but not in the brain influences sound processing

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In a previous study, we found that brain derived neurotrophic factor (BDNF) influences the IHC characteristics and auditory brainstem responses (ABR) differentially in the intact and injured cochlea [1]. In the present study we question if these influences are a result of BDNF expression in the auditory periphery or in the central nervous system (CNS).

A conditional BDNF KO mouse line was generated using the Cre-LoxP system under the TrkC promoter know to be expressed in most brain neurons as e.g. in the hippocampus, cerebral cortex and the cerebellum [2]. By crossing the trkC-Cre mice with Rosa26 mice as well as by northern blot and western blot we could verify the deletion of BDNF in cochlear neurons as well as in tissue from inferior collicolus (IC) and auditory cortex (AC). The hearing function was measured before and 1-3 weeks after a moderate noise exposure by Click-evoked ABR thresholds, tone-burst evoked ABR thresholds and DPOAEs. Therefore ABR wave amplitude growth functions were calculated for increasing stimulus levels with reference to the ABR thresholds and analyzed. Peripheral and central molecular markers of plasticity were also measured and analyzed.

We present first data that indicate a special function of BDNF in the peripheral organ for central sound processing and vulnerability after acoustic injury.

Keywords: BDNF, trkC, ABR, DPOAEs, noise exposure

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Hearing and comorbidities in patients with professional hearing loss

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Introduction: The relevance of professional sensorineural hearing loss (SNHL) does not decrease with time. Installation of modern equipment, the improvement of personal protective equipment can only reduce the harmful effect of the noise factor, which is associated with damage to the auditory function. The manufacturing process requires workers concentration, physical endurance. This load in noise causes fatigue, exhaustion of internal compensation mechanisms of the body.

Methods: We examined 70 patients with professional SNHL in age from 19 to 57 years who had a record of service in noise conditions. The diagnosis was confirmed by tonal threshold audiometry, audiometry in the extended frequency range, the study of uncomfortable loudness levels.

Results: By age all patients were divided into three groups: under 30 years (37,5%), from 30-39 years (29,4%), 40 years and more (33%). With increasing age and length of a recode of service the professional hearing loss increased, while the number of those without comorbidities, decreased: in group 1, there were 8 patients (11,4%), in second group – 5 patients (7,1%),and in third group -1 patient. (1,4%). Comorbidities were identified in 86,7% of the subjects. After age 40 in patients there were registered 3 and more comorbidities (43,3% cases). Between comorbidities we observed more common diseases of the eye and adnexa (35,7% of cases), respiratory (28,6%), blood circulation system (20%).

Conclusion: By the time noise renders its negative effects not only on auditory function, but also on the condition of the body as a whole, that confirms the growth of comorbidity. With age under the influence of harmful factors the depletion of compensatory mechanisms probably takes place.

Keywords: professional hearing loss, disease, noise

Synergistic effect of Styrene exposure and acoustic trauma: a functional and morphological study in a model of noise induced hearing loss (NIHL)

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Introduction: In the western world the chemical processing industry is the third largest industry counting, only in Europe, about 1,2 million workers. The study of risk factors related to occupational exposures in this context assumes a relevant significance and the health effects caused by organic solvents have long been investigated. Among them styrene is extensively used in the chemical industry. It is an organic compound widely used in the industrial production of plastics and resins, currently classified as an ototoxic agent and a possible carcinogen in humans. It is also known the synergistic effect of styrene exposure and numerous other toxic substances and/or environmental insults such as noise exposure. The later aspect is particularly important since, in industrial working environments, occupational exposure to the first factor implies very often the coexistent exposure to the last one.

In this study we evaluated the synergistic effect between exposure to styrene and chronic acoustic trauma on cochlear cell damage pattern.

Materials and Methods: Wistar rats were exposed to styrene by gavage and to chronic noise exposure (100 dB SPL, 10 kHz pure tone, 1h/day for 10 days). The induced hearing loss in treated groups was functionally assessed by auditory brainstem responses (ABRs) and the cochlear damage and oxidative mechanisms was quantified by immunohistochemical analyses.

Results: In the rat model, styrene exposure causes a significant hearing loss and cochlear damage that induces the activation of oxidative stress mechanisms. Concomitant noise exposure significantly increases styrene.

Conclusion: Based on results, we speculate that the association between noise and organic solvent exposure in industrial working represent a risk factor for the health workers.

POSTER PRESENTATIONS P-62

Review of TNF blockers and other biological therapy agents in autoimmune inner ear disease

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Objectives: Evaluation of the results achieved in clinical studies that have tested TNF-α blockers and other biological therapy agents in patients with autoimmune inner ear disease (AIED) and sensorineural hearing loss secondary to other autoimmune diseases.

Methods: A systematic search was performed for studies that analyze the response to TNF-α blockers and other biological therapy agents in patients with AIED, with special emphasis on inclusion and exclusion criteria, the characterisitics of the patients studied, response criteria, outcome measures and type of clinical study.

Results: Fifteen relevant articles were identified among 207 articles in PUBMED (last review in April, 2013). Four articles analyzed the response to etanercept, 3 to infliximab, 2 to adalimumab, 1 to a combined etanercept plus methotrexate therapy, 3 to anakinra and 2 to rituximab. The response rate was higher than 70% in most of the articles reviewed. It is important to emphasize that poorer response was achieved in those studies that evaluated short-term response and those that included patients already refractary to other treatments such as corticosteroids or ciclophosphamide.

Conclusions: The results achieved so far with biological therapy agents are encouraging. However, since to date very few randomized clinical trials have been conducted, more studies are needed to confirm these results. The outcome is better in those patients with recent hearing loss so that it would be advisable to initiate treatment with TNF blockers and other biological therapy agents promptly in those patients who do not achieve or maintain good results with corticosteroids, or when these are contraindicated.

Keywords: Autoimmune inner ear disease, systematic review, TNF-α blockers, biological therapy agents.

Efficacy of etanercept in an animal model of autoimmune labyrinthitis

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Objectives: Evaluation of the efficacy of etanercept, a TNF-α blocker, to prevent or restore the functional and morphological changes in the guinea pig cochlea in an experimental labyrinthitis model with keyhole limpet hemocyanin (KLH). To compare these results with those achieved with corticosteroids in the same animal model.

Methods: Thirty female albino Dunkin-Hartley guinea pigs weighing between 300 and 700 g were employed. Twenty five animals were immunized with subcutaneous and intracochlear KLH. Of these animals, 20 were treated intraperitoneally with either 1 mg/kg of 6-methylprednisolone (n=10) or 2.5 mg of etanercept (n=10) 30 minutes before the cochlear perfusion and 3 days after it, and 5 animals did not receive any treatment. Five animals received PBS (subcutaneous and intrachoclear) and constituted the control group. Auditory brainstem response (ABR) tests were performed on both ears of each animal before and after immunization. Scanning microscopy was used to study pathological changes in the inner ear.

Results: Hearing loss was lower in the control group (p=0.001) and in both treatment groups (6-methylprednisolone, p=0.007; etanercept, p=0.001) than in the untreated animals, but the threshold shifts were not significantly different between the two treatment groups. The loss of hair cells was lower in the control group (p=0.02) and the etanercept group (p=0.026) than in the untreated animals. There was no significant difference between the two treatment groups.

Conclusions: There is a significant reduction in the ABR shifts and loss of hair cells after etanercept treatment in this animal model. Etanercept is at least as effective as glucocorticoids to block inner ear damage after autoimmune challenge.

Keywords: Experimental labyrinthitis, guinea pig, etanercept, scanning microscopy, hair cells.

Guidance of spiral ganglion cell neurites using a nanomatrix *in vitro*

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In severe hearing loss or deafness auditory function can partially be restored by a cochlear implant (CI) which electrically stimulates the auditory nerve, mimicking lost inner ear sensory cells. A limiting factor in CI performance is the anatomical gap between the electrode array in scala tympani and the stimulated regions of the auditory nerve in Rosenthal's canal. One approach to create a gapless interface is to initiate growth of spiral ganglion cell dendrites towards the CI electrode. Since these neurites do not grow into fluid-filled spaces, the scala tympani may be bridged with a skeleton that allows neurite growth towards the implant. Therefore, we set out to test injectable gels that may provide scaffolds for outgrowing neurites and also serve as a reservoir for growth- and guidance factors.

Protocols to form stable nanomatrix droplets were established to test commercially available 3-D nanomatrices for their ability to allow neurite growth in an organotypic culture model of the postnatal (P3-5) mouse spiral ganglion. Spiral ganglion explants were placed next to these nanomatrices. Spouting of neurites was stimulated by the application of brain-derived neurotrophic factor (BDNF). Neurite growth was analyzed using a confocal laser scanning microscope allowing three-dimensional reconstruction of the nanomatrix and the sprouting neurites. Multiple candidates for fiber forming nanomatrices were evaluated for their stability, toxicity and ability to serve as reservoir for neurotrophic factors. Two approaches to facilitate neurite sprouting within the matrix were tested; including various peptide/polymer contents and the generation of a positive BDNF gradient to attract neurites to the nanomatrix network.

The postnatal mouse spiral ganglion explant model allows to evaluate 3-D nanomatrices regarding their ability to allow neurite outgrowth. Furthermore, nanomatrices can be tested regarding possible toxic effects, their ability to form fiber networks, and their ability to serve as a repository for neurotrophic factors.

Keywords: cochlear implant; spiral ganglion; 3-D nanomatrix; neurite outgrowth

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Methylprednisolone injection to the inner ear: an alternative to the systemic therapy

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Objectives: Since corticosteroids are believed to exert their activity through a powerful suppression of inflammatory and immunological response, increases the K+ secretion of the stria vascularis improving endolymphatic ion balance and can reach the perilymph via the round window, the aim of this communication is to evaluate the clinical outcomes achieved with intratympanic methylprednisolone for the treatment of several inner ear disorders.

Methods: Under topical anesthesia 40mg/2mL intratympanic methylprednisolone was administered slowly through a myringotomy in the posteroinferior quadrant with a 27-gauge needle to fill the middle ear cavity. Patients stayed in lateral position without swallowing. Consent inform was obtained from all patients and were asked to give a score between 0 to 10 for their symptoms, corresponding 0 to no bothering symptom and 10 to the most annoying or incapacitating (AVS)

Results: Thirty eight patients were included: 15 female and 23 male (mean age: 49.8 years; range: 24-87 years).The diagnoses were: 13 sudden hearing loss (34.2%), 11 tinnitus (28.9%), 11 Meniere´s disease (28.9%) and 3 autoimmune inner ear disease (7.9%). The mean of number of injections was 4 for sudden hearing loss, autoimmune inner ear disease and tinnitus and 6.8 for Meniere´s disease. With the scale we found that the mean of hearing loss before the drug was 8.35 and after the steroid, was 5.65. The mean of tinnitus before the drug was 8.0 and after the steroid was 4.72. The mean of vertigo before the drug was 8.57 and after the steroid was 3.79. The mean of descent corresponding to hearing loss, tinnitus and vertigo were: 2.69(CI 95% 1.47-3.91); 3.27 (CI 95% 2.23-4.32); 4.78 (CI 95% 2.88-6.68).

Conclusions: Intratympanic injections have increased in clinical use to prevent the side effects associated with systemic therapy, becoming an effective alternative to systemic steroids administration. Moreover, intratympanic injections provide the highest inner ear concentrations and the longest duration within the perilymph. However, this approach can be influenced by the anatomy of the round window, the limited permeability due to mucous thickening, or swallowing movements during the procedure.

Keywords: Intratympanic injection, Meniere´s disease, methylprednisolone

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POSTER PRESENTATIONS P-66

Fibrin-collagen coating of cochlea implant electrodes for transplantation of adipose-derived stem cells

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Cochlea implants (CI) are the state-of-the-art therapy in profoundly hearing impaired patients. However, one persisting limitation is the bioelectric coupling of the electrode contacts to the spiral ganglion neurons (SGN). This leads to poor selectivity of stimulation and restricted channel separation. It has been shown that *adipose-derived stem cells* (ASC) produce neurotrophins and other growth factors that protect neurons and influence neurite outgrowth [1, 2]. For cell transplantation several hydrogel scaffolds have been tested with encouraging results [3]. The aim of this study is to improve the bioelectric coupling by developing a fibrin-collagen hydrogel coating with encapsulated ASCs for CI electrodes.

Therefore ASC were cultivated in hydrogels containing fibrin and/or collagen in varying fractions for 14 days. Proliferation was analyzed by means of histology, measurement of ds-DNA-content and MTT-assay (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay). At different instants of time concentrations of BDNF and Laminin were measured in supernatants by ELISA. Test-electrodes were coated with a fibrin-collagen mixture by adding the hydrogel to the electrodes into a mold. Insertion forces of coated electrodes and control electrodes were measured in artificial cochlea model with a well-established experimental setup.

A homogenous cell distribution and increase of cell density was observed in cryosections over the time. Rising dsDNA-contents of the hydrogels and increased extinction values in MTT assay indicated a good cell proliferation and viability. Results of the ELISA showed that BDNF and Laminin were produced increasingly by ASC in all hydrogels over the time. Slightly elevated insertion forces were determined for the coated electrodes compared to the uncoated control electrodes. Before insertion and after extraction photos were taken of the coated electrodes showing a persisting adhesion but inhomogeneous distribution of the hydrogel. In summary growth and neurotrophin production of ASC in fibrin-collagen hydrogels could be proved over 14 days. Insertion forces of fibrin-collagen coated electrodes have been increased slightly.

Keywords: cochlea implant, adipose-derived stem cells, neurotrophin, fibrin, collagen

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A novel method for transplantation of terminally differentiated neurons derived from pluripotent stem cells into the cochlea

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Objectives: In the inner ear, regeneration of spiral ganglion neurons using pluripotent stem cells including iPS cells has been investigated [1], and the results indicate a possibility for functional recovery [2, 3]. As a next step, we should translate such basic findings into the clinic. In translation into clinic, there are several issues to be resolved for the safety and efficacy as a therapeutic treatment. A risk of tumorigenesis is included in a list to be resolved. Use of terminally differentiated neurons can be one possible resolution. In this study, we examined the method for neural induction and preparation of human iPS cells for cochlear transplantation as terminally differentiated neurons.

Methods: According to the method reported previously [4], human iPS cells were differentiated into neural stem cells (NSCs) without use of feeder cells. Then, NSCs derived from human iPS cells were seeded on a 3D collagen matrix and differentiated into neurons on it for transplantation. We examined expressed markers at different culture stages using immunostaining and RT-PCR, and the potential of iPS cell-derived NSCs for differentiation into neurons in vitro. We also tested the survival of iPS cell-derived neurons cultured on a 3D collagen matrix after transplantation into guinea pig cochleae.

Results: Human iPS cell-derived NSCs exhibited expression of NSC markers and no expression of undifferentiated cell-markers. In addition, iPS cell-derived NSCs enabled expansion without losing neuronal differentiation potency. Following 14-day culture in a differentiation condition, the majority of cultured cells expressed a marker for glutamatergic neurons. After seeding on a 3D collagen matrix, iPS cell-derived NSCs differentiated into neurons and elongated neuritis on a collagen matrix. After transplantation into normal guinea pig cochleae, we identified the survival of human iPS cell-derived neurons in the cochlea.

Conclusions: The present findings have indicated that our method for neural induction is highly efficient for generation of glutamatergic neurons from human iPS cells, and that culture of iPS cell-derived neurons on a 3D collagen matrix is a useful method for preparation of transplants.

Keywords: iPS cell, neural induction, glutamatergic neuron, collagen matrix, transplantation

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Survival of human iPS cell-derived neurons after transplantation into guinea pig cochleae

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Objectives: We previously demonstrated the potential of mouse ES cell-derived cell transplantation into the cochlea for functional restoration of spiral ganglion neurons [1]. In addition, a recent report has shown the restoration of auditory function by transplantation of human ES cell-derived cells [2]. Based on these findings, we aim to restoration of auditory function by transplantation of human iPS cell-derived cells. Here we report the survival of human iPS cell-derived neurons in guinea pig cochleae.

Methods: Guinea pigs were used as recipient animals similarly to our previous studies [1,3,4]. As transplants, human iPS cell-derived neurons were chosen because of low risk for tumor formation. We transplanted a 3D collagen matrix on which human iPS cell-derived neurons had been cultured into the scala tympani of the basal turn of guinea pig cochleae. The animals were divided into two groups; one received an intra-venous injection of guinea pig bone marrow stromal cells (BMSCs) together with systemic administration of an immune-suppressant during their survival, and the other were applied only an immune-suppressant. One week after transplantation, cochleae of experimental animals were provided for histological assessments. We examined the survival of transplants in cochleae and infiltration of inflammatory cells into cochleae.

Results: The survival of iPS cell-derived neurons was found in animals received BMSCs treatment, while no iPS cell-derived neurons were identified in animals without BMSCs application. For infiltration of inflammatory cells into cochleae, obvious infiltration of CD45-positive cells was observed in cochlear specimens without BMSCs treatment. In specimens treated with BMSCs, infiltration of CD45-positive cells was limited. A quantitative analysis has revealed a significant difference in numbers of CD45-positive cells in the basal turn of cochleae between animals treated with BMSCs and those without BMSCs treatment.

Conclusion: Present results have demonstrated that human iPS cell-derived neurons are capable for the survival in guinea pig cochleae, although adequate control of inflammatory responses is required. In the near future, we will examine the potential of transplantation of human iPS cell-derived neurons for functional restoration of guinea pig cochleae.

Keywords: Xenograft, Human iPS cell, iPS cell-derived neurons, Collagen matrix, Bone marrow stromal cells

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Molecular optical imaging enables visualization of exogenous stem cells in the intact guinea pig cochlea

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Introduction: It is generally accepted that preservation of functional auditory neurons is crucial with regard to cochlear implant performance. Growing evidence suggests that stem cell therapy may be applied to replace degenerated afferent neurons in the auditory nerve of patients suffering from severe hearing impairment. However, to achieve functional repair, survival of stem cells is a prerequisite. This calls for *in vivo* visualization of these cells and long-term monitoring of their migration and survival after transplantation into the deafened cochlea.

Methods: We have investigated if molecular optical imaging enables visualization of exogenous (stem) cells within intact guinea pig cochleas. Human embryonic kidney (HEK293) cells and epidermal neural crest stem cells (EPI-NCSCs) were used that stably express fluorescent (copepod green fluorescent protein; copGFP) and bio-luminescent (green click beetle luciferases; CBG99) reporters at an equimolar ratio. Experiments were performed on complete heads and intact tympanic bullae from guinea pig cadavers, which had been obtained from non-related experiments.

Results: Following injection of copGFP- and luciferase-expressing HEK293 cells into the internal auditory meatus of intact bullae, a bright and distinctly localized bioluminescent signal was passing through the bony wall of the bullae, and was observed emitting from the cochleas after opening the bullae. Fluorescence due to copGFP could not be detected. In another series of experiments, the bullae were left *in situ* in the heads and opened via a retro-auricular surgical approach, and copGFP- and luciferase-expressing HEK293 cells and EPI-NCSCs were injected through the round window membrane into either the scala tympani or the modiolus of the basal turn. The bioluminescent signal was invariably located near the concha of the auricle, indicating that the light emitted by the cochlea was passing through the tympanic membrane and the external auditory meatus. The fluorescent signal could not be detected. A dilution series was performed to detemine the amount of cells (n=10,000-25,000) needed to reach signal threshold.

Conclusions: This feasibility study demonstrates that bioluminescent imaging enables visualization of exogenous (luciferase-expressing) stem cells in the intact guinea pig cochlea. Studies are in progress to reproduce these results in living animals.

Keywords: molecular optical imaging; luciferase; guinea pig; stem cells; cochlea

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Mondini dysplasia: conceptual new and new electrode designed for cochlear implantation

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Introduction: Incomplete partition type II (Sennaroglu classification) is the type originally described by Mondini cochlea. The triad is: modiolus defect in the apical part of the cochlea and its corresponding septum intercalar, minimally dilated vestibule and enlarged vestibular aqueduct. It could be presented with Gusher, a profuse clear liquid output by opening the inner ear by a defect of variable size between CAI and ear malformations. According to literature gusher incidence malformation in case of inner ear is between 40 - 50%.

Material and method: We report the case of a 2 year old girl diagnosed with bilateral profound hearing loss with bilateral incomplete partition type II. The extensive bone defect at the bottom side of the Internal Auditory Canal(IAC), predicted a profuse surgicalGusher.

We present a video of the cochlear implant surgery using a new electrode specially designed to minimize the risks of surgical Gusher.

Sennaroglu recently designed a special electrode to prevent leakage of cerebral spinal fluid after insertion of the electrode. The electrode has the feature of having a silicone plug where the insert ends. The electrode is designed to block preventively and effectively the outflow (Gusher) in cochleostomy.

Results: We described surgery and implantation technique for this particular electrode. During surgery there was a very heavy gusher. We did a small cochleostomy to the right size to fit the diameter of the silicone plug and enhance its effectiveness.

We used small grafts of fascia to achieve the highest possible hermeticism seal. The result was favorable

Conclusions: The gusher is the result of a bone defect in the bottom side of the CAI. When is profuse, often presented a wide communication between subarachnoid space and the inner ear, which has a higher risk of postoperative meningitis. Although conventional electrodes can get a proper seal, we consider laudable any attempt innovation and improvement of the surgical technique or the design of the electrodes in order to minimize this risk.

Keywords: Cochlear Implant, cochlear malformation, gusher, profound hearing loss

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Application of new generation technologies (NGS and aCGH) to the integral diagnosis and investigation of inherited hearing loss

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The prevalence of the different subtypes of nonsyndromic hereditary hearing loss (NSHL) is still extensively unknown because of its large genetic heterogeneity, and that is the reason why genetic diagnostic based on traditional practices is not cost-effective. In this study, we have developed and validated two methods based on new generation technologies (NGS and aCGH) to be used in combination for the diagnostic and researching of inherited hearing loss. The first method consists on a modular CGH array based on Agilent technology which allows us to evaluate the existence of pathogenic copy number variations (CNVs) in all NSHL loci. With this tool it is possible to analyse the genomic integrity of more than 700Mb of DNA sequence corresponding to more than 100 NSHL mapped loci. This array has been validated using DNA of patients with pathogenic CNVs previously diagnosed by MLPA technique.

The second method consists on the design and validation of a modular liquid-phase target enrichment system using Nimblegen technology focused on the genomic regions, full exonic and intronic sequences, of more than 70 NSHL genes currently known. The validation of this method on a Solid 5500 NGS platform shows a mean coverage of 140-200X, in which 90% of reads maps in the target region, and 90% of the captured sequence has a coverage higher than 10X. This tool has been validated with already diagnosed patients by Sanger method and in all cases the pathogenic mutation has been identified. The combined application of these tools is making possible the comprehensive analysis of highly heterogeneous genetic pathologies such as NSHL in a cost-effective way due to the fact that they result on more than 90% savings compared to traditional techniques; it is also providing a database of strategic interest about the variations of potential pathogenicity in previously unexplored genomic regions such as the intronic sequences of the NSHL genes or the genomic interval of deafness loci with no gene identified. Altogether they conforms a strong and feasible integral tool for the diagnostic of the inherited hearing loss forms.

Keywords: nonsyndromic hereditary hearing loss, NGS, aCGH

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Highly efficient diagnostic testing in patients with hereditary hearing loss using Panel-based Next Generation Sequencing

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Genetic heterogeneity complicates the molecular diagnosis of hereditary hearing loss (HHL). Although a multitude of genes are attributed to HHL, patients are routinely tested only for mutations in *GJB2*, *GJB3* and *GJB6* (~5-10% of HHL). Thus, screening of all known hearing loss genes in parallel by high-throughput sequencing methods (next generation sequencing technology) is the most promising tool for a comprehensive detection of causative mutations, especially after negative testing for *GJB2*.

Here we present the methodology of the 'hearing loss' diagnostic panel comprising 95 genes associated with non-syndromic and syndromic HL (Target enrichment, NGS library preparation and sequencing on the SOLiD 5500xl platform, bioinformatic analysis and medical evaluation). A group of 120 patients with profound hearing loss were analyzed in a clinical setting. Multiplexed samples were sequenced with high coverage per base. Combined with bioinformatic analyses, single base substitutions, small deletions and insertions in known genes of genetic hearing loss can reliably be detected.

In conclusion, we have established a panel-based NGS pipeline which is a highly sensitive, fast and cost efficient tool for the genetic diagnostics of HHL. NGS-based mutation analysis allows us to detect causative mutations in >60% of HHL patients. These results imply consequences for counseling of patients and families and can also be the foundation for novel gene- or even mutation-specific treatment options in hearing loss.

Keywords: Next-Generation Sequencing; NGS; hereditary hearing loss; diagnostic testing

SVT-AP-99: inhibition of Apaf-1 as a potential therapeutic strategy to prevent cisplatin-induced hearing loss

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Objectives: Cisplatin is a highly effective chemotherapeutic agent used for the treatment of various solid tumors. Their benefits are partially offset by their toxic effects, including ototoxicity. There is a considerable need for the discovery of new protective agents since there are not currently approved products to prevent hearing loss associated with Cisplatin treatment. At the molecular level, Cisplatin trigger the production of reactive oxygen and nitrogen species inducing cell death by apoptosis of inner hair cells. Apaf-1 plays a key role in the apoptosis signaling cascade being a key component of the apoptosome. The results of this work show that its inhibition prevents ototoxicity caused by the administration of Cisplatin through the prevention of apoptosis.

Methods: The efficacy of Apaf-1 inhibitors on the prevention of Cisplatin induced ototoxicity was analyzed *in vitro* in the HEI-OC1 (organ of Corti cell line) and *in vivo* in a Zebrafish and a rat model of Cisplatin induced ototoxicity.

Results: Treatment with SVT-AP-99 prevents Cisplatin induced apoptotic cell death in HEI-OC1 cell line, decreasing cytochrome C release and caspase 3 activation. Apaf-1 inhibitor markedly decrease the Cisplatin induced loss of auditory neuromasts in the Zebrafish embryos. Finally, intratympanic administration of Apaf-1 inhibitor in a rat model of Cisplatin induced ototoxicity was protective against hearing loss, as determined by auditory brainstem responses threshold shift (ABR). The ABR threshold in the SVT-AP-99 group was significantly lower than the Cisplatin group in all frequencies tested. In addition, gene expression of pro-apoptotic (p53), ototoxicity (KIM-1) and inflammation markers (TNFα) in rat cochlea were also diminished upon SVT-AP-99 administration. These results prove that the compound shows efficacy as a protective agent against Cisplatin induced cell death and inflammation.

Conclusion: Apaf-1 inhibitor prevents ototoxicity caused by Cisplatin and therefore hearing loss, indicating this product could be an effective potential otoprotectant.

Keywords: Apoptosis; Cisplatin; ototoxicity; otoprotectant; drug

Plucked human scalp hair follicles may serve inner ear cell- based therapy

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Objectives: There is an increasing interest in the therapeutic potential of autologous stem cells. Advantages include minimizing the need for systemic immunosuppression while reducing ethical and regulatory issues [1]. Neural crest stem cells (NCSC) are considered highly suitable for autologous stem cells therapy, for they persist during adulthood and can be harvested from easily accessible sources. NCSCs have great potential in the development of a cell-based therapy to treat deafness, for the neural crest and the otic placode share similar molecular events during neurogenesis [2]. Moreover, NCSCs have recently been identified in the developing otic vesicle, contributing not only to the glia lineages but also to sensory and neuronal progenies [3]. It has been shown that stem cells from the neural crest are located in the adult hair follicle (HF) bulge [4]. These cells are multipotent and can differentiate into neurons, melanocytes and glia [4]. HF stem cells, due to their NC-origin and accessibility, may be of great use in the development of an autologous cell-based therapy to treat deafness. In that perspective, the use of plucked HFs will increase the practical application of HF stem cells.

Methods: We developed a technique to pluck, almost painlessly, HFs and established protocols for fast isolation, expansion and cryopreservation of the stem cells. We tested viability after cryopreservation and subsequent syringe needle flow, *in vitro* NCSC-characteristic protein profile, homing and neural differentiation.

Results: The yield of stem cells is, on average, 3x 10^4 cells/follicle 1 month after the start of the culture. Therefore the cellular yield of 6-10 HFs would be enough for transplantation purposes. Cell viability after syringe-mediated disaggregation (30 Gauge, 0.5µL/min.) and cryopreservation was 82.2%±2.3%. The NCSC protein profile was similar to previous results from HF obtained from skin biopsies [5] i.e., cells were Nestin^{+ve}, SOX9^{+ve}, SOX10^{-ve}, SLUG^{+ve}, AP-2a+ve. Establishment of HF stem cell homing is ongoing. Neural differentiation (B3Tub^{+ve}, NeuF^{+ve} cells) is achieved within 4 weeks after neural induction.

Conclusion: Stem cells from plucked HF can easily be cultivated, expanded and kept frozen until needed, while keeping NCSC characteristics. This allows practical application of HF stem cells for inner ear cell based therapy.

Keywords: plucked hair follicles, cell based therapy, neural crest, inner ear

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Analysis of the effect of stimulated cGMP cascade on the vulnerability for hearing loss after noise exposure during aging

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It has been shown that stimulating of cGMP cascade partially prevent noise-induced hearing loss after traumatising noise exposure (Jaumann et al., 2012). The goal of the present study was to find out whether the stimulation of cGMP cascade is also protective for slowly progression hearing loss with age.

Wistar rats were hosted up to 26 months in the animal facility in standard conditions according to guidelines and regulations of Helsinki declaration. Regular observation of the veterinarian was performed for every animal. Functional hearing measurements (acoustic brainstem response (ABR) and otoacoustic emission (DPOAE)) were performed before and up to 5 months after a single moderate noise exposure of young (3-4 months old) and aged (16-20 months old). Starting from the third day after noise exposure during 5 months rats were treated with cGMP cascade modulating drug pressed in dry food or vehicle as a control. We analyzed defined plasticity markers in the periphery and brain. For all animal groups the hearing thresholds and above-treshold responses of ABRs before and after noise exposure were analyzed in details.

The results will help to clarify a presumptive otoprotective capacity of cGMP cascade modulating drug for presbyacusis in the context for a search of promising preventive therapies for aged-related hearing loss.

Keywords: age-related hearing loss, ribbon synapses, temporary tresholdshift, waveform analysis

Acknowledgements: Supported by Action on Hearing Loss, RNID G45 (Rü).

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Preventive effect of polyphenols on hearing loss comparing evoked potentials hearing of brain stem responses and stable state hearing in experimental animals age-related

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Hearing loss associated with aging is called presbycusis. Cell damage that is observed with age affects the auditory system in its entirety. Oxidative stress also appears to increase with age, causing cell damage in numerous tissues. Polyphenols have antioxidant properties. Prevent various diseases associated with oxidative stress, such as cancer, inflammatory diseases, cardiovascular disease and aging.

The audiometric standard remains the goal to quantify and describe hearing loss in people who are able to respond and cooperate. Other methods are necessary to assess hearing in an objective are young children, patients with developmental delay or neurological disorders, patients who require medical evaluation and legal hearing and experimental animals. Auditory evoked potentials (AEP) in response to acoustic stimulation have been used and, in particular, the auditory brainstem response (ABR) and steady-state evoked potentials (ASSR) have become most widely used test.

The aim of this study is to determine the preventive effect of polyphenols on treatment-related hearing loss with age and determine which of the two techniques used, brainstem evoked potentials (ABR) and steady-state potentials (ASSR) to measure hearing loss associated with age in an animal model is more objective. Methods: Male Sprague-Dawley rats (n = 100), grouped in different ages: 3,6,12,18 and 24 months, each group in turn were divided into control and treated with a mixture of polyphenols, 100 mg / kg bw / day dissolved in water for 4 months. The rats were measured auditory function using the ASSR and ABR.

Results: 1) two techniques has some hearing loss with age; 2) when comparing groups obtained ABR hearing thresholds and frequency ASSR from 500 to 16000 Hz, we observe that the thresholds obtained with the ASSR have values lower than those of ABR; 3) has been determined that polyphenols prevent hearing loss related to age, obtaining lower hearing thresholds with ASSR.

Our results conclude that treatment with polyphenols prevent hearing loss with age and are ASSR obtained with more objective measures and that it is a more sensitive technique to assess the hearing loss.

Keywords: presbycusis, Polyphenols, auditory brainstem response (ABR), steady-state evoked potentials (ASSR).

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Autoantibodies and sudden hearing loss

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Objectives: Cisplatin is a highly effective chemotherapeutic agent used for the treatment of various solid tumors. Their benefits are partially offset by their toxic effects, including ototoxicity. There is a considerable need for the discovery of new protective agents since there are not currently approved products to prevent hearing loss associated with Cisplatin treatment. At the molecular level, Cisplatin trigger the production of reactive oxygen and nitrogen species inducing cell death by apoptosis of inner hair cells. Apaf-1 plays a key role in the apoptosis signaling cascade being a key component of the apoptosome. The results of this work show that its inhibition prevents ototoxicity caused by the administration of Cisplatin through the prevention of apoptosis.

Methods: The efficacy of Apaf-1 inhibitors on the prevention of Cisplatin induced ototoxicity was analyzed *in vitro* in the HEI-OC1 (organ of Corti cell line) and *in vivo* in a Zebrafish and a rat model of Cisplatin induced ototoxicity.

Results: Treatment with SVT-AP-99 prevents Cisplatin induced apoptotic cell death in HEI-OC1 cell line, decreasing cytochrome C release and caspase 3 activation. Apaf-1 inhibitor markedly decrease the Cisplatin induced loss of auditory neuromasts in the Zebrafish embryos. Finally, intratympanic administration of Apaf-1 inhibitor in a rat model of Cisplatin induced ototoxicity was protective against hearing loss, as determined by auditory brainstem responses threshold shift (ABR). The ABR threshold in the SVT-AP-99 group was significantly lower than the Cisplatin group in all frequencies tested. In addition, gene expression of pro-apoptotic (p53), ototoxicity (KIM-1) and inflammation markers (TNFα) in rat cochlea were also diminished upon SVT-AP-99 administration. These results prove that the compound shows efficacy as a protective agent against Cisplatin induced cell death and inflammation.

Conclusion: Apaf-1 inhibitor prevents ototoxicity caused by Cisplatin and therefore hearing loss, indicating this product could be an effective potential otoprotectant.

Keywords: Apoptosis; Cisplatin; ototoxicity; otoprotectant; drug

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Methods for hearing threshold measurements with ABR in guinea pigs

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For a long time the guinea pig has been a convenient model in hearing research for the human inner ear, because the anatomical structure of the inner ear of a guinea pig - especially the cochlea- is very similar to the human inner ear. In this abstract, two kinds of methods for spotting the hearing thresholds of a normal hearing guinea pig are described in detail: "closed-field"-measurement (CF) and "open-field"-measurement (OF). These two options of hearing measurement, which differentiate oneself from the acoustic supply, are compared with and advantages as well as disadvantages are discussed. Furthermore, the influences of cross-hearing on the results of these measurements are investigated.

Auditory-brainstem-responses (ABR) were recorded by use of ten ears of normal hearing guinea pigs each. The hearing thresholds of every ear were evaluated by repeating these measurements ten times. For each measurement the guinea pig was re-positioned below the sound source as well the electrode was re-wired. The CF-measurements were accomplished by using a sound tube, which functioned as a connection piece between the electric-acoustic transducer and the auditory meatus. In case of OF-measurements however, the electric-acoustic transducer was positioned directly above the auditory meatus in a well-defined distance without a connection piece.

The values of standard-deviation of the CF-serial were greater than those of the OF-serial. Consequently, the determination of hearing thresholds in closed-field features a greater variability than by using the open-field method. It can be assumed that an accurate position of the sound tube is of particular importance. Only if the sound tube is inserted properly into the acoustic meatus, you can get convincing and reproducible values to determine hearing thresholds of a guinea pig. A small obliteration of the sound tube can influence the usability considerably. Besides, the influx of cross-hearing was regarded in the interpretation of the results: The crosstalk-attenuation was greater by using the closed-field method than the open-field-measurement. This has to be taken into consideration in cases of asymmetrical hearing, e.g. models for single sided deafness.

Keywords: guinea pig; hearing threshold; ABR; asymmetric hearing loss

Influence of cochleostomy and implant insertion on drug gradients following intratympanic application in guinea pigs

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Locally-applied drugs can protect residual hearing following cochlear implantation. However the pharmacokinetics associated with the surgical procedures of cochleostomy and electrode insertion are not well understood, leading to uncertainty about drug distribution in cochlear fluids and thus dosage protocols. The influence of cochlear implantation on drug levels in scala tympani (ST) was investigated *in-vivo* in guinea pigs using the marker trimethylphenlyammonium (TMPA), wherein TMPA concentration was measured in real-time with TMPA-selective microelectrodes sealed into the basal or second turn of ST. *In-vitro* experiments were conducted using microcapillary tubes filled with lactated Ringer's solution and Fluorescein. The results revealed that implantation procedures can have a substantial influence on drug levels in the basal turn. The method used for performing the cochleostomy can affect localized TMPA distribution in the basal turn and electrode insertion does not redistribute intracochlear TMPA toward the apex. TMPA distribution in the second turn was less affected by implantation procedures. The ramifications of these results will be discussed.

Keywords: Cochleostomy, perforation, cochlear implantation, electrode, pharmacokinetics, drug distribution.

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Change in endocochlear potential during experimental insertion of a simulated cochlear implant electrode in the guinea pig

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Objective: Preservation of residual hearing during cochlear implantation is important. This study investigated changes in endocochlear potential (EP) during simulated cochlear implant (CI) electrode insertion.

Study Design: Laboratory animal study.
Setting: Academic hospital laboratory.

Subjects and Methods: Guinea pigs were divided into 4 groups: cochleostomy only (4 animals), suction after cochleostomy (5 animals), simulated CI electrode insertion parallel to the longitudinal axis of the scala tympani without suctioning (7 animals), and simulated CI electrode insertion toward the modiolus without suctioning (7 animals). The EP was measured from the second turn of the cochlea, and the values after 20 minutes were compared.

Results: The EP showed little change at 20 minutes after cochleostomy with a nearly normal value of 84.83 ± 2.12 mV. Suctioning of the perilymph from the cochleostomy site caused a slight acute reduction in EP by about 6 mV, and the value at 20 minutes after cochleostomy was 78.64 ± 4.42 mV. Insertion of the simulated CI electrode parallel to the longitudinal axis of the scala tympani caused a slight decrease in EP to 78.91 ± 5.06 mV. Insertion toward the modiolus caused a marked decrease in EP to 54.13 ± 4.42 mV at 20 minutes after the treatment, significantly lower compared to the other 3 groups.

Conclusion: EP was well preserved during carefully performed surgical procedures of simulated CI electrode insertion, but it decreased significantly if the simulated CI electrode was inserted toward the modiolus. Careful attention is necessary to ensure the correct direction of CI electrode insertion to preserve residual hearing. Use of suction should be minimized if possible.

Functional and histological outcomes following cochlear implantation in the mouse

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Extending the use of cochlear implants to patients with residual hearing entails an increased understanding of their effects at a cellular and molecular level. Animal models are the only means of assessing such consequences. Mice are an excellent model for auditory research and this is in part due to the range of naturally occurring and genetically-modified strains which mimic human deafness. To date, very few studies of cochlear implantation (CI) in mice exist. Our aim was to create a mouse model of CI and subsequently assess the effects of implantation on cochlear ultrastructure and function. The round window was accessed and implantation performed in C57Bl6 mice (early-onset hearing loss model) using either a dummy implant (n=16) or a specialized electrode array (n=8). The contralateral cochlea acted as a control. Auditory brainstem response (ABR) audiometry prior to and at time-points following CI was undertaken. Following sacrifice, cochleae were harvested and prepared for histological examination. Postoperatively, a general trend towards greater mean threshold shifts in the implanted ear was noted. There were no cases of complete hearing loss. Correct implant placement was confirmed on light microscopy and cone beam computed tomography. Histological analysis revealed encapsulation of the implant in tissue with features suggesting the presence of fibrosis. Immunolabelling using CD45 (leucocyte marker) revealed significantly greater numbers of positively labeled cells within the basal turn of implanted cochleae compared to controls (Mann-Whitney U Test, $p<0.05$). This was particularly seen within the region of the spiral ligament, scala tympani and basilar membrane. Our results demonstrate that mouse CI via the round window is feasible and holds many translational benefits. Further investigation into the presence of inflammation and fibrosis using this mouse model will help identify measures to potentially reduce these effects. The model will also allow for future research using eluting electrode arrays as a means for drug delivery to cochleae, ultimately enhancing patient outcomes.

Keywords: Cochlear implantation, mouse model, implantation effects.

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Laser assisted cochleostomy in guinea pigs

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Background: In otosclerosis surgery different lasers can be used to make the fenestration in the stapes footplate to insert the prosthesis (stapedotomy). A possible downside when using lasers is the possibility of inflicting harm to inner ear structures. Potential risks differ between lasers, depending on wavelength, absorption spectra and laser settings.

Aim: To determine which laser gives least damage to inner ear structures and function.

Material and methods: The basal turns of guinea pig cochleas were fenestrated using four types of lasers (Thulium, KTP, CO2, diode). At three different time points (prelaser, directly postlaser and 4 hours postlaser) acoustically evoked compound action potentials were recorded at five frequencies (2, 4, 8, 11.3, 16 kHz) and at different sound pressure levels in order to determine hearing thresholds. A control group was added to correct for influence of the surgery alone. A repeated measures ANOVA was used to investigate differences between groups. Histologic preparations of the cochlea were evaluated for ultrastructural damage.

Results: In all laser groups, hearing thresholds are significantly elevated in the higher frequencies. The Thulium laser group shows the most detrimental effects on cochlear function. Histologic preparations of cochleas from all laser groups showed no damage to the Organ of Corti, but haemorrhages in the scala tympani of the basal turn of the cochlea.

Conclusion: All lasers investigated are relatively safe for use in stapedotomy. The Thulium laser, however, gives most damage to inner ear structures and deteriorates cochlear function in the basal turn.

Keywords: Cochlear implantation, mouse model, implantation effects.

A comparison of two vasoactive/vasodilative agents in combination with corticosteroid for treatment of sudden sensorineural hearing loss

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Idiopathic sudden sensorineural hearing loss or sudden deafness is considered an otologic emergency. In spite numerous investigations its cause and treatment remain uncertain. Various medications have been used to enhance recovery of hearing but none of them showed any significant difference from the placebo treatment. Vascular hypothesis is one of the accepted etiologic theories and vasodilator agents have been used to treat this medical condition.

In our preliminary retrospective study we compared the efficiency of two vasoactive/vasodilative agents (pentoxyfilline and betahistine) separately and together for treatment of sudden deafness. Corticosteroid (methylprednisolone) was used in all three groups as well. In the study we included patients who were hospitalized for sudden unilateral hearing loss with no identified cause. The exclusion criteria were any known reasons of conductive or sensorineural hearing loss. Hearing threshold was measured and pure tone average (PTA) was calculated on admission, at first and second follow up. PTA was calculated as arithmetic mean of the hearing thresholds at 4 frequencies (0,5; 1; 2 and 4 kH). Best recovery rate and improvement in PTA was seen in pentoxyfilline + steroid group, less in pentoxyfilline + betahistine + steroid group and in betahistine + steroid group. A possible effect of steroid was also taken into consideration. Patients who received higher dose of methylprednisolone had greater PTA improvement than those who received a lower dosage of steroid. There was no statistically significant difference between all three groups so none of the treatment schemes proved to be better for therapy of sudden deafness. The amount of steroid given was not found to be statistically significant for the treatment outcome.

The question of the right treatment is obviously still open to debate. New therapies are therefore encouraged to be developed as well as new diagnostic tools to be proposed. To determine the actual cause of sudden hearing loss would aid immensely in finding of the cure.

Keywords: betahistine; pentoxyfilline; sensorineural hearing loss; treatment outcome

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High speed imaging in laser-assisted stapedotomy

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Background: Different lasers are used in stapedotomy, e.g. KTP (532nm wavelength), diode (980nm), Thulium (2 µm), and CO_2 (10.6 µm). The individual characteristics of these lasers differ, theoretically causing different effects (and trauma) to the vestibule. Short wavelengths, as KTP and diode lasers produce, will not be absorbed in perilymph Thulium and CO_2 on the other hand will be absorbed in perilymph, causing heating. During this process, mechanical effects, e.g., bubble or pressure wave formation can occur.

Aim: Visualisation of the thermal and mechanical effects in the vestibule, during laser-assisted stapedotomy.

Material and methods: An inner ear model, with human cadaver stapes, was used. With different lasers, holes were created in the stapes. High Speed Schlieren en thermal imaging was used to capture thermal and mechanical effects under the footplate. With a hydrophone sound effects were recorded and analysed.

Results: Decelerated high speed movie clips will be shown, clearly showing differences in effects between lasers. Both thermal and mechanical effects are highest with the Thulium laser. Sound production is lowest with the KTP laser (48 dBA) and CO_2 laser (66 dBA). It is highest for the thulium laser 82 dB (A), but still less than conventional technique using a skeeter drill (95 dBA).

Conclusion: High speed imaging is a good technique to capture effects occurring during stapedotomy. All lasers are safe for stapedotomy, however the thulium laser seems least favourable, since both thermal and mechanical effects were most pronounced.

Keywords: Laser, stapedotomy, high speed imaging, thermal imaging

Characterization of neural stem cells in the rat cochlear nucleus

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Introduction: The cytoarchitecture of the cochlear nucleus (C.N.) depends on the stimulation applied. In recent years, neural stem cells have been indentified in this nucleus [1] [2] of the brainstem. The C.N. consists of three anatomical subregions: the anteroventral (AVCN), the posteroventral (PVCN) and the dorsal (DCN) cochlear nucleus. Since the subregions have been attributed to specialized auditory functions a better understanding of the particular neurogenic potential ofbthese subregions is of importance.

Methods: C.N. of P9 rats were microscopically dissected, cultured for 4 weeks in stem cell medium (Neurobasal, GlutaMAX, B27, EGF, FGF-2), and evaluated on the ability to expand by formation of neurospheres. In addition progenitor cell markers were identified in neurospheres. After dissociation of the spheres, single cells were plated in a differentiation medium (Neurobasal, GlutaMAX, B27, retinoic acid). Consecutively neuronal and glial markers were used to identify the maturated cells.

Results: The investigations showed that the stem cell potential of the individual subregions of the C.N. differed significantly. The highest sphere forming capacity could be detected in the PVCN. Progenitor cell markers were evident in neurospheres of all subregions. All dissociated cells were able to differentiate into neurons and glial cells.

Conclusion: The present results display that all different subregions of C.N. have a neurogenic potential. For the future it is of interest if changes of the neurogenic potential, for example by pharmacological treatment, might alter the cytoarchitecture of the C.N.

Keywords: auditory pathway, cochlear nucleus, neural stem cells, neurospheres, progenitor cells

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Study of the role of miRNAs in the development of the auditory portion of the inner ear

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Congenital deafness is a heterogeneous disorder that affects 1 in 800 babies with a wide range of causes. In human, the hearing loss results from the loss of sensory hair cells in the inner ear which are beyond the scope of regeneration. Therefore, a better understanding of the molecular regulation of the inner ear development could provide a strategy to improve the regeneration or preservation of the auditory function.

In this study, we address the possible role of microRNAs (miRNAs) in the early development of the organ of Corti by using mouse model with a conditional loss of Dicer (cKO-*Dicer*), the miRNA-maturating enzyme. In this mutant mouse, we observe a severe hypoplasia of the cochlea, the vestibule and the cochleovestibular ganglion at E12. A labeling of pH2AX indicates that the absence of miRNAs induces severe DNA damages (double strand breaks) responsible for an activation of the p53 pathway [1]. We also observe defects in proliferation and differentiation in progenitor cells of the organ of Corti.

Finally, a transcriptomic comparison of E12 wild type and CKO-*Dicer* otic vesicles reveals an upregulation of Wnt pathway inhibitors such as *Sfrp4* in cKO-Dicer and a downregulation of Wnt ligands and target genes, including *Eya1*, a gene involved in inner ear development [2]. *Luciferase assay* demonstrated that miR-124, a miRNA expressed during inner ear development [3], target the 3'untranslated-region (3'UTR) of *Sfrp4*.

Thus, we suggest that miR-124 regulates Wnt pathway through *Sfrp* and could play a role in cell proliferation, cell survival and differentiation of the inner ear.

Keywords: miRNA; cochlea; sfrp4, wnt signaling pathway.

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First steps towards a gapless interface between auditory neurons and multi-electrode arrays *in vitro*

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Background: Cochlear implants (CIs) have become the gold standard treatment for deafness. Despite all the success, some limitations remain. Our project NANOCI (www.nanoci.org) aims at developing a new generation of CIs where the auditory neurons directly grow onto the implant to form a gapless interface between the neurites and the electrode contacts in the cochlea. In theory, this strategy should result in *i)* a better auditory resolution and *ii)* lower energy consumption, two of the main limitations of current CI systems. As a first step towards this ambitious goal, we aimed at developing a gapless interface between auditory neurons and multi electrode arrays (MEAs) *in vitro* to study parameters influencing the interface including neuron density, neuron morphology and surface structure.

Methods: First, the optimum cell preparations of rodent auditory neurons in vitro were comparatively assessed with respect to morphology and physiological response profiles. Four different types of preparations were studied: a) primary dissociated, b) primary partially dissociated explant cultures (micro explants), c) undissociated spiral ganglion explant cultures and d) stem cell generated spiral ganglion-derived neuronal cells. Response profiles of auditory neurons were evaluated using MEAs. In a second step, glass slides were modified using nanotechnology to induce guided and selective growth towards electrode pads.

Results: All primary spiral ganglion cultures, dissociated, partially dissociated and undissociated were superior in terms of plating density, cell morphology and response profiles compared to stem cell-derived neuronal cell types. On our MEAs, auditory neurons were not spontaneously active, but upon electrical stimulation, they gave rise to action potentials. On glass slides, neurons could efficiently and selectively be guided along poly-l-lysine and laminin coated micro channels.

Conclusion: In the first year of the NANOCI-project, we were able to establish functionally mature spiral ganglion neuron cultures on multi electrode arrays. In addition, the nanostructurization of glass surfaces allowed for a selective and precise growth along micro channels. Although still preliminary, these results will lay the foundation to stimulate and record from auditory neurons via a gapless interface in vitro, and ultimately for future in vivo applications.

Keywords: Inner Ear Biology, cochlea implant, auditory neurons, nanotechnology

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Autophagy is required for apoptotic cell clearance and neural differentiation in early otic development

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Autophagy is a highly regulated program of self-degradation of the cytosolic constituents that has key roles during early development and in adult cell growth and homeostasis. To investigate the role of autophagy in otic neurogenesis, we studied the expression of autophagy genes in early stages of chicken inner ear development and the consequences of inhibiting the autophagic pathway in organotypic cultures of explanted chicken otic vesicles. Here we show the expression of autophagy-related genes Beclin-1, Atg5 and LC3B during early development of the chicken inner ear. The otic epithelium shows intense lysosomal activity and numerous autophagic vesicles, especially at the neuroblasts exit zone. The inhibition of the transcription of LC3B by using both genetic and pharmacological approaches causes an aberrant morphology of the otic vesicle with accumulation of apoptotic cells. Moreover, inhibition of autophagy provokes the misregulation of the cell cycle in the otic epithelium, impaired neurogenesis and poor axonal outgrowth. Finally, the addition of methyl pyruvate abrogated the consequences of autophagy inhibition. Therefore, our results indicate that autophagy provides the energy required for the clearing of neuroepithelial dying cells and suggest that it is required for the migration of otic neuronal precursors. Taken together, our results show for the first time that autophagy is an active and essential process during early inner ear development.

Keywords: apoptosis; autophagy; 3-MA; otic neurogenesis

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TIS21 is involved in the development of spiral ganglion cells

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Background: TIS21 gene belongs to the member of BTG/Tob family with antiproliferative properties. TIS21 has function to induce the neuronal differentiation at cerebellum of mice (Canzoniere, Neurosci., 2004). We have already revealed that TIS21 is expressed in the spiral ganglion cells (SGCs) in embryonic day16 (E16) to postnatal day7 (P7) (Hayashida, Neuroreport, 2010). We set out to perform further developmental analysis utilizing TIS21 knock-in mice.

Methods: Wild type mice (TIS21(+/+)), heterozygous TIS21-GFP knock-in mice (TIS21 (+/-GFP)), homozygous TIS21-GFP knock-in mice (TIS21 (-GFP/-GFP)) were used for this experiment. TIS21-GFP knock-in mice is gift from Professor WB. Huttner in Max Planck Institute of Molecular Cell Biology and Genetics (Dresden, Germany). We performed cryosectioning for immune-histological studies. The mouse frozen heads were sliced at a thickness of 8μm using a cryostat along the modiolus of the cochlea. After Tuj1 and Hoechst stainings, we counted the number of the SGCs and measured the area of the Rosenthal canal of each turn of TIS21 (-GFP/-GFP) mice and wild type mice at E15.5, E18.5 and P4.

Results: GFP was expressed at the SGCs of TIS21 (+/-GFP) mice. The number of the SGCs in TIS21 (-GFP/-GFP) mice significantly decreased when compared with that of TIS21 (+/+) mice at E15.5, E18.5 and P4. The area of Rosenthal canal in TIS21 (-GFP/-GFP) mice decreased at E15.5 and P4.

Conclusion: The lack of TIS21 gene induces the decrease of the number of the SGCs and the decrease of the area of the Rosenthal canal in TIS21 (-GFP/-GFP) mice. These data suggest that TIS21 is necessary for the development of the SGCs and the Rosenthal canals.

Keywords: TIS21, development, spiral ganglion cells

Inhibition of matrix metalloproteinase-2 but not metalloproteinase-9 influences spiral ganglion neurons *in vitro*

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The evidence that matrix metalloproteinases are important in neurite elongation and axonal guidance in the central nervous system and the retina during development is increasing over the past decade. In the present study, we investigated the effects of matrix metalloproteinase inhibition on spiral ganglion neurons *in vitro* by blocking matrix metalloproteinases involved in axonal guidance, neurite elongation and apoptosis in other neuronal systems. Spiral ganglion explants from 5-day-old Wistar rats were treated with different concentrations of the general matrix metalloproteinase inhibitor GM6001, a specific matrix metalloproteinase-2 and a specific matrix metalloproteinase-9 inhibitor *in vitro*. The effect of the matrix metalloproteinase inhibitors on neurite outgrowth and length of neurites was then measured and subsequently analyzed. We observed a reduction in the neurite outgrowth as well as in the length of neurites after matrix metalloproteinases general inhibition. Specific inhibition of matrix metalloproteinase-2 showed a reduction in the number and the length of neurites in a dose-dependent manner. On the other hand, the inhibition of matrix metalloproteinase-9 had no effect neither on the neurite length nor on it's number. These findings suggest an influence of the matrix metalloproteinases on neuronal survival and neurite elongation in the mammalian inner ear *in vitro*.

Keywords: extracellular matrix, inner ear, matrix metalloproteinase, spiral ganglion neurons

The regulation of Hes/Hey factors during sensory development of the chicken inner ear

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The Notch pathway plays an essential role in the specification of the prosensory patches and in the determination of hair cells of the inner ear. The latter results from *lateral inhibition* mediated by the Notch ligand Delta1 (Dl1) and Jagged2/Serrate2 (Jag2/Ser2)). These ligands are expressed in hair cells that repress Notch ligands in neighbouring cells preventing their differentiation and promoting a salt and pepper gene expression pattern. However, the prosensory function of Notch relies on lateral induction and is mediated by Jagged1 (Jag1, also Serrate1/Ser1). In *lateral induction* the signal sending cell, the one that expresses the Notch ligand, activates the expression of the Notch ligand in the neighbouring cells resulting in a coherent pattern of gene expression. What underlies these two seemingly opposing modes of operation? We have explored this question by analysing the expression and function of Hes/Hey factors during inner ear development in the chick. The results show that Hey1 corresponds well with Jag1 expression in the prosensory patches. The cellular pattern of Hey1 expression is homogeneous within the patches as expected from lateral induction. On the contrary, Hes5 expression is speckled and delayed with respect to Hey1. It overlaps with Dl1 expression, and both parallel lateral inhibition during neurogenesis and hair cell determination. Hes1 is expressed weakly in prosensory and sensory patches and Hey2 is mainly expressed in the periotic mesenchyme. Respectively, Hey1 and Hes5 expression depends partially or completely on Notch activation as judged by the effects of gamma-secretase inhibitors DAPT and LY411575. The forced expression of hJag1 in the otic cup induces Hey1, but less efficiently Hes5, while Dl1 induces both. Overexpression of NICD induces Hey1 and Hes5, but with different thresholds. Hey1 is induced al low NICD levels and shows little concentration-dependence, while Hes5 expression requires higher dosage of NICD and is proportional to NICD dosage. The results suggest that Hey1 and Hes5 are good readouts of the prosensory and determination states of sensory the sensory epithelium. We are currently analysing the mechanism of this differential expression and the effects of the overexpression of Hes1 and Hes5.

Keywords: Notch, hair cell, sensory development

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Netrin-4 promotes and modulates inner ear spiral ganglion neurite outgrowth *in vitro*

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Netrins are a family of extracellular proteins that direct cell and axon migration during embryogenesis. Three secreted netrins (netrin 1, 3 and 4), and two glycosylphosphatidylinositol (GPI) -anchored membrane proteins, netrins G1 and G2, have been identified in mammals.1 Recently the Netrin-4 gene has been isolated and Netrin-4 mRNA is widely expressed in many regions of the adult brain, suggesting a role of Netrin-4 in the nervous system [2]. Functional studies demonstrated that Netrin-4 promotes neurite elongation from olfactory bulb explants, and that Netrin-4 may modulate and fine-tune the outgrowth and shape of emergent epithelial buds [1, 3]. In contrast to the situation in the olfactory system and the nervous system, the role of Netrin-4 in the auditory system is unknown.

To explore the potential for Netrin-4 to provide guidance to developing spiral ganglion neurons, spiral ganglion explants of p5 rats were cultured on Netrin-4 coated culture plates. Uniform coating of the whole surface or 100 um stripes of Netrin-4 were evaluated

We found that Netrin-4 promotes spiral ganglion neurite outgrowth in a dose dependant matter in vitro. Moreover, Netrin-4 acts as an attractant and modulates spiral ganglion neuron outgrowth in the alternate choice stripe assay in vitro. Our results suggest that Netrin-4 may play a role during the innervation of the inner ear.

Keywords: Axonal guidance; Spiral ganglion neurons; Netrin-4.

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Spiral ganglion-derived fibroblast cell culture

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In current research it is aimed at a direct connection between regrowing neurites and stimulating electrode contacts of cochlear implants. To achieve this, it might be necessary to distinguish electrically between neurons and other cell types. To investigate the effects of different substances on survival and regeneration of spiral ganglion cells (SGC) a cell culture of freshly isolated SGC is well established. This culture contains also glial cells and fibroblasts. To differentiate between the influences of the different cell types, a purified fibroblast preparation would be very helpful.

Spiral ganglia were isolated from newborn rats (P3). After mechanical dissociation, cells were seeded at a density of $3x10^5$ cells in fibroblast specific medium and passaged at confluence. Cell types were distinguished by using antibodies against the 200kD neurofilament (SGC), vimentin (fibroblasts and glial cells) and an S100 antibody (glial cells). Fibroblasts and glial cells were separated by FACS using extracellular staining (p75 for glial cells and Thy1 for fibroblasts. Characterization of the fibroblasts was then started by staining against Connexin 26 and carboanhydrase II to compare them with fibroblasts as described for the spiral ligament.

Using this approach, we can distinguish fibroblasts, glial cells and SGC form the spiral ganglion. Using fibroblast specific medium and sub-cultivation removed the SGC and enriched the fibroblasts in the cell culture. Cell sorting resulted in a purification of fibroblasts between 94 and 99 %. Most preparations are free of detectable glial cells.

According to current results fibroblasts from the spiral ganglion might be similar to fibroblasts type 1 from the spiral ligament or form an own type of fibroblasts in the inner ear. These purified fibroblasts can be stored frozen and cultured further after thawing.

Therefore, a purified cell culture of freshly isolated fibroblasts from the spiral ganglion is now available for further investigations.

Keywords: spiral ganglion, immunostaining,

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Rolipram improves survival of spiral ganglion cells *in vitro*

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Drug- and noise-induced trauma of the inner ear leads to the damage of hair cells that is followed by a degeneration of the SGN due to the loss of electrical activation and support with trophic factors released from the hair cells. The damage of the hair cells is often connected to an impairment of hearing, which can be moderated by cochlea implants. However, the benefit of the cochlear implant depends on the number and excitability of the SGN. Therefore, current research focuses on the identification of agents that will preserve SGN after implantation. In this project we investigated the neuroprotective effect of Rolipram as a promising agent to improve the viability auf the auditory neurons. Rolipram is a pharmaceutical agent that mediates its effect by selective inhibition of the phophodiesterase 4 leading to an increase in cyclic AMP (cAMP) [1]. Different studies reported a neuroprotective effect of Rolipram [1, 2, 3]. However, the significance for the survival of SGN has not been reported so far. To investigate the neuroprotective effect of Rolipram, we isolated spiral ganglion cells of neonatal rats for cultivation with different Rolipram concentrations and determined the survival rate of the SGN. Furthermore, we examined immunocytologically distinct proteins that might be involved in the neuroprotective signalling pathway of Rolipram.

Our results showed that the application of Rolipram improved survival of SGN in vitro, when applied at a concentration of 0,1 nM. According to previous studies [1, 3, 4], our immuncytological data showed that Rolipram application induces the phosphorylation and thereby activation of the transcription factor CREB that can be activated by the cAMP-PKA-signalling pathway as well as the activation of ERK as a part of the MAP-kinase pathway. We conclude that Rolipram has the potential to improve the vitality of auditory nerve cells and we hypothesize that it will protect the neurons after lesion of the hair cells and cochlear implantation from secondary degeneration.

Keywords: CREB, ERK, neuroprotection, Rolipram; spiral ganglion cells

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Extracellular matrix from human mesenchymal stem cells for cell-based drug delivery into the inner ear

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Innovative cell-based drug delivery systems may provide long-term application of i.e. growth factors to improve the electrode-nerve-interface of auditory implants. An approach for drug delivery is the coating of electrode surfaces with lentivirally modified NIH3T3 cells secreting BDNF considering potential proliferation of the cells nearly all over the electrode surface and, thus, providing growth factors to the spiral ganglion neurons (SGN) throughout all cochlear turns [1]. However, coverage of the electrode surface with cells is subject to a random distribution of the cells on the surface resulting in differing cell numbers and, thus, in varying BDNF concentration provided to the SGN. Additionally, insertion of the cell coated electrode into the cochlea results in partial disruption of the cell layer. To address these problems adhering of the genetically modified cells to extracellular matrix (ECM) may improve the growth conditions on the electrode and prevent the immobilised cells against mechanical stress. In this study an *in vitro* model for adhering recombinant NIH3T3 cells and SGN to the ECM was established. For that decellularized ECM substrates were generated from mesenchymal stem cells (MSC), which secreted their matrix molecules onto a glass cover slips for ten days. Murine NIH3T3 cells and SGN enzymatically dissociated from the spiral ganglions of postnatal rats were seeded on these ECM substrates and cultivated in high glucose DMEM and panserin, respectively, for 48 h at 5 % CO_2 and 37°C. The NIH3T3 cells were microscopically tracked by GFP fluorescence and the SGN were immunocytochemically detected using anti-neurofilament-antibody and DAB staining. The fibroblasts showed adhesion and growth along the ECM structures forming uniform meandering pattern, instead randomly distributed cell cluster as observed in control assays without ECM. Furthermore, an increase of the proliferation activity could be found. The SGN also showed neurite outgrowth along the ECM grid, however, a significant increase of the survival rate on the ECM in comparison to the control assay without ECM was not found. In conclusion we could demonstrate beneficial effects of MSC-derived ECM on the proliferation and morphology of the fibroblasts as well on the survival and neurite outgrowth of the SGN.

Keywords: Cell-based drug delivery, extracellular matrix (ECM); spiral ganglion neurons (SGN), green fluorescent protein (GFP)

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Differentiation of Boettcher's cells during postnatal development of rat cochlea

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Contrary to the highly specialized epithelial cells of the mammalian auditory organ, little is known about the surrounding cells, and in particular Boettcher's cells (BC). Our morphological studies show that in rats, they begin their differentiation around P8 to reach maturity around P20, when they are completely covered by Hensen's and Claudius' cells. Moreover, tight junctions were noted near the apex of BC as long as they are in direct contact with the endolymphatic space, i.e. between around P8 and P16. We observed gap junctions between BC and the adjacent cells before the end of the covering process suggesting that BC could also be involved in potassium recycling into endolymph. Adherens junctions were also seen between BC all along their maturation. Importantly, we noticed cytoplasmic secretory granules and an accumulated material, probably a secretion, in the intercellular space, between P8 and P25. These results seem to indicate that BC could basally take part in the secretion of the extracellular matrix of the basilar membrane. Finally, we show that their basolateral microvilli are longer and more tighlty grouped at maturity and harbour urea transporters as soon as P18. Together, our observations support the view that BC could perform several functions.

Keywords: connexin-26, microvilli, secretion, ultrastructure, urea transporter

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