51st Inner Ear Biology Workshop
Programme & Abstract Book

51st Inner Ear Biology Workshop

The University of Sheffield
Sheffield, United Kingdom
30th August – 2nd September 2014

Programme & Abstract Book

Welcome

Dear friends and colleagues,

Welcome to Sheffield, venue of the 51st Inner Ear Biology Workshop.

Sheffield is located in the region of Yorkshire in northern England, surrounded by the beautiful scenery of the Peak District National Park. Yorkshire has played a key role in many historical events that moulded England; the War of the Roses coming to mind as a central one. Sheffield, a city built on a rich tradition of industrial ingenuity and innovation, is the seat of two large Universities and vibrates with academic activity and student life.

The workshop will be held at The Edge, the Conference Centre of the University of Sheffield, from August 31st to September 2nd 2014. As is now becoming traditional for this event, the workshop will be preceded by a one-day translational Symposium on August 30th introducing the latest developments in *Cochlear Implantation and the Future Scope of the Technology*.

The Workshop programme covers a wide range of topics in inner ear research, ranging from sensory cell physiology to central pathway biology, and including regeneration, development and cellular biology. It will be focussed on translational developments, aiming to bridge the gap between basic science and its clinical application.

Our gratitude goes to our sponsors and collaborators that have supported this event in different ways and above all to you, the participants. Through your active engagement, and by sharing your latest research and scientific enthusiasm, have allowed this event to run for more than half a century!

On behalf of the Organizing Committee, I am delighted to welcoming you to Sheffield, and am looking forward to a most enjoyable exchange of knowledge and ideas.

Marcelo Rivolta

IEB 51st Workshop

Conference Chair:

- Marcelo Rivolta

Organising Committee:

- Matthew Holley
- Stuart Johnson
- Walter Marcotti
- Marta Milo
- Tanya Whitfield

International Scientific Committee:

- Jonathan Ashmore (UCL, UK)
- Anthony W. Gummer (Univ. Tübingen, Germany)
- Gaetano Paludetti (Catholic Univ. of the Sacred Heart, Rome, Italy)
- Isabel Varela-Nieto (IIBM 'Alberto Sols', Madrid, Spain)
- Gerard O'Donoghue (Queen's Medical Center, Nottingham, UK)

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- Isabel Varela-Nieto (IIBM 'Alberto Sols', Madrid, Spain)
- Anna R. Fetoni (Catholic Univ. of the Sacred Heart, Rome, Italy)
- Marlies Knipper(University Hospital, Tübingen, Germany)

Organising Secretariat:

- Rose Nightingale (Training & Events Manager)
- Tabasum Nisa (Training & Events Manager)

Sponsors and Collaborators

Main Sponsors:

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Collaborators:

Otostem
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Targear
www.targear.eu

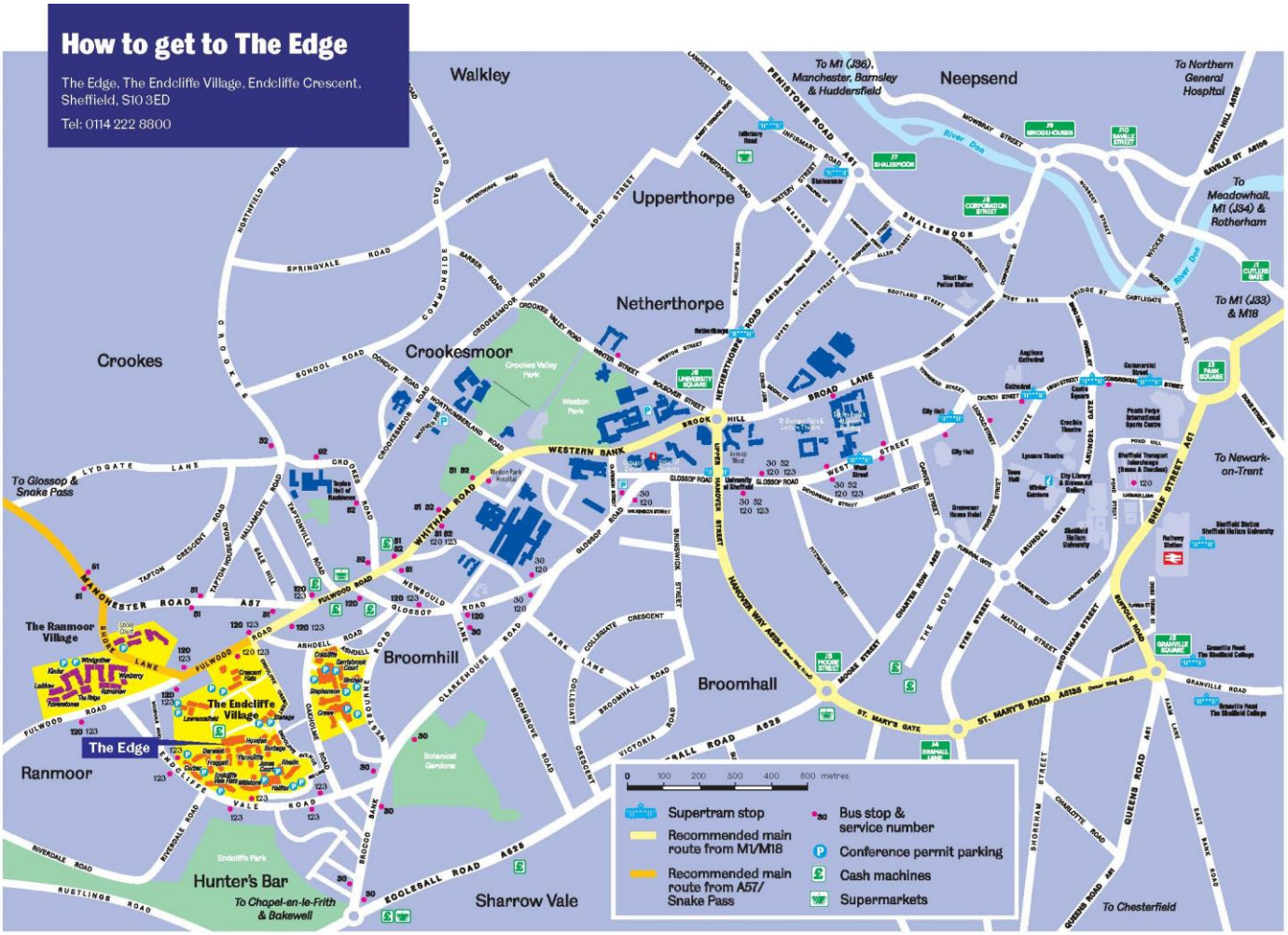
Centre for Stem Cell Biology
www.cscb.shef.ac.uk

Welcome to Sheffield
www.welcometosheffield.co.uk

General Information

A. Conference Venue

The Workshop and Symposium will be held in The Edge at the University of Sheffield (The Edge, Endcliffe Village, 34 Endcliffe Crescent, Sheffield, S10 3ED). The venue is located within 20 minutes walking distance of the city centre and just 2 minutes from the accommodation.



B. Registration

Registration for all delegates will be taken at The Edge Hub which is situated next to the Dining area. If you are unsure of this area, please make you way to The Edge Reception and they will inform you where to go.

C. Internet Access

Internet access will be available at The Edge and Wifi access codes will be available on arrival.

D. Useful Information

Lunches and Coffee Breaks:
Lunches and coffees will be provided from Saturday to Tuesday free of charge to registered participants. All food and drinks will be served either in the dining area or the bar at The Edge.

Breakfast Times:

Halifax Hall
Weekday – 7am until 10am
Weekend – 7.30am until 10.30am

Student Accommodation – taken in The Edge Dining Room
Weekday & Saturday's – 7.30am until 9am
Sunday's – 8.30am until 9.30am

The Edge Bar opening times:
Saturday 30th August – You will be provided with an outdoor BBQ at The Edge at 7pm, the bar will be open from 6pm until Midnight.

Sunday 31st August – After your Reception at the Town Hall, you will be brought back to The Edge Bar for approximately 9.30pm, bar open until 11.30pm.

The bar WILL NOT BE OPEN on Monday 1st September.

Coaches:
Coaches will arrive on Encliffe Vale Road approximately 6.30pm on Sunday 31st August for all guests for the Reception at the Town Hall. Coaches will then collect delegates and bring back to the Endcliffe Campus approximately 9.30pm.

Coaches will arrive on Endcliffe Vale Road approximately 7.00pm on Monday 1st September for those delegates booked on to the dinner at Hassop Hall. Coaches will then collect delegates and bring back to the Endcliffe Campus approximately by midnight.

Check out times:
Please note that the check out time for both the student accommodation and Halifax Hall is 10am on the day of departure.

Luggage may be kept in The Edge Bar; this is however at your own risk as the room is not lockable.

Arrival refreshments:
Please be aware that the arrival refreshments will be taken in The Edge bar from 8.30am until 9am on all days due to breakfast service in the dining room. All other refreshment and lunch breaks will be taken in The Edge Dining area.

Oral Presentations

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

Presentations are only accepted in PowerPoint formats. Please bring your presentation on 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. There will be a PC (NOT a Mac) with MS PowerPoint 2007 and 2010 at the venue.

Speakers should contact the AV technician 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. Those with Apple-based presentations should make sure they are saved in a PC-compatible format.

PLEASE NOTE THAT IN ORDER TO KEEP TO THEIR TIME ALLOCATION, SPEAKERS ARE URGED TO UPLOAD AND TEST THEIR PRESENTATION WELL IN ADVANCE.

SPEAKERS WILL NOT BE ALLOWED TO USE THEIR OWN LAPTOP.

Posters

Poster board dimensions are 100 cm wide (39-inch), 170 cm (67-inch) high (portrait orientation). Prepare your poster accordingly.

PLEASE NOTE THAT THE POSTERS WILL ONLY BE IN THE PORTRAIT CONFIGURATION

Posters must be set up at least ½ hour in advance of the first Poster Session (scheduled for August 31st, at 12:45), and should remain up until the last session (scheduled for September 1st, at 17:30). Posters should be promptly removed immediately after this session. There will be no poster session on September 2nd.

The presenting author is responsible for setting up and removing the poster. The IEB Workshop is not responsible for posters and materials left after the session. Ideally, authors should be at their posters during every poster presentation session. However, it would be recommended they leave a note at their posters indicating their availability when visiting other posters.

Index

Inner Ear Biology Workshop
51st Annual Conference, 30 Aug – 2 Sep 2014

Symposium Program

Saturday, August 30

Cochlear Implantation and the future scope of the technology

A selection of speakers, leaders in their fields, will cover the state-of the art of cochlear implantation and introduce the current challenges the technology is facing. Our Plenary speaker is Prof. Ingeborg Hochmair, winner of the 2013 Lasker-DeBakey Clinical Medical Research Award.

Venue: High Tor 2 Lecture Theatre, The Edge, Endcliffe Village, 34 Endcliffe Crescent, Sheffield, S10 3ED

08:30 Open Registration

08:30 Arrival refreshments

08:45 Opening of the symposium by Marcelo Rivolta

08:50 Welcome address
Prof Anthony Ryan, OBE
Pro Vice Chancellor, Faculty of Science, University of Sheffield

09:00 ***Cochlear implants: Fundamentals***
Chairperson – Gerard O'Donoghue

09:00 S1 **Introduction to cochlear implants**
Gerard O'Donoghue

09:30 S2 **On the anatomy of the human cochlea relevant for cochlea implantation**
Helge Rask-Andersen

10:00 S3 **Single-sided deafness affects binaural interactions**
Andrej Kral

10:30 - 11:00 Coffee Break

11:00 ***Cochlear implants: New advances and complementary technologies***
Chairperson – Helge Rask-Andersen

11:00 S4 **Electric Acoustic Stimulation (EAS) and structure preservation cochlear implantation**
Paul Van de Heyning

11:30 S5 **Optogenetic development of cochlear implantation**
Anna Gehrt

12:00 S6 **Functional Magnetic Resonance Imaging (fMRI) and brain plasticity in cochlear implantation**
Deb Hall

12:30 - 14:00 Lunch

14:00 ***Nanotechnology-based Cochlear Implants***
Chairperson – Takayuki Nakagawa

NANOCI consortium public workshop

14:00 S7 **NANOCI - Nanotechnology based cochlear implant with gapless interface to auditory neurons**
Pascal Senn

14:10 S8 **NANOCI -Nanotechnology used in NANOCI**
Herbert Keppner

14:30 S9 **NANOCI - Testing innovative bio-funcionalized compounds for the ear**
Marcus Mueller

14:50 S10 **NANOCI - Fusing Nanotechnology and Biology into a Pilot Product- the Manufacturer's Point of View**
Carolyn Garnham

15:10 S11 **Stem cells meet cochlear implants: a gerbil 'double ablation' model**
Leila Abbas

15:30 -16:00 Coffee Break

16:00 – 17:00 ***Plenary Lecture (PL)***
Introduced by Marcelo Rivolta

Cochlear implantation: past, present and what lies ahead
by Ingeborg Hochmair

19:00 - 21:00 Welcome Reception at The Edge

Workshop Program

Sunday 31 August

Workshop Program Overview

08:30 Open Registration

08:30 Arrival refreshments

08:50 Welcome address

09:00 ***Molecular and anatomical bases of hearing and hearing loss***
Chairpersons – Allen Ryan and Anneliese Schrott-Fischer

09:00 LS1 **Lead Speaker: Guy Richardson**
Structure, function and development of the tectorial membrane: an extracellular matrix of the inner ear

09:30 O1 The role of G protein isoforms in hearing in adult mice
Sze Chim Lee, Novakovic A, Beer-Hammer Sandra, Montcouquiol Mireille, Nürnberg Bernd, Rüttiger Lukas and Knipper Marlies

09:45 O2 Response of type I and type II spiral ganglion neurons to guidance molecule gradients
Allen F Ryan, Andy Kamgar-Parsi, Kwang Pak, Gary Housley and Yves Brand

10:00 O3 Type I IFN is produced in supporting cells against virus infection of the cochlear sensory epithelium via RIG-I like receptor signalling pathway
Yushi Hayashi, Koji Onomoto, Ryo Narita, Mitsutoshi Yoneyama, Hiroki Kato, Akiko Taura, Takayuki Nakagawa, Juichi Ito, Kimitaka Kaga and Takashi Fujita

10:15 O4 Beta 5 Tubulin and 15-protofilament microtubules appeared in supporting cells of the organ of Corti during development in rodents
Justine Renauld, Nicolas Thelen, Nicolas Johnen and Marc Thiry

10:30 - 11:00 Coffee Break

11:00 ***Cellular physiology of hearing and hearing loss***
Chairpersons – Laura Corns and Stuart Johnson

11:00 LS2 **Lead Speaker: Helen Kennedy**
Calcium induced calcium release during action potential firing in developing inner hair cells

11:30 O5 Endocytic activity of the outer hair cell
Csaba Harasztosi, Susanne Lauber, Emese Harasztosi and Anthony W. Gummer

11:45 O6 Expression and localization of somatostatin receptors subtype 3, 4 and 5 in the wild type and knockout mouse cochlea and a protective role of somatostatin
Adrijana Perkovic, Vesna Radojevic and Daniel Bodmer

12:00 O7 Calcium waves in the adult mouse organ of Corti.
Piotr Sirko, Jonathan Gale and Jonathan Ashmore

12:15 O8 Maturation of molecular state memory within SLCA26 following membrane insertion
Feng Zhai, Lei Song, Jun-Ping Bai, Chunfu Dai and *Joseph Santos-Sacchi*

12:30 – 12:45 Group Photo

12:45 – 14:00 Lunch and Poster Session

14:00 ***Systems Biology and Gene Networks / Ototoxicity I***
Chairpersons – Marta Milo and Anna Fetoni

14:00 LS3 Lead Speaker: Michael Lovett
A Comparative Analysis of the Regenerative Transcriptome in the Avian Utricle and Cochlea after Aminogycoside-Induced Damage

14:30 O9 Neither necessary nor sufficient: Hair cell development without ATOH1 and neuronal development with ATOH1
Bernd Fritzsch, Ning Pan and Israt Jahan

14:45 O10 Small-RNA deep sequencing analysis in Human Fetal Auditory Stem Cells (hFASCs) identifies novel miRNAS with a potential role in the development of the human inner ear
Lucia Borreguero, Matías Morín, Antonio Rueda, Angeles Mencía, Ricardo Romero-Guevara, Objoon Trachoo, Ximo Dopazo, Ana Aransay, M. Laura Garcia-Bermejo, Edurne Ramos, Javier Santoyo, Marcelo Rivolta and Miguel Angel Moreno Pelayo

15:00 O11 Protective effect of metformin against gentamicin-induced auditory hair cell loss in vitro
Andrea Glutz, Katharina Leitmeyer, Cristian Setz, Soledad Levano, Vesna Radojevic, Yves Brand and Daniel Bodmer

15:15 O12 Inhibition of mTOR by Rapamycin results in auditory hair cell damage and decreased number and length of spiral ganglion neurites in vitro
Katharina Leitmeyer, Andrea Glutz, Cristian Setz, Soledad Levano, Vesna Radojevic, Daniel Bodmer and Yves Brand

15:30 – 16:00 Coffee Break

16:00 ***Ageing: Presbyacousis and Vestibular Conditions***
Chairpersons – Michael Bowl and Isabel Varela-Nieto

16:00 LS4 **Lead Speaker: Karen Steel**
Clues to the molecular basis of progressive hearing loss from mouse mutants

16:30 O13 Trombone- A model of age-related hearing loss suggests a novel deafness gene SLC4A10 is required for normal auditory function
Prashanthini Shanthakumar, Carlos Aguilar, Sue Morse, Vincent Michel, Andrew Parker,Lauren Chessum,Femke Stelma, Michelle Simon, Sara Wells, Sally J. Dawson, Christine Petit, Paul Potter, Steve DM Brown and Michael R Bowl

16:45 O14 BDNF deletion in the cochlea/lower brainstem leads to central plasticty changes over age
Campanelli D, Lee SC, Varakina K, Bing D, Zuccotti A, Singer W, Rüttiger L, Schimmang T and Knipper M

17:00 O15 Hearing and ageing: a multistep genomic strategy to identify new genes/variants in European and Central Asian populations
Girotto G, Dawson S, Concas MP, Ciullo M, Nutile T, Cappellani S,Loukola A, Bahl A, Pirastu M, Gasparini P and Vuckovic D

17:15 O16 Utricular hair cells and transitional cells reciprocally regulate endolymphatic cation transport via purinergic stimulation
Sung Huhn Kim, Jin Young Kim and Jae Young Choi

17:30 – 18:30 **Poster Session**

18:30 – 21:00 **Welcome Cocktails and Reception by the Lord Mayor at the Sheffield City Town Hall**

Monday 1 September

08:30 Open Registration, Poster display

08:30 Arrival refreshments

09:00 ***Development of the ear***
Chairpersons – Bernd Fritzsch and Sarah Baxendale

09:00 LS5 **Lead Speaker: Andrea Streit – Towards a gene regulatory network for inner ear specification**

09:30 O17 Unravelling the roles of lysine acetylation by ELP3 during inner ear development
Susana Mateo Sánchez, Laurence Delacroix, Sophie Laguesse, Sandra Huysseune, Alain Chariot, Laurent Nguyen and Brigitte Malgrange

09:45 O18 Tenascin-C affects the growth behaviour and the differentiation of auditory neurons
Monika Kwiatkowska, Nina Kraft, Jacqueline Reinhard, Andreas Faissner, Stefan Dazert and Stefan Volkenstein

10:00 O19 Septin 7 contributes to the formation of the inner ear gross morphology
Norio Yamamoto, Hiroko Torii, Makoto Kinoshita, Takayuki Nakagawa and Juichi Ito

10:15 O20 Semicircular canal formation in zebrafish
Sarah Baxendale, Esther C. Maier, Rachael Lawrence and Tanya T. Whitfield

10:30 – 11:00 Coffee Break

11:00 ***Stem Cells and Regeneration***
Chairpersons – Andy Forge and Sarah Boddy

11:00 LS6 Lead Speaker: Pascal Senn
OtoStem: A Framework 7 European consortium to develop human stem cell applications for the treatment of hearing loss

11:30 O21 Conditioning the cochlea to facilitate survival and integration of exogenous cells into the auditory epithelium
Yong-Ho Park and Yehoash Raphael

11:45 O22 Localization and expression profiles of adipose-derived stem cells in a model of cochlear injury induced by acoustic trauma in the guinea pig
Anna R. Fetoni, Wanda Lattanzi, Sara M.L. Eramo, Rolando Rolesi, Fabiola Paciello, Marta Barba, Diana Troiani and *Gaetano Paludetti*

12:00 O23 Cochlear hair cell generation from LGR5-positive supporting cells
Albert Edge

12:15 O24 Hair cell regeneration by ATOH1 gene therapy in the cochlea of mature deafened guinea pigs
Rachael T Richardson, Patrick J Atkinson, Brianna O Flynn, Bryony A Nayagam and Andrew K Wise

12:30 – 13:30 Lunch and Poster Session

13:30 ***Spoendlin Award Presentation and Lecture***
Chairperson – Isabel Varela-Nieto
Prediction of mechanical effect due to a cochlear implant – Guanjin Ni

14:00 ***Genetic basis of human deafness***
Chairpersons – Antonio Lopez-Escamez and Miguel Angel Pelayo

14:00 LS7 Lead Speaker: Maria Bitner-Glindzicz
Genetic deafness in humans in the 'Next Generation' era: the pleasure and the pain

14:30 O25 Novel loci in chromosomes 2 and 6 associated with bilateral Meniere's disease may define autoimmune inner ear disease
Sonia Cabrera, Lidia Frejo, Teresa Requena, Carmen Martín-Sierra and Jose Antonio Lopez-Escamez

14:45 O26 Exome sequencing identifies FAM136A and DTNA as candidate genes in familial Meniere's disease
Teresa Requena, Sonia Cabrera, Carmen Martín-Sierra, Lidia Frejo, Anna Lysakowski, Jose Antonio Lopez-Escamez

15:00 O27 Molecular dynamic simulations highlight structural and functional alterations in deafness-related M34T mutation of Connexin 26
Damiano Buratto, Francesco Zonta and Fabio Mammano

15:15 O28 New hereditary hearing loss (HHL) genes/mutations identified by high throughput technologies in the Italian and Qatari populations
G.Girotto, D.Vozzi, E.Rubinato, A.Morgan, K.Abdulhadi, D.Vuckovic, M.Di Stazio, A. D'Eustacchio, M. La Bianca, R. Badii and P.Gasparini

15:30 – 16:00 Coffee Break

16:00 ***Noise-induced hearing loss / Ototoxicity II***
Chairpersons – Guy Richardson and Agnieszka J. Szczepek

16:00 LS8 Lead Speaker: Christopher Plack
Hidden hearing loss: causes and consequences

16:30 O29 cGMP Generators in the inner ear: which ones may be involved in otoprotection after noise damage?
Lukas Rüttiger, Mahdieh Alinaghikhani, Nicole Eichert, Dorit Möhrle, Ksenia Varakina, Evanthia Mergia, Doris Koesling, Hannes Schmidt and Marlies Knipper

16:45 O30 STAT3 and otoprotection
Agnieszka J. Szczepek, Elisabeth Gerschner; Heidi Olze and Birgit Mazurek

17:00 O31 Chemotherapy for the future: Novel aminoglycosides dissecting antibacterial activity and ototoxicity
Dmitri Shcherbakov, Su-Hua Sha, Déborah Perez-Fernandez, Rashid Akbergenov, Heithem Boukari, Stefan Duscha, Pietro Freihofer, Srinivas R. Dubbaka, Jing Xie, Andrea Vasella, V. Ramakrishnan, *Jochen Schacht* and Erik C. Böttger

17:15 – 18:15 Poster Session

19:00 – 00:00 Dinner at Hassop Hall

Tuesday 2 September

08:30 Open Registration

08:30 Arrival refreshments

09:00 ***New therapeutics and experimental models for hearing loss***
Chairpersons – Pascal Senn and Leila Abbas

09:00 LS9 Lead Speaker: Takayuki Nakagawa
Topical IGF-1 therapy using a gelatine hydrogel: pre-clinical study, clinical trials and mechanisms

09:30 O32 Isolation and characterization of nasal and middle ear epithelial cells for the development of an otopathogenic infection model
Apoorva Mulay, Michael Cheeseman, Steve Brown, Khondoker Akram, Lynne Bingle and Colin Bingle

09:45 O33 Future trends regarding the bioelectrical interface of cochlear implants
Stefan Volkenstein, Monika Kwiatkowska and Stefan Dazert

10:00 O34 Development of an in vitro bioassay to analyze interface-dependent response profiles of auditory neurons on multi-electrode arrays
Stefan Hahnewald, Marta Roccio, Emanuele Marconi, Carolyn Garnham, Teresa Melchionna, Anne Tscherter, Jürg Streit, Julien Brossard, Alexandra Homsy, Herbert Keppner, Hans-Rudolf Widmer and Pascal Senn

10:15 O35 A comparison of ear-canal Distortion Product Otoacoustic Emissions with inferred intra-cochlear distortion products
Brenda L. Lonsbury-Martin, Glen K. Martin and Barden B. Stagner

10:30 – 11:00 Coffee Break

11:00 ***Central perception and tinnitus***
Chairpersons – Marlies Knipper and Tony Gummer

11:00 LS10 Lead Speaker: Ian Forsythe
Nitric Oxide signalling in auditory processing

11:30 O36 State-dependent information processing in auditory thalamocortical circuit
Shuzo Sakata

11:45 O37 BDNF-dependent auditory fibers set brains baseline for sound
Marlies Knipper, Tetyana Chumak, Lukas Rüttiger, Sze Chim Lee, Dario Campanelli, Annalisa Zuccotti, Wibke Singer, Jiří Popelář, Katja Gutsche, Sebastian Philipp Schraven, Mirko Jaumann, Jing Hu, Thomas Schimmang, Ulrike Zimmermann and Josef Syka.

12:00 O38 PIP2 determines excitability of spiral ganglion neurons via regulation of Kv1-containing heteromeric channels
Lorcan Browne, Katie Smith, David McAlpine, David Selwood and Dan Jagger

12:15 O39 Comparison of dorsal cochlear nuclei microgliosis in different rat tinnitus models
Venturino Alessandro, Oda Adriano, Losurdo Morris, Colombo Gloria, Pizzala Roberto and *Perin Paola*

12:30 – 13:00 Business Meeting and Closing Ceremony

End of Workshop

Poster Program

Posters must be set up at least ½ hour in advance of the first Poster Session (scheduled for August 31st, at 12:45), and should remain up until the last session (scheduled for September 1st, at 17:30). Posters should be promptly removed immediately after this session. There will be no poster session on September 2nd.

I. MOLECULAR AND ANATOMICAL BASES OF HEARING AND HEARING LOSS

P1. VOLTAGE AND CALCIUM IMAGING IN THE DEVELOPING COCHLEA OF WILD TYPE AND DFNB1 MOUSE MODELS
Federico Ceriani and Fabio Mammano

P2. HUMAN TECTORIAL MEMBRANE: AN IMMUNOHISTOCHEMISTRY ANALYSIS AND ELECTRON MICROSCOPIC STUDY
Hisamitsu Hayashi, Wei Liu, Francesca Atturo, Kristian Pfaller, Rudolf Glueckert, Anneliese Schrott-Fischer and Helge Rask-Andersen

P3. DIFFERENTIAL EXPRESSION PATTERNS OF ATP6B1 IN THE INNER EAR LATERAL WALL OF COMMON MARMOSET AND MOUSE
Makoto Hosoya, **Masato Fujioka, Hideyuki Okano and Kaoru Ogawa**

P4. MORPHOLOGY, IMAGING AND LOCAL SURGICAL ANATOMY OF THE TEMPORAL BONE OF COMMON MARMOSET *(CALLITHRIX JACCHUS)*
Masato Fujioka, Keigo Hikishima, Ken-ichiro Wakabayashi, Makoto Hosoya, Hirotaka James Okano, Toshio Itoh, Hideyuki Okano and Kaoru Ogawa

P5. MEASURING GLUTATHIONE CONTENT IN THE ORGAN OF CORTI USING LIVE IMAGING
Paromita Majumder, Michael Duchen and Jonathan Gale

P6. IMMUNOHISTOCHEMISTRY OF METHYL METHACRYLATE EMBEDDED GUINEA PIG AND MOUSE COCHLEAE
Peter Bako, Mohamed Bassiouni, Imre Gerlinger, Claudia Frick, Hubert Löwenheim and Marcus Müller

P7. IMAGING OF ANIMAL AND HUMAN INNER EARS WITH X-RAY MICROTOMOGRAPHY
Rudolf Glueckert, Anneliese Schrott-Fischer, Lejo Johnson Chacko, Martin Gloesmann and Stephan Handschuh

P8. HUMAN BASILAR MEMBRANE: AN IMMUNOHISTOCHEMISTRY AND ELECTRON MICROSCOPIC STUDY
Wei Liu, Francesca Atturo, Robair Aldaya, Peter Santi, Sebahattin Cureoglu, Rudolf Glueckert, Kristian Pfaller, Annelies Schrott-Fischer, Pascal Senn, Hubert Löwenheim and Helge Rask-Andersen

P9. DETAILED ANALYSIS OF HISTONE MODIFICATION IN THE SPIRAL GANGLION OF THE MOUSE COCHLEA
Ken-ichi Watanabe, Wilhelm Bloch

P10. ULTRASTRUCTURAL LOCALIZATION OF TMHS IN MOUSE COCHLEAR HAIR CELLS
C.M. Hackney, S. Mahendrasingam, D.N. Furness, M. Beurg and R. Fettiplace

P11. MELATONIN'S EFFECT ON THE INNER EAR CELLS' ULTRASTRUCTURE
Natalya N. Petrova, *Vera N. Perevozchikova*, Ekaterina V. Petrova

P12. RESIDUAL CRE RECOMBINASE EXPRESSION CORRELATES WITH HEARING LOSS AND REDUCED CONNEXIN 26 TRANSCRIPT LEVEL IN THE CX30$^{\Delta/\Delta}$ MOUSE MODEL
Veronica Zorzi, Giulia Crispino, Andrea Carrer, Pietro Scimemi and Fabio Mammano

II. CELLULAR PHYSIOLOGY OF HEARING AND HEARING LOSS

P13. EPS8 REGULATES K^+ CURRENT EXPRESSION IN MOUSE COCHLEAR INNER BUT NOT OUTER HAIR CELLS NOR IN VESTIBULAR TYPE I AND TYPE II HAIR CELLS
Elisa Tavazzani, Giancarlo Russo, Paolo Spaiardi, Marco Manca, Ivo Prigioni and Sergio Masetto

P14. CALCIUM ENTRY INTO STEREOCILIA DRIVES ADAPTATION OF THE MECHANOELECTRICAL TRANSDUCER CURRENT IN MAMMALIAN COCHLEAR HAIR CELLS
Laura F. Corns, Stuart L. Johnson and Walter Marcotti

P15. SENSORY TRANSDUCTION IN OUTER HAIR CELLS DOES NOT ACCOUNT FOR THE HEARING LOSS CAUSED BY HAPLOINSUFFIENCY OF TRANSCRIPTION FACTOR GATA3
Tanaya Bardhan, Walter Marcotti and Matthew Holley

P16. ZEBRAFISH: AN EMERGING MODEL FOR INVESTIGATING HAIR CELL PHYSIOLOGY AND FUNCTION.
Jennifer Olt, Stuart L. Johnson and Walter Marcotti

III. OTOTOXICITY

P17. PROTECTIVE EFFECT OF RESVERATROL AND N-ACETYLCYSTEINE COMBINATION ON KANAMYCIN-INDUCED OTOTOXICITY IN RATS
Silvia Murillo-Cuesta, Fernando García-Alcántara, Raquel Martínez-Vega, Marta Milo, Isabel Varela-Nieto and Teresa Rivera

P18. TARGETING TRANSLATION DURING CELLULAR STRESS: PHARMACOLOGICAL MANIPULATION OF STRESS GRANULE FORMATION IN THE OC-2 CELL LINE
Ana Cláudia Gonçalves, Sally Dawson and Jonathan Gale

P19. MECHANISM OF PROTECTIVE EFFECT OF LOW-LEVEL LASER THERAPY ON HEI-OC1 CELLS AFTER GENTAMICIN-INDUCED OTOTOXICITY
So-Young Chang, Chung-Ku Rhee, Myung-Whan Suh, Phil-Sang Chung, *Jae Yun Jung*

P20. *IN VIVO* PROTECTIVE EFFECT OF QTER AGAINST STYRENE OTOTOXICITY AND ACOUSTIC TRAUMA: A FUNCTIONAL AND MORPHOLOGICAL STUDY IN A MODEL OF NOISE INDUCED HEARING LOSS (NIHL).
Rolando Rolesi, Annarita Fetoni, Fabiola Paciello, Sara ML Eramo, Massimo Ralli, Diana Troiani and Gaetano Paludetti

IV. AGE-RELATED HEARING LOSS

P21. THE HARWELL AGING SCREEN IDENTIFIES NOVEL MOUSE MODELS OF EARLY-ONSET AND AGE-RELATED HEARING LOSS
Michael R Bowl, Carlos Aguilar, Sue Morse, Joanne Dorning, Prashanthini Shanthakumar, Ruairidh King, Shelley Harris, Andrew Blease, Michelle Simon, Andy Parker, Sara Wells, Paul Potter, Steve DM Brown

P22. ROLE OF *ACSL4* AND LIPID METABOLISM IN HIGH FREQUENCY HEARING LOSS
Elisa Martelletti, Annalisa Buniello, Neil Inghamand Karen P. Steel

P23. AGE-RELATED HEARING LOSS IS AFFECTED BY PERIPHERAL IMMUNE SYSTEM
Akosua Agyemang-Prempeh, Carl Verschuur and Tracey Newman

P24. KINESIN LIGHT CHAIN-2 (*Klc2*) :A NOVEL GENE INVOLVED IN PROGRESSIVE HEARING LOSS
Neil J. Ingham, Seham Ebrahim & Karen P. Steel

P25. LONG-TERM EFFECT OF OMEGA-3 FATTY ACID SUPPLEMENTATION IN A MODEL OF AGE-RELATED HEARING LOSS
Raquel Martinez-Vega, Teresa Partearroyo, Gregorio Varela-Moreiras, María A. Pajares Isabel Varela-Nieto.

P26. WHITE MATTER INTEGRITY CHANGES IN AGE-RELATED HEARING LOSS: TRACT BASED SPATIAL STATISTICS STUDY
Fahad Alhazmi and Vanessa Sluming

V. VESTIBULAR CONDITIONS

P27. DIFFERENCE OF AUTOANTIBODY BETWEEN MENIERE'S DISEASE AND BILATERAL SUDDEN HEARING LOSS: IS THERE A DIFFERENT AUTOIMMUNITY MECHANISM?
Eun Sung Kim, Sung Huhn Kim, Jin Young Kim, Jee Eun Ryu and Jae Young Choi

P28. VESTIBULAR ANATOMY MODELLING AND SHAPE VARIATION ANALYSIS IN TEN FULLY SEGMENTED HUMAN TEMPORAL BONES USING MICRO-CT
Lj Chacko, S. Handschuh, M. Glosmann, R. Glueckert and A. Schrott-Fischer.

P29. A CASE OF A 7-YEAR OLD BOY WITH SENSORINEURAL HEARING LOSS, VERTIGO AND TINNITUS
Lara Angelle Micallef, Anthony J. Fenech

P30. 3D CULTURE OF HUMAN VESTIBULAR NERVE
Fredrik Edin, Wei Liu; Hao Li and Helge Rask-Andersen

P31. AUTHENTIC BIOPHYSICAL PROPERTIES OF $I_{K,L}$ IN MAMMALIAN VESTIBULAR TYPE I HAIR CELLS REVEALED AFTER CALYX REMOVAL
Paolo Spaiardi, Elisa Tavazzani, Marco Manca, Jacopo Magistretti, Giancarlo Russo, Ivo Prigioni and Sergio Masetto

P32. AUTOIMMUNITY AS A CANDIDATE FOR THE ETIOPATHOGENESIS OF MENIERE'S DISEASE: DETECTION OF AUTOIMMUNE REACTIONS AND ITS CLINICAL CORRELATION WITH SYMPTOMS.
Sung Hun Bae, Sung Huhn Kim, Jin Young Kim, JeeEun Ryu, Jae Young Choi

P33. GENE EXPRESSION ANALYSIS OF THE VESTIBULAR ORGAN OF *IGF1*-/- AND *IGF1*+/+ MICE USING AFFYMETRIX WHOLE TRANSCRIPT ARRAYS.
Lourdes Rodriguez de la Rosa, Silvia Murillo, Julio Contreras, Joaquin Dopazo, Isabel Varela-Nieto and *Marta Milo*

VI. DEVELOPMENT OF THE EAR

P34. A REPORTER MOUSE AND CELL LINE FOR THE TRANSCRIPTION FACTOR GATA3 IN THE INNER EAR
Tanaya Bardhan, Jasmine Kuah, Lisa Hambley and Matthew Holley

P35. OTOLITH TETHERING IN THE ZEBRAFISH INNER EAR
Georgina A. Stooke-Vaughan, Nikolaus Obholzer, Rachael Lawrence, Sarah Baxendale, Sean G. Megason, Jaydip Ray and Tanya T. Whitfield

P36. THE QUANTITATIVE ANALYSIS OF AQUAPORIN EXPRESSION LEVELS IN THE INNER EAR
Takushi Miyoshi, Norio Yamamoto, Taro Yamaguchi, Yosuke Tona, Kiyokazu Ogita, Takayuki Nakagawa and Juichi Ito

P37. MODELLING AXIAL POLARITY IN THE DEVELOPING ZEBRAFISH EAR
Ryan D. Hartwell, Nick Monk and Tanya T. Whitfield

P38. REGULATION OF THE ATOH1 ENHANCER IN THE INNER EAR: A LINK BETWEEN DEVELOPMENT AND REGENERATION OF HAIR CELLS
Héctor Gálvez, Jelena Petrovic, Joana Neves, Fernando Giraldez and Gina Abelló

VII. STEM CELLS AND REGENERATION

P39. MONITORING OTIC DIFFERENTIATION USING AN OTIC PLACODE-SPECIFIC HUMAN EMBRYONIC STEM CELL REPORTER LINE
Darrell M. Barrott, Sarah Jacob Eshtan and Marcelo Rivolta

P40. HETEROGENEITY OF THE STEM CELL COMPARTMENT AND ITS IMPACT ON THE GENERATION OF OTIC PROGENITORS FROM HUMAN EMBRYONIC STEM CELLS
Sarah Jacob Eshtan, Darrell M. Barrott and Marcelo N. Rivolta

P41. GENERATION OF OTIC PROGENITORS AND SENSORY LINEAGES FROM HUMAN INDUCED PLURIPOTENT STEM CELLS
Sarah L. Boddy, Ricardo Romero-Guevara, Christian Unger, Paul Gokhale, Laura Corns, Walter Marcotti, Peter W. Andrews and Marcelo N. Rivolta

P42. GROWTH FACTOR-INDUCED DIFFERENTIATION OF HUMAN ADIPOSE-DERIVED STEM CELLS TOWARDS OTIC FATE
Stephanie Juniat, Mingyi Zhou, Tanel Ozdemir, Patrick O'Shea, Jonathan Gale

P43. MULTIMODAL VISUALIZATION OF MOUSE HAIR-FOLLICLE-BULGE-DERIVED STEM CELLS
Timo Schomann, Laura Mezzanotte, John C.M.J. de Groot, Johan H.M. Frijns, Margriet A. Huisman

P44. EAR IN A DISH: DEVELOPMENT OF AN *IN VITRO* ASSAY FOR OTOTOXIC AND OTOPROTECTIVE DRUG SCREENING
Aurélie Dos Santos, Andreas Eckhard, Fiona Speichinger, Ngoc-Nhi Luu, Marcus Müller and Hubert Löwenheim.

P45. CONTROLLED DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS TO AN OTIC PROGENITOR CELL FATE
Hanae Lahlou, Arnaud Fontbonne, Christian Chabbert, Jean Michel Brezun, François Feron, Azel Zine

P46. EFFECT OF EXTRACELLULAR MATRIX ELASTICITY UPON STEM CELL DIFFERENTIATION
Margriet A. Huisman, Timo Schomann, Tirsa T. van Duijl, Carlijn N.E. Peerboom, Karien E. de Rooij, John C.M.J. de Groot and Johan H.M. Frijns

P47. LGR5+ COCHLEAR STEM/PROGENITOR CELLS EXPAND *IN VITRO* UPON WNT SIGNALING ACTIVATION USING A SMALL MOLECULE INHIBITOR.
Marta Roccio, Stefan Hahnewald, Pascal Senn

VIII. GENETIC BASES OF HUMAN DEAFNESS

P48. POLYMORPHISMS IN GENES INVOLVED IN THE IMMUNO-INFLAMMATORY PROCESS IN PATIENTS WITH MÉNIÈRE'S DISEASE
Masaaki Teranishi, Yasue Uchida, Naoki Nishio, Ken Kato, Hironao Otake, Tadao Yoshida, Michihiko Sone, Saiko Sugiura, Fujiko Ando, Hiroshi Shimokata and Tsutomu Nakashima

P49. EXOME SEQUENCING AND BIOINFORMATIC ANALYSIS IN IDENTICAL TWINS WITH MÉNIÈRE'S DISEASE
Carmen Martín-Sierra, Teresa Requena, Sonia Cabrera, Lidia Frejo, Jose Antonio Lopez-Escamez

P50. FUNCTIONAL STUDIES OF KCNQ4 MUTATIONS REVEAL THREE DIFFERENT PATHOGENIC MECHANIMS
Fernando Mayo, Matías Morín, Daniel González-Nieto, Luciana Serrao de Castro, Ilaria Fasciani, Ignacio del Castillo, Felipe Moreno, Luís C. Barrio and Miguel Ángel Moreno-Pelayo

P51. MITOCHONDRIAL MUTATIONS M.A3243G AND M.A1555G IN THE SLOVAK HEARING IMPAIRED POPULATION
Ivica Mašindová, Lukáš Varga, Martina Škopková, Juraj Staník J., Lucia Valentínová L., Miloslava Hučková, Iwar Klimeš, Milan Profant, and Daniela Gašperiková

P52. DFNA15 CAUSED BY POU4F3 MUTATIONS IS ONE OF THE MOST FREQUENT FORMS OF HEREDITARY DEAFNESS IN SPAIN WITH DIFFERENT UNDERLYING PATHOGENIC MECHANISMS
Luciana Serrao-de-Castro, Matias Morín, Fernando Mayo, Lucía Borreguero, Irene Valenzuela, Ignacio del Castillo and Miguel Angel Moreno-Pelayo

P53. MOLECULAR STUDY OF BRAZILIAN PATIENTS WITH AUDITORY NEUROPATHY
Priscila Zonzini Ramos, Guilherme Machado de Carvalho, Alexandre Caixeta Guimarães, Arthur Menino Castilho and Edi Lúcia Sartorato

IX. NOISE-INDUCED HEARING LOSS

P54. JAK2/STAT3 IS INVOLVED IN THE NOISE-INDUCED STRESS RESPONSE IN THE INNER EAR
Alfred L Nuttall, Sarah Foster, Irina Omelchenko, and Teresa Wilson

P55. ROSMARINIC ACID PROTECTS AGAINST NOISE INDUCED HEARING LOSS ENHANCING ENDOGENOUS ANTIOXIDANT RESPONSES
Fabiola Paciello, Sara L. M. Eramo, Rolando Rolesi, Massimo Ralli, Anna Rita Fetoni, Diana Troiani and Gaetano Paludetti

P56. THE EFFECTS OF ENDOTHELIN RECEPTOR BLOCKER ON NOISE-INDUCED COCHLEAR INJURY IN MICE
Ah-Ra Ryu, Sung-Tae Seo, Yong-Ho Park, *Jin Woong Choi*

P57. RECRUITMENT STUDY OF WORKERS IN FURNITURE COMPANIES
Natalya N. Petrova, Viya S., Panshina, Ekaterina V. Petrova

P58. ROLE OF P66SHC IN NOISE INDUCED HEARING LOSS AND PRESBYACUSIS
Sara Letizia Maria Eramo, Anna Rita Fetoni, Fabiola Paciello, Diana Troiani, Gaetano Paludetti and Giovambattista Pani

X. NEW THERAPEUTICS AND EXPERIMENTAL MODELS FOR HEARING LOSS

P59. AGE-RELATED EFFECTIVENESS OF 7,8,3'-TRIHYDROXYFLAVONE ON NEURITE OUTGROWTH FROM SPIRAL GANGLION EXPLANTS
Claudia Frick, Marcus Müller and Hubert Löwenheim

P60. THERMOSENSITIVE DRUG DELIVERY SYSTEMS: A PREFERRABLE SHUTTLE FOR NANOPARTICLES TO THE INNER EAR?
Elisabeth Engleder, Clemens Honeder, Maximilian Breyer, Michael Wirth Christoph Arnoldner and Franz Gabor

P61. THE NEUEAR PROJECT: DEVELOPING A NEUROTROPHIC ENCAPSULATED CELL IMPLANT FOR SEVERE HEARING LOSS
Suvarna Dash-Wagh, Lovisa Franzén, Jens Tornøe, Lars Wahlberg and Mats Ulfendahl

P62. EVALUATION OF HEARING AID'S SUCSESS PROBABILITY BASED ON AIDED AUDITORY STEADY STATE RESPONSE THRESHOLDS
Sardari Sara, Sameni Seyed Jalal, Jafari Zahra

P63. METAMORPHOSIS OF WAVE PROCESSES IN INNER EAR: PART 1. FROM VIBRATIONS TO WAVES AND BACK
Evgeny L. Ovchinnikov, Sergey S. Yashin

P64. METAMORPHOSIS OF WAVE PROCESS IN INNER EAR: PART 2. SOUND ENERGY WAYS AT PRE-RECEPTOR LEVEL
Evgeny L. Ovchinnikov, Sergey S. Yashin

P65. A FRAMEWORK FOR MULTIDISCIPLINARY PROJECT WORK WITH COCHLEAR IMPLANT USERS: INVOLVING CI USERS IN DEVELOPING REHABILITATION TOOLS TO IMPROVE PERFORMANCE IN MULTI-SPEAKER CONVERSATION
Harriet Crook, Erica Bradley, Guy Brown, Bill Wells, Amy Beeston and Emina Kurtic

P66. IMPACT OF HYPERBARIC OXYGEN THERAPY ON PROLIFERATION AND BDNF-RELEASE OF GENETICALLY MODIFIED NIH3T3 FIBROBLASTS
Jennifer Schulze, Gerrit Paasche, Hans Lamm, Thomas Lenarz and Athanasia Warnecke

XI. CENTRAL PERCEPTION AND TINNITUS

P67. DELAYED DEVELOPMENT OF CEREBRAL WHITE MATTER IN CHILDREN WITH CONGENITAL DEAFNESS
Kye Hoon Park, Won-Ho Chung, Hunki Kwon and Jong-Min Lee

P68. DIFFERENTIAL REGULATION OF RESPONSIVENESS IN CENTRAL CIRCUITRIES TO AUDITORY DEPRIVATION UNDERLIE THE GENERATION OF HYPERACUSIS AND TINNITUS
Bing D, Lee SC, Möhrle D, Campanelli D, Singer W, Rüttiger L and Knipper M

Symposium Presentations

S1. INTRODUCTION TO COCHLEAR IMPLANTS

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The cochlear implant represents the most successful attempt at replacing a human sensory end-organ. Over 300,000 individuals worldwide have been the recipients of these devices and demand for the technology continues to grow across the globe. The principles underpinning the functioning of these devices has changed little since their inception ie that of encoding the speech signal via an externally worn sound processor and transmitting this information to implanted electronics that stimulate the auditory nerve directly, by-passing the mechano-electric functions of the normal cochlea. Cochlear implants do not restore normal hearing but provide meaningful access to the speech signal with definitive success being heavily dependent on the recipient's sensory processing capacity in both auditory and visual domains. For deaf children implants provide access to speech which paves the way to developing competence in spoken language, facilitating educational achievement, employability and social engagement. For deaf adults, implants relieve their sense of auditory isolation and restore effective communication in their daily lives enabling them to function in the workplace and enjoy their social lives.

Cochlear implants have distinct limitations as it is not surprising that a small number of electrodes cannot replace the function of thousands of exquisitely tunes hair-cells in the cochlea. The spatial selectivity of electrical stimulation is compromised, being limited by geometric considerations within individual cochleas, a paucity or absence of neural elements amenable to stimulation (or 'dead regions'), the inevitable gap between electrodes and their target structures as well as such factors as fibrosis, ossification and malformation. In addition, the responsiveness of the central auditory pathways to the impoverished information delivered by cochlear implants varies across subjects and is particularly poor in those deaf individuals with cognitive impairment. Thus, the quest for biological solutions for deafness – both at the periphery and for central sensory processing, remains an absolute imperative if the massive global disease burden caused by disabling deafness is to be alleviated.

Keywords: cochlear implants.

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S2. ON THE ANATOMY OF THE HUMAN COCHLEA RELEVANT FOR COCHLEAR IMPLANTATION

Helge Rask-Andersen[1], Wei Liu[1], Fredrik Edin[1], Hao Li[1],Hisamitsu Hayashi[1,5], Francesca Atturo[1, 2], Annelies Schrott-Fischer[3], Rudolf Glueckert[3] and Kristian Pfaller[4]

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Objectives: *"From a physiological point of view cochlear implants can simply not work"* was a statement by a renowned inner ear physiologist in the early era of cochlear implantation. I must confess I had a similar view. Evolution has been dramatic. The human cochlea is an intriguing structure, still secret, vulnerable and difficult to analyze deep in the hard bone.

Methods: We investigated unique, well fixed, surgically obtained human cochlea using electron microscopy, micro-dissections and confocal immunohistochemistry after mild Na-EDTA decalcification. Spiral ganglion neurons, lateral wall and basilar membrane structure were focused on.

Results and Conclusions: Results show that there are captivating structural and macromolecular differences compared to experimental animals; particularly the auditory nerve. These are relevant considering degeneration pattern following hair cell loss and deafness in man and raises important issues about CI surgery in patients with long-time deafness. Hybrid principles combining acoustic and electric hearing sets new demands on surgeons' acquaintance with the cochlear anatomy; particularly the "hook" region and round window membrane. Hearing preservation surgery gives new possibilities to treat inner ear disorders through intra-cochlear drug delivery and even gene-therapy. However, the cochlear function is not unaffected by introduced foreign bodies near the basilar membranes. Electric currents spread along functional residual inner hair cells/terminals may be harmful. The tympanic covering layer harbours a fibronectin/β-integrin trans-membrane receptor system that can initiate inflammation and fibrotic reactions around the electrode. Additional progress in CI electrode array designs adapted to the individual shape of the human cochlea is warranted.

Keywords: Cochlear implant, Human Anatomy, TEM, SEM, IHC

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S3. SINGLE-SIDED DEAFNESS AFFECTS BINAURAL INTERACTIONS

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Previous studies have shown that early single-sided deafness leads to a reorganization of aural preference in the brain (Kral et al., 2013, Brain; Kral et al., 2013, Front Syst Neurosci). Here we investigated sensitivity to binaural cues in the auditory cortex and compared binaural and monaural properties of neurons with cochlear implant stimulation in adult normal hearing cats (HCs), congenitally deaf cats (CDCs) born deaf on both ears, and cats born with unilateral deafness but normal hearing on the other ear (uCDCs). In CDCs the monaural response thresholds, dynamic ranges and spontaneous activity were significantly reduced compared to HCs. There were fewer excitatory-excitatory (EE) responses and more 0E responses, but fewer binaural facilitation in CDCs. The highest spontaneous firing rate was found in uCDCs, followed by HCs and CDCs. uCDCs showed weaker responses to the deaf ear compared to the hearing ear. The monaural and binaural responsiveness depended on the relation of the recorded cortex and the hearing ear in uCDCs. The cortex ipsilateral to the hearing ear reorganized extensively, with more EE and less E0 responses. The cortex contralateral to the hearing ear demonstrated more E0 responses and more suppressive interactions. Facilitatory binaural interactions were similarly reduced in CDCs and uCDCs. ITD sensitive units were rare in uCDCs and mostly observed in the contralateral cortex. The ipsilateral cortex had more flat or non-classified ITD responses. In total, unilateral deafness prevented nonspecific deficits in responsiveness, but reorganized the hemispheres differently, with more extensive reorganizations at the cortex ipsilateral to the hearing ear. Finally, binaural interactions and ITD sensitivity were extensively reduced in unilateral deafness. These results demonstrate significant loss of binaural hearing following single-sided deafness and a hemisphere-specific reorganization as a consequence of the adaptation to single-sided deafness.

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S4. ELECTRIC ACOUSTIC STIMULATION (EAS) AND STRUCTURE PRESERVATION COCHLEAR IMPLANTATION

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Combined electric acoustic stimulation (EAS) of the auditory system is the concept of using CI technology and acoustic amplification in the same ear and is a relatively new treatment for patients with a considerable amount of residual hearing in the low frequency range. In EAS the aim is to preserve low frequency hearing after cochlear implantation which can be used for acoustic amplification, while a CI provides electric stimulation to the auditory system in the high frequency range to compensate for the hearing loss in the high frequencies[1]. In 1999 von Ilberg et al.[2] first discussed the possibility of using electric and acoustic stimulation simultaneously in patients without losing functional residual hearing in the low frequencies. This realized by the combination of surgical technique with an approach through the round window membrane or a low cochleostomy, dedicated low traumatic low volume electrodes, atraumatic slow insertion, and systemic and topical corticoids. Meanwhile many studies have shown a high preservation rate of residual hearing. Recently a standard to report on residual hearing was published by Skarzynski et al [3].

The author will give an overview of the state of the art of EAS with details of surgical technique, selection of patients and assessing residual hearing[4], fitting highlights, results and future perspectives.

Keywords: Cochlear implantation, Electric acoustic stimulation, hearing preservation, partial deafness, residual hearing.

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S5. OPTOGENETIC DEVELOPMENT OF COCHLEAR IMPLANTATION

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When hearing fails, speech comprehension can be restored by auditory prostheses. However, sound coding with current electrical prostheses shows limited frequency resolution due to broad spread of current. Optical stimulation can be spatially confined and may therefore improve frequency resolution. Here, we provide an initial characterization of optogenetic stimulation of the auditory pathway using the light-gated ion channel, channelrhodopsin-2 to render spiral ganglion neurons (SGNs) light-sensitive. Optogenetic stimulation of SGNs activated the auditory pathway, as demonstrated by recordings of neuronal population and single neuron responses, and was able to restore auditory activity in deaf mice. By recording local field potentials in the inferior colliculus we approximated the spatial spread of cochlear excitation for suprathreshold optical, acoustic, and electrical stimuli, which suggested a better frequency resolution for optogenetic than for monopolar electrical stimulation. Virus-mediated expression of the channelrhodopsin-2-variant CatCh in SGNs reduced the light requirement and allowed spiking to follow stimulation up to at least 60 Hz. In summary, our study demonstrates the feasibility of optogenetic stimulation of the auditory pathway in rodents and lays the groundwork for future applications of optogenetics in auditory research and prosthetics.

Keywords: cochlear implant; optogenetic stimulation;

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S6. FUNCTIONAL MAGNETIC RESONANCE IMAGING (FMRI) AND BRAIN PLASTICITY IN COCHLEAR IMPLANTATION

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During the last two decades or so, auditory neuroscience has made significant progress by enabling the in vivo study of human central auditory function. Functional MRI (fMRI) has become the tool of choice for addressing many research questions concerning central auditory plasticity. Our team uses fMRI to understand the functional organization of the auditory system in normal-hearing listeners and patients with sensorineural hearing impairments. Our recent fMRI work has focused on normal hearing listeners to optimise and validate methods for assessing tonotopic (frequency) maps in the auditory cortex. We have been quantifying frequency-sensitive activity in the tonotopic map across primary auditory cortex; to be suitable as a localiser of tonotopic cortical fields within individuals or to make quantitative comparisons between maps in dedicated tonotopic mapping studies. Despite these advances, there are significant issues using this technique in individuals with a cochlear implant because of the magnetic components in the implanted device. At present, other groups have used fMRI in paediatric candidacy evaluation for cochlear implantation. My talk will end with a briefly review of some of this work.

Keywords: Primary auditory cortex, tonotopy, stimulus, scanning paradigm, human

S7. NANOCI - NANOTECHNOLOGY BASED COCHLEAR IMPLANT WITH GAPLESS INTERFACE TO AUDITORY NEURONS

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Cochlear implants have become the gold standard treatment for deafness worldwide. Despite their tremendous success, some limitations remain. The bottleneck for optimal stimulation is caused by the anatomical gap between the electrode array and the auditory neurons in the inner ear. As a consequence, current devices are limited through (i) low frequency resolution, hence poor sound quality and (ii), strong signal amplification, hence high energy consumption responsible for significant battery costs and for impeding the development of fully implantable systems. Recent findings indicate that auditory nerve fibres can grow under neurotrophin stimulation towards the electrodes, which opens the door to address all issues simultaneously.

The EU-FP7 – NMP- funded collaborative project NANOCI aims at developing a neuroprosthesis with a gapless interface to auditory nerve fibres. The initial attraction of neurites will be provided by an innovative, nanostructured gel-matrix containing diffusible and surface-bound neurotrophins. The long-lasting operation without interface degradation, reduced biofouling and improved conductivity will be achieved by nanostructuring the array surface using (i) various functional nanomaterials, including carbon nanotubes, combined with (ii) structuration methodologies such as ion implantation and sacrificial nanoparticle embedding in parylene, SOLID (solid on liquid deposition) encapsulation, and sonochemistry. Components will be validated using appropriate bioassays including human auditory neurons in vitro. In parallel, software models will be developed to exploit the bidirectional, gapless interface. Fusing all developments, an animal-grade, pilot nanoCI-device is manufactured and tested in vivo. This will allow assessment of the feasibility of future, cost-efficient, and fully implantable neuroprostheses with substantially improved sound quality.

Keywords: cochlear implant, man:machine interface, nanotechnology, stem cells, neurotrophins, inner ear regeneration, future CI technology.

Acknowledgements: This work has been sponsored by the EU in the 7th framework programme (NANOCI, grant agreement no. 281056)

S8. NANOCI - NANOTECHNOLOGY USED IN NANOCI

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The modification of a state of the art electrode array for cochlear implants to fulfill the requirements for the NANOCI project requires several innovative technology inputs looking at micro / nanotechnology. Oriented growth of the neurons towards the electrodes will be arranged by creating a steady-state gradient of neurotrophin-concentration between the existing nerve and the electrode. The gradient will be established by controlled release of using a neurotrophin containing well. The well consists of a microlitre size droplet that is hermetically sealed by a thin Parylene layer (Poly-para Xylylene; Solid on liquid deposition [1]). Subsequent laser- or ion-beam machining of the layer creates pores, allowing defined release of the neurotrophin molecules. The time-scale where the gradient has to be kept must be in step with the dynamics of cell growth. As soon as the neuron arrives at the implant, anchoring of the cells onto the electrode (carrying the acoustic information in form of electrical potentials) must be achieved at high probability. Anchoring sites may exist in form of appropriate surface roughness of the metallic electrode, but also in form of hybrid nanoparticles covalently bonded to polymer films by local UV irradiation [2]. All promising solutions will be looked at on the way of finding the best compromise between low-impedance electrical contacting, neurophilicity, and anchoring performance. As a special innovative technique, ultra-sound induced embedding of nanoparticles on the implant will be applied. This technique is a large and powerful platform for solving several issues: One activity looks at the creation of conducting anchoring sites, another will apply highly efficient anti-bacterial nanoparticles around the entire implant allowing to protect it from bacterial attack at long term.

Keywords: man:machine interface, nanotechnology, solid on liquid deposition, sono-chemistry, Ion-beam machining, laser micro-machining, sol-gel chemistry, carbazole chemistry.

Acknowledgements: This work has been sponsored by the EU in the 7th framework programme (NANOCI, grant agreement no. 281056)

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S9. NANOCI – TESTING INNOVATIVE BIO-FUNCTIONALIZED COMPOUNDS FOR THE EAR

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Ultimate goal of the NANOCI-project is to induce neuronal growth inside the cochlea, guided by an innovative bio-functionalized 3D nanomatrix. For the development of these bio-functionalized nanomaterial and bioactive compounds, test-systems were designed and used to measure stimulation, guidance, attraction and anchoring of neurites. The consortium utilizes test-systems on the level of cells, organs and ultimately in animals. Following 3-R principles we wished to reduce animal experiments.

An organotypic culture model of the postnatal mouse spiral ganglion was used to analyze neurite outgrowth. Quantification was made by a customized Sholl analysis using ImageJ. The assay allows detailed observations on chemically induced growth in nanomatrices and on cochlear implant materials in vitro. For example, dissolving growth factor (BDNF) in the nanomatrices led to neurite outgrowth demonstrating release of sufficient BDNF from the matrix into the culture medium.

Multielectrode arrays (MEAs) are used to record extracellular electrical activity from multiple sites of neuronal cell cultures. MEA recordings include single-unit, multi-unit and local field potential signals. Upon electrical stimulation, neuronal cell cultures gave rise to action potentials. We have established functionally mature spiral ganglion neuron cultures on multi electrode arrays.

The extraction of neural progenitor cells from surgically-removed human inner ears allows observing the growth cone behavior of human auditory neurons during chemical stimulation. Information on the biochemical and molecular background of axonal sprouting, steering and growth cone guidance can be achieved. The use of postmortem autopsy temporal bones as a source for inner ear progenitor cells or differentiated cell types is a completely new approach.

Guinea pigs are used to test the nanomatrix prior to electrode insertion in a surgical procedure. In our model a complete hair cell loss is induced by local application of kanamycin and furosemide into the middle ear. Response properties to electrical stimulation are recorded and compared with those from animals implanted with a conventional electrode. Cochlear histology is conducted to evaluate general cochlea morphology, nerve growth and tissue reaction using refined immunohistological techniques.

Together these test-systems allow the definition and validation of an animal-grade nanoCI-device. This will allow assessment of the feasibility of a future, cost-efficient, and fully implantable neuroprosthesis with substantially improved sound quality.

Keywords: cochlear implant, nanomatrix, stem cells, Multielectrode arrays, neurotrophins, future CI technology.

Acknowledgements: This work has been sponsored by the EU in the 7th framework programme (NANOCI, grant agreement no. 281056)

S10. FUSING NANOTECHNOLOGY AND BIOLOGY INTO A PILOT PRODUCT – THE MANUFACTURER'S POINT OF VIEW

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Participation in the challenging EU-FP7 - funded collaborative project NANOCI is a privilege for researchers in a cochlear implant company. Several decades of technological progress in CIs has allowed continued improvements in sound quality, ergonomics, structure preservation and power consumption with the MED EL Synchrony system representing the current state of the art. The NANOCI project hypothesises that reducing the physical barrier between the auditory nerve and the electrode array through outgrowth of peripheral processes will allow improved information transfer into the auditory periphery, by achieving temporal control over precisely controlled, and potentially smaller, populations of neurons during a single stimulus pulse sequence. In this sense it could more closely represent the processing carried out by a normal hearing cochlea. The ability to cause a defined temporal firing pattern in a small, spatially defined neural population is very attractive to sound coders working in the CI field.

Currently many CI users achieve very impressive levels of hearing performance. For full enjoyment of music, and effortless hearing in challenging environments, there is still room for improvement. Furthermore the peripheral status of each deaf cochlea is unique. The field of the brain-machine interface seeks to provide huge information transfer through close coupling of large numbers of electrodes, and is beginning to show some very interesting results. However, the cochlea is not structured for such close coupling, therefore NANOCI must also look to developments in regenerative medicine, and nanotechnology.

The challenges to implementation of such a concept are obvious: to name a few, the small size of the array; the significant area taken up by the electrode contacts; the inert nature of implant grade silicone; the need to maintain electrode flexibility and biocompatibility; ergonomics; sterilization and regulatory compliance. Furthermore massively increasing channel number requires either more wires or embedded electronic components. MED EL's contribution to the project has focused so far on the incorporation of some of the technologies into the implant array; modelling of the neural interface; consideration of new telemetry and coding challenges; and initial work in the exploitation of new drug delivery and pressure sensing technologies.

Keywords: cochlear implant, man:machine interface, nanotechnology, stem cells, neurotrophins, inner ear regeneration, future CI technology.

Acknowledgements: This work has been sponsored by the EU in the 7th framework programme (NANOCI, grant agreement no. 281056)

S11. STEM CELLS MEET COCHLEAR IMPLANTS: A GERBIL 'DOUBLE ABLATION' MODEL

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Objectives: The development of animal models for human deafness is key for the exploration of potential therapies. Using the ouabain-induced neuropathy model described by Lang et al[1], we have shown that hearing function can be restored by a transplant of human embryonic stem cell-derived otic neural progenitors, hONPS[2]. By developing an animal in which both neurons and hair cells can be selectively abrogated, we would have a model in which a cochlear implant can be introduced alongside hONPs and any interaction between the two assayed. The gerbil is an ideal model given their auditory frequency range and robust recovery post-surgery. In spite of its well-established use in hearing research, a simple and reliable protocol for hair cell damage is not yet available for this species.

Methods: Hair cell loss was explored by different paradigms of aminoglycoside application. Allowing a week for recovery, neuropathy was induced by the topical application of ouabain to the round window (RW). Animals then received a transplant of hONPs (or a vehicle injection) and a custom-built cochlear implant. In other groups, an implant was inserted in animals with a kanamycin/furosemide lesion only, or in untreated, naïve animals.

Results: The most effective hair cell damage was obtained by a single, systemic administration of kanamycin and furosemide. This procedure destroyed IHCs and OHCs but was not followed by a secondary neuronal degeneration, as observed in other species. As expected, sequential ouabain application to the RW led to a severe loss of spiral ganglion neurons. Preliminary data after cochlear implantation shows that the extent of fibrosis in a naïve implanted animal is minimal, with a thin 'sleeve' of tissue surrounding the implant and no evidence of necrosis or ossification detected by microCT, 3D deconvolution or histological analysis.

Conclusions: Aminoglycoside treatment induces a selective loss of hair cells with preservation of auditory neurons in the gerbil. A subsequent application of ouabain allows a controlled damaged of the neurons. This two-stage treatment offers an appropriate system to explore the interaction of a cochlear implant with transplanted stem cells.

Keywords: Cochlear implantation, stem cells, ototoxicity, gerbil.

Acknowledgements: This work has been supported by the Medical Research Council and Cochlear.

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Plenary Lecture

PL. COCHLEAR IMPLANTATION: PAST, PRESENT AND WHAT LIES AHEAD

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In Vienna in 1977, collaboration between Ingeborg and Erwin Hochmair, and Kurt Burian, led to the implantation of the world's first multi-channel microelectronic cochlear implant. This implant included a long, flexible electrode, which could, for the first time, deliver electric signals to the auditory nerve along a large part of the cochlea. With a modified version of this device, the next milestone in cochlear implant development was reached in 1979: the understanding of words and sentences without lip-reading in a quiet environment via a small, body-worn sound processor.

The Hearing Implant Company MED-EL was founded in the eighties with a vision that would ultimately bring cutting-edge applications to life in more than 100 countries and 100,000 patients. Intense and continuous innovation followed, including the development of the world's first behind-the-ear (BTE) cochlear implant audio processor in 1991. The next major advancement was the development of a high stimulation rate cochlear implant designed to faithfully implement a new speech coding strategy developed by Blake Wilson. This became the first device with which the majority of postlingually deaf adults achieved more than 50% monosyllabic word understanding within 6 months after implantation.

During more recent years, MED EL developed, acquired and continuously improved a growing family of hearing implants. It supported pioneering work on (still) current topics such as the enhanced benefit of bilateral implantation, combined electric and acoustic stimulation, cochlear implants for single-sided deafness, and compatibility with medical procedures such as magnetic resonance imaging. Respect for the cochlea and its delicate structures have guided MED EL's research and development activities towards a highly flexible electrode array preserving hearing and the delicate structures of the cochlea despite deep insertion into the low frequency apical region. Current work involves further CI enhancements; reaching out to older adults, the partially hearing and unilaterally deaf; an implantable vestibular prosthesis; fitting electrode arrays to the variable cochlea; drug elution into the cochlea and remote support and objective measures. At the same time MED EL supports basic research for the future of the field; for example into the future of drug delivery and regenerative medicine, and optogenetic stimulation.

Keywords: Cochlear implant; History of cochlear implants; Cochlear implant research; future CI technology.

Lead Speaker Abstracts

LS1. STRUCTURE, FUNCTION AND DEVELOPMENT OF THE TECTORIAL MEMBRANE, AN EXTRCELLULAR MATRIX OF THE INNER EAR

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The tectorial membrane (TM) of the mammalian inner ear is composed of collagens (Types II, IX and XI) and a number of non-collagenous proteins (Tecta, Tectb, Ceacam16, otogelin, otogelin-like and otolin). These proteins are secreted into the lumen of the cochlear duct during development and polymerise to form a spiral ribbon that is, in mature mice, ~6 mm long and is graded in both its thickness and width along the length of the cochlea, with the cross-sectional area increasing more than 10 fold from base to apex. The collagen fibrils of the mature TM are imbedded in striated-sheet matrix, the formation of which depends on Tecta, Tectb and Ceacam16[1], and are oriented, with a slight offset towards the apex, across the radial axis of the TM. Other notable features of the TM, the covernet fibrils, the marginal band and Hensen's stripe run principally along its longitudinal axis.

Quite how the proteins of the tectorial assemble to form such a precisely graded and highly-organized structure remains an open but important question. Using light and electron microscopy we find a tectorin-based matrix forms ~2 days before the emergence of the collagens filaments. The collagens filaments first appear within this matrix at embryonic day (E) 15.5 but are randomly oriented. Within the space of 24 hours they become aligned with one another and form fibrils that are arranged radially across the cochlear duct. Despite some initial defects in bundling and alignment, radially-oriented collagen fibrils still form on the surface of the greater epithelial ridge in *TECTA*$^{-/-}$*/TECTB*$^{-/-}$ double null mutant mice. These results suggest that some of the other non-collagenous proteins or signals present on the epithelial surface determine the orientation of the collagen fibrils during cochlear development.

Keywords: Tectorial Membrane; Tectorins; Collagens; Development.

Acknowledgements: Sponsored by The Wellcome Trust and Action on Hearing Loss

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LS2. CALCIUM INDUCED CALCIUM RELEASE DURING ACTION POTENTIAL FIRING IN DEVELOPING INNER HAIR CELLS

Radu Iosub[1], Daniele Avitabile[3], Lisa Grant[1], Krasimira Tsaneva-Atanasova[2] and *Helen J Kennedy*[1]

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In the mature auditory system inner hair cells (IHCs) convert sound induced vibrations into electrical signals that are relayed to the CNS via auditory afferents. Before the cochlea can respond to normal sound levels, developing IHCs fire calcium based action potentials that disappear close to the onset of hearing [1]. Action potential firing triggers transmitter release from the immature IHC that in turn generates experience-independent firing in auditory neurons [2]. These early signalling events are thought to be essential for the organization and development of the auditory system and hair cells.

A critical component of the action potential is the rise in intracellular calcium that activates both small conductance potassium channels essential during membrane repolarisation, and triggers transmitter release from the cell [3, 4]. Whether this calcium signal is generated by calcium influx or requires calcium induced calcium release (CICR) is not yet known. IHCs can generate CICR, but to date its physiological role has remained unclear [5].

Here, we used high and low concentrations of ryanodine to block or enhance CICR to determine whether calcium release from intracellular stores affected action potential waveform, inter-spike interval or changes in membrane capacitance during development of mouse IHCs. Blocking CICR resulted in mixed action potential waveforms with both brief and prolonged oscillations in membrane potential and intracellular calcium. This mixed behaviour is captured well by our mathematical model of IHC electrical activity. We perform two-parameter bifurcation analysis of the model that predicts the dependence of IHCs firing patterns on the level of activation of two parameters, the SK2 channels and CICR rate. Our data demonstrate that CICR forms an important component of the calcium signal that shapes action potentials and regulates firing patterns, but is not involved directly in triggering exocytosis. These data provide important insights into the calcium signalling mechanisms involved in early developmental processes.

Keywords: development, calcium induced calcium release, inner hair cells, mathematical modelling

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LS3. A COMPARATIVE ANALYSIS OF THE REGENERATIVE TRANSCRIPTOME IN THE AVIAN UTRICLE AND COCHLEA AFTER AMINOGYCOSIDE-INDUCED DAMAGE

Michael Lovett[1], Nicole Renaud[2], Yuan Chieh Ku[2], Anastasia Filia[1] and Mark E. Warchol[2].

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We recently described the first comprehensive transcriptome analysis of hair cell (HC) regeneration in the chick utricle[1]. We have now completed a similar study of the regenerating chick cochlea. Our objective is to identify candidate genes that might be useful tools in engineering human HC regeneration. We employed mRNA-seq of pure sensory epithelia (SE) from antibiotic-damaged *in vitro* organotypic culture systems. Samples were collected at 24hour intervals across a seven day regenerative time period and were compared to matched (undamaged) controls. Multiple replicates and multiple qRT-PCR validations were performed. The same filtering criteria were applied to both the utricle and cochlea datasets to identify differentially expressed (DE) genes. This yielded ~3,600 DE genes in the utricle and ~4,600 DE genes in the cochlea across the entire time course. The overlap between the two regenerating SE was ~1600 DE genes, which are heavily enriched for genes known to affect sensory function, neuronal development and synaptic transmission. Such data comprise a new source of many, as yet unexplored, candidates that may constitute the core "regenerome" of HC replacement. The two time courses also reflect different modes of HC replacement. Data from the utricle emphasize HC production via SCs division, while cochlear data indicate that the predominant method of HC replacement is phenotypic conversion. Analysis of *NOTCH* signalling components clearly reflects these different programs and allows us to cluster additional DE genes that showed remarkable co-expression or reciprocal expression with genes such as *ATOH1*. These types of unbiased statistical pattern matching tools also allowed us to identify subgroupings of DE genes that are enriched for particular biological processes. Predominant among these are genes encoding transcription factors (TFs). We have identified ~90 TFs that are shared parts of both regenerative programs and a group of ~20 that very specific to one or other of the two SE. These prioritization methods have allowed us to identify a list of ~200 candidates that are now being tested by knockdown and over-expression analysis for their affects upon HC regeneration.

Keywords: Hair cell regeneration, Next Gen RNA-sequencing, cochlea, utricle, sensory epithelia, transcriptomes.

Acknowledgements: This work was supported by grants from the NIH and the Hearing Health Foundation to ML and MEW.

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LS4. CLUES TO THE MOLECULAR BASIS OF PROGRESSIVE HEARING LOSS FROM MOUSE MUTANTS

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Progressive hearing loss is very common in the human population and can start at any age from the first decade of life onwards. Single gene mutations have been implicated in progressive hearing loss in a handful of extended families where linkage analysis can be used to pinpoint the causative mutations, but for most cases there are no clues to the causes. It is likely that a combination of environmental factors and genetic predisposition underlies hearing loss in many cases, making it difficult to study directly. Mouse mutants offer an alternative approach to identifying genes that are essential for maintenance of normal hearing.

We have generated a large number of new mouse mutants with known genes inactivated and screened them for hearing deficits by Auditory Brainstem Response recording (ABR) at 14 weeks old. Out of the first 800 new mutant lines screened, 21 have shown raised thresholds. Several of these have been followed up with ABR at different ages and show progressive increases in thresholds with age. Examples of primary defects in the hair cells, in synapses below inner hair cells, and in maintenance of endocochlear potential have been discovered, emphasising the heterogeneous nature of progressive hearing loss. These genes represent good candidates for involvement in human progressive hearing loss.

Key words: mouse mutants; genetic deafness; progressive hearing loss.

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

LS5. TOWARDS A GENE REGULATORY NETWORK FOR INNER EAR SPECIFICATION

Monica Tambalo[1], Jingchen Chen[1], Meyer Barembaum[2], Marianne Bronner[2] and *Andrea Streit*[1]

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The vertebrate inner ear arises early in development from a thickened epithelium, the otic placode. Specification towards an otic fate requires diverse signals and complex transcriptional inputs that act sequentially and/or in parallel. To uncover and integrate novel genes with known molecular players in the gene regulatory network (GRN) underlying otic development, we have performed transcriptome analyses of the presumptive otic region at sequential stages of commitment toward inner ear identity. These results reveal dynamic changes in gene expression as a function of time during the transition from progenitor to committed otic state. Modulation of otic inducing signals or of known otic transcription factors establishes a genetic hierarchy amongst these genes that underlies this critical transition. Our results not only provide an in depth characterization of the otic transcriptome, but also identify new links in the GRN that controls otic specification.

Keywords: otic commitment; transcriptional networks; cell fate specification

Acknowledgements: This work is supported by the BBSRC and NIH.

LS6. OTOSTEM: A FRAMEWORK 7 EUROPEAN CONSORTIUM TO DEVELOP HUMAN STEM CELL APPLICATIONS FOR THE TREATMENT OF HEARING LOSS

Pascal Senn[1,2] , Marcelo N. Rivolta[3], Azel Zine[4,5], Helge Rask-Andersen[6], Stefan Heller[7], Albert Edge[8], Helmuth van Es[9], Michael Bös[10], Peter Ulrich[11] and Hubert Lowenheim[12]

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Hearing impairment is the most frequent human sensory deficit and is mainly caused by the irreversible loss of neurosensory cells in the cochlea. The lack of human otic cell models represents a significant roadblock that has hampered the development of drug-based or cell-based therapies for the treatment of hearing loss. In a collaborative effort under this proposal we wish to devise approaches to generate human otic progenitors and differentiated otic cells from different human stem cell sources. We have devised guidance protocols for mouse and human embryonic and reprogrammed stem cells toward inner ear cell types that make use of principles of early germ layer formation and otic induction. A limitation is the efficacy of otic progenitor cell generation. Purification techniques for human otic progenitors from ES/iPS cell sources and in addition from native human otic tissues from fetal and adult stages will serve the dual purpose for one to enable the development of novel bioassays for drug screens, as well as generating cells with decreased tumorigenicity for cell transplantation studies in animal models *in vivo*. New hit compounds identified from screening efforts will be tested and validated further in established organ culture models. The identification of relevant candidate compounds will be further developed as lead drug candidates in noise and ototoxic drug-induced *in vivo* models. The scope of this stem cell technology development requires a collaborative team effort, with groups that have substantial combined experience in human ES/iPS cell work, inner ear stem cell biology, high-throughput assay development, and in translating research findings into the clinic as well as into the biotechnology realm. Within the consortium exists an established translational route from bench to bedside for the commercial development of human otic stem cell derived technology towards inner ear medical applications aiming at the restoration of hearing function.

Keywords: Human stem cells, hearing loss.

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LS7. GENETIC DEAFNESS IN HUMANS IN THE 'NEXT GENERATION' ERA: THE PLEASURE AND THE PAIN.

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Over the last two decades, genetic studies of human deafness have provided insights into the molecular architecture, development and function of the inner ear. However genetic diagnosis has lagged behind research and little has been translated into improved diagnosis or management of deafness in patients. Recent technological advances in DNA sequencing ('next generation' or 'massive parallel sequencing') means that hundreds and even thousands of genes can be analyzed simultaneously at relatively low cost leading to unprecedented levels of gene discovery in all areas of biology and medicine. This promises the advent of truly personalized medicine, from better diagnosis, better information on response to interventions and better information about prognosis.

We discuss the use and potential benefits of the data emerging from these studies in human deafness and give examples from our lab. Using a limited 'gene panel' approach to diagnosis in patients referred for genetic testing, our diagnostic rate has increased to over 40%. Research testing using a whole exome approach has been trialed in an East London British- Bangladeshi community and indicates that there are still many rare deafness-causing genes yet to be described but highlights some of the potential ethical issues involved in exome and genome sequencing. We give examples of gene identification within these families and communities and of gene discovery both in people with pure non-syndromic as well as rare and complex syndromic forms of deafness, and those that will benefit from whole genome approaches such as the Genomics England 100,000 Genomes project.

Keywords: Next Generation Sequencing (NGS); Massive Parallel Sequencing; deafness; genomics

LS8. HIDDEN HEARING LOSS: CAUSES AND CONSEQUENCES

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Dramatic results from recent animal experiments suggest that noise exposure can cause substantial cochlear synaptopathy and neuropathy without affecting sensitivity to quiet sounds [1]. The neuropathy seems to be selective for high-threshold auditory nerve fibres [2]. Hence, although this "hidden" hearing loss may not be measurable by conventional pure tone audiometry, it may have consequences for the coding of sounds at moderate and high levels, and may also be related to tinnitus [3]. It has been known for several years that some individuals with normal audiograms have difficulty in hearing speech in noisy environments, although evidence for a direct link to noise exposure is scant. Preliminary results from a study on occupational noise exposure suggest that listeners with normal hearing who work in a noisy factory have impaired speech discrimination in modulated spatial noise compared to office workers from the same company, even when controlling for audiometric threshold and cognitive ability. In another study, we used the electrophysiological frequency-following response (FFR) to characterize neural temporal coding. We found a substantial decline in FFR strength in young listeners with a history of recreational noise exposure (night clubs and live music events), despite no elevation in audiometric threshold compared to the control group. Animal experiments also reveal that the ageing process itself, in the absence of significant noise exposure, is associated with cochlear neuropathy [4]. Evidence from human temporal bone studies [5] suggest that this form of hidden loss is common in humans, and may have perceptual consequences, in particular regarding the coding of the temporal aspects of sounds. Taken together, the results suggest that the effects of noise-induced hidden hearing loss on neural coding are measurable in humans, and that this loss has significant consequences for perception in real-world environments.

Keywords: Hidden hearing loss, cochlear neuropathy, noise, ageing.

Acknowledgements: This work has been sponsored by the Medical Research Council (MR/L003589/1), Action on Hearing Loss, and the Colt Foundation.

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LS9. TOPICAL IGF-1 THERAPY USING A GELATIN HYDROGEL: PRE-CLINICAL STUDY, CLINICAL TRIALS, AND MECHANISMS

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Insulin-like growth factor-1 (IGF-1) has been known to play a crucial role in the development and the maintenance of the cochlea. We therefore focused on IGF-1 as a therapeutic candidate for topical application. However, achieving sustained delivery of growth factors to the cochlear fluid remains as a fundamental hurdle for the use of growth factors in cochlear treatment. We resolved this issue using a drug delivery system using the gelatin hydrogel. After confirmation of the efficacy and safety of topical IGF-1 treatment using gelatin hydrogels in animal models, a prospective clinical trial of this treatment for patients with sudden deafness refractory to systemic steroids was performed. The results indicated the efficacy and safety of topical IGF-1 therapy for treatment of patients with sudden deafness. We subsequently conducted a randomized, controlled clinical trial of topical IGF-1 therapy in patients with refractory sudden deafness, which indicate superior efficacy and safety of topical IGF-1 therapy to intratympanic injection of dexamethasone. Interestingly, hearing recovery following topical IGF-1 treatment appeared very slowly, which suggess involvement of regenerative mechanisms for hearing recovery. We, thus, performed animal experiments to investigate the capacity of IGF-1 for regeneration of synaptic contacts between inner hair cells and spiral ganglion neurons. Analyses in explant cultures of mouse cochleae demonstrated the promotion of regeneration of ribbon synapses by IGF-1, which was also demonstrated in a guinea pig model with noise-induced hearing loss. These findings indicate that regeneration of ribbon synapses may be involved in hearing recovery that was observed in patients treated with topical IGF-1 therapy. In the near future, we will examine the potential of topical IGF-1 therapy using gelatin hydrogels for treatment of acute tinnitus and age-related hearing impairment, because recent publications suggested an involvement of ribbon synapses in the etiology of acute tinnitus and age-related hearing impairment.

Keywords: Insulin-like growth factor-1; Drug delivery; sudden deafness; acoustic trauma.

Acknowledgements: This work has been sponsored by a Grant-in-Aid for Young Scientists (B) (25861551) and a Grant-in-Aid for Scientific Research (S) (23229009) from the Ministry of Education, Culture, Sports, Science and Technology in Japan and by the Japan Society for the Promotion of Science, and by a Grant-in-Aid for Researches on Sensory and Communicative Disorders from the Japanese Ministry of Health and Welfare (H21-Kankaku-ippann-006).

LS10. NITRIC OXIDE SIGNALLING IN AUDITORY PROCESSING

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In order to maximize information transmission, the excitability of a target neuron must adapt to on-going synaptic activity. In the auditory brainstem, spontaneous firing rates are around 30Hz and sound evoked activity can be over 300Hz. Specialized giant synapses in this region are adapted for high frequency firing, but show little long term plasticity, suggesting that other mechanisms of control may be important. Concepts of synaptic plasticity are highly developed, but the factors and mechanisms that influence postsynaptic excitability are less well defined. Voltage-gated potassium channels are crucial mediators of neuronal excitability, influencing resting membrane potentials, action potential waveform and firing rates (Johnston *et al.*, 2010). We have used whole-cell patch recording from *in vitro* slices of the mouse brainstem to examine auditory processing. Nitric oxide (NO) is generated in response to synaptic activity and can influence potassium channel currents through guanylyl cyclase/cGMP signalling. The first problem is to understand how nitrergic signalling is activated, and then to identify the ion channels and signalling pathways involved. We have focused on Kv2 and Kv3 channels both of which are highly expressed in the auditory brainstem. Synaptic stimulation of the calyx of Held induces suppression of Kv3 currents and induction of Kv2 currents in the postsynaptic neuron – the principal neurons of the medial nucleus of the trapezoid body (MNTB) - that is mediated by NO (Steinert *et al.*, 2011). This switch in the delayed rectifiers mediating AP repolarization enhances the ability of MNTB neurons to maintain firing at high frequencies. But NO also modulates glutamatergic EPSCs, voltage-gated calcium currents, potassium chloride co-transporter KCC2 (Kopp-Scheinpflug *et al.*, 2011; Yassin *et al.*, 2014) and the hyperpolarization-activated non-specific cation current, I_H. Our data demonstrates that NO mediates activity-dependent modulation of postsynaptic neuronal excitability in the auditory brainstem.

Keywords: Intrinsic plasticity; voltage-gated K^+ currents, activity-dependent.

Acknowledgements: This work was supported by MRC, Wellcome Trust, Action on Hearing Loss and Rosetrees Trust.

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Spoendlin Award Lecture

PREDICTION OF MECHANICAL EFFECT DUE TO A COCHLEAR IMPLANT

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The effect of a cochlear implant on residual, low frequency, hearing is complex and poorly understood. This research focuses on the mechanical effect of a cochlear implant on the cochlear mechanics by comparing the predicted basilar membrane, BM, response before and after the implantation. Audiograms measured from pre- and post-implant users are used as input of a computational model of the passive cochlea, proposed by Elliott *et al.* [1], which are then used to study the mechanical effect of the implantation. In the model, a short cochlea implant, designed to electrically stimulate the basal regions at high frequencies while allowing normal hearing at low frequencies [2], is introduced into the lower cochlear fluid chamber. The active amplification of the cochlea is not considered, since a passive cochlear model whose response is not dependent on stimulus level can reasonably well represent the cochlea for subjects with hearing impairment. The results for the BM coupled response show that the volume change in the fluid chambers due to the implant has a negligible effect, less than about 0.1 dB, on the vibration of the modeled cochlea at low frequencies. A more extreme condition, in which the cochlear implant is assumed to touch the BM at some or whole basal positions and thus impeded its motion, is also studied. Although no travelling wave can propagate in the basal region in the latter case, the remainder of the cochlea is still coupled to the stapes by incompressible fluid. The BM response at low frequencies is relatively unaffected by the blocking of the BM motion in the basal region, although the effect is more dramatic for excitation frequency whose characteristic place is close to the end of the implant. Although this work does not model every aspect of the cochlear implantation, it does provide a way of predicting the possible mechanical effects of the implantation on the cochlear passive mechanics and the residual hearing.

Keywords: Cochlear model, cochlear implant, mechanical effect.

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Oral Presentations

O1. THE ROLE OF G PROTEIN GA_I ISOFORMS IN HEARING IN ADULT MICE

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During the development of the mammalian cochlea, precise migration of the primary cilium of the apical surface of the hair cells and the control of the positions of the stereocilia in hair cells is mediated by heterotrimeric G_i-protein-dependent signaling [1]. However, the consequences of these developmental steps for the hearing function in adults remain elusive. In the present study, mice with tissue-specific deletion of heterotrimeric G protein $G\alpha_i$ isoforms, i.e. $G\alpha_{i2}$ and $G\alpha_{i3}$, were generated and the functional, cochlear, as well as neuronal phenotypes of these mice were investigated.

We analyzed the auditory function by auditory brainstem response (ABR) and otoacoustic emission of the tissue-specific KO mice. The results are discussed in the context of the role of G-protein-dependent signaling and auditory function during hair cell development.

Keywords: auditory brainstem response; G protein; $G\alpha_i$ isoforms; otoacoustic emission.

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O2. RESPONSE OF TYPE I AND TYPE II SPIRAL GANGLION NEURONS TO GUIDANCE MOLECULE GRADIENTS

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Objective: Type I and type II spiral ganglion neuron (SGN) dendrites follow distinct paths to their hair cell (HC) targets in the organ of Corti, which includes highly significant and differential directional responses. The factors that determine this guidance remain poorly understood. To test the hypothesis that guidance molecule gradients contribute to pathfinding by developing SGNs, we evaluated the response of fibers extending from explants of neonatal spiral ganglion to gradients of selected guidance molecules.

Methods: Using molds generated by photolithography, guidance factors were applied to tissue culture surfaces as 100 µm stripes on a poly-L-lysine (PLL) background. The stripes were generated with longitudinal gradients. Neonatal (postnatal day 1-2) mouse spiral ganglia were harvested, divided into explants, and cultured on the gradient stripes. Termination and stripe tracking of type I and II neurites, identified by anti-neurofilament, anti-beta tubulin or anti-peripherin immunolabeling, were evaluated. In addition, directional responses to gradients were evaluated for fibers approaching stripes at angles from 80-110 degrees, to determine whether neurites exhibited a preference for increasing or decreasing concentrations of laminin, semaphorin 3a (Sema3a) or semaphorin 5a (Sema5a). A microfluidic device was used to create gradients of NT-3 or BDNF in solution.

Results: Both type I and type II neurites exhibited avoidance behavior when presented with stripes of laminin, Sema3a or Sema5a, terminating significantly more often on PLL, and tracking PLL stripes. Type I neurites exhibited no directional response to laminin gradients, turning with equal frequency toward lower or high concentrations of the adjacent laminin stripes. However, type II neurites turned significantly more often toward increasing laminin concentrations. For Sema3a, both type I and type II neurites turned toward lower concentrations of the stripes, while neither exhibited directional responses to Sema5a stripes. No responses to control stripes of BSA were observed. Neurites exhibited directional responses to NT3 gradients, but not to BDNF gradients.

Conclusions: The results suggest that while several guidance molecules can attract or repel SGN neurites, laminin, Sema3a and NT-3 have the potential to influence the direction of neurite growth through gradients.

Keywords: neurite guidance, neutrophins, semaphorin, spiral ganglion, trophic gradients

O3. TYPE I IFN IS PRODUCED IN SUPPORTING CELLS AGAINST VIRUS INFECTION OF THE COCHLEAR SENSORY EPITHELIUM VIA RIG-I LIKE RECEPTOR SIGNALING PATHWAY

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The inner ear has been regarded as an immunoprivileged site because of isolation by the blood-labyrinthine barrier. Several reports have indicated the existence of immune cells in the inner ear, but there are no reports showing immunocompetence of the cochlear tissue. In this report, we examined the potential involvement of retinoic acid inducible gene-I (RIG-I) and melanoma differentiation-associated gene 5 (MDA5), which are critical for initiating antiviral innate immune responses. We found that RIG-I and MDA5 are expressed in the mouse cochlear sensory epithelium, including Hensen's and Claudius' cells. Ex vivo viral infection using Theiler's murine encephalomyelitis virus revealed that the virus replicates in these cells and that protein levels of RIG-I and MDA5 are up-regulated. Furthermore, the critical antiviral transcription factor, interferon (IFN) regulatory factor-3, is activated in the infected cells as judged by its nuclear translocation and the accumulation of type I IFN transcripts. These results strongly suggest that not auditory hair cells but supporting cells like Hensen's and Claudius' cells play an important role in innate immunity against virus infection.

Keywords: Innate immunity; Retinoic acid inducible gene-I family; Cochlear sensory epithelium; Supporting cells; Hensen's and Claudius' cells

Acknowledgements: This project is supported by KAKENHI (a Grant-in-Aid for Research Activity Start-up (25893299) to YH.

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O4. B 5 TUBULIN AND 15-PROTOFILAMENT MICROTUBULES APPEARED IN SUPPORTING CELLS OF THE CORTI'S ORGAN DURING DEVELOPMENT IN RODENTS

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A feature of the organ of Corti's supporting cells is the presence of an abundant cytoskeleton which is mainly composed of microtubules. These supporting cells have also been shown to contain a minor mammalian tubulin, the β5-tubulin [1], recently related as a biomarker for cancer cells [2] and cell proliferation [3]. It was shown that a β-tubulin isoform can specified the microtubule architecture, such as the expression of the Moth β2 in the Drosophila testes imposed the 16 protofilaments (16pf) structure on the corresponding subset of Drosophila microtubules, which normally contain 13pf [4]. Moreover, supporting cell microtubules are formed by 15pf instead of the canonical 13, a unique fact among vertebrates [5]. Such a protofilament configuration has been observed in *C. elegans*' neurons which are responsible for the mechanosensory sense of touch [6]. It was also shown that these 15pf microtubules were essential to the proper functioning of these mechanosensory neurons [6].

To determine the role of this particular tubulin in the auditory organ and its possible involvement in the formation of the unusual 15pf microtubules of supporting cells, we studied the spatiotemporal localization of β5-tubulin during development in rats from embryonic day 18 until P25 (25th postnatal day). We also analyzed the localization of β5-tubulin mRNA expression in the Corti's organ. Then we examined the fine structure of microtubules at the electron microscope level. For these experiments, we used an early postnatal stage and a late postnatal stage.

Our results showed that β5-tubulin, contrary to other β-tubulins, had a unique distribution in the cochlea. This β-tubulin appeared at a postnatal stage, before the opening of the Corti's tunnel and being restricted to supporting cells, especially in pillar and Deiters cells,. The same localization of β5-tubulin mRNA was observed by *in Situ* Hybridization. Electron microscopy indicated further that Pillar and Deiters cells were composed by 15-protofilament microtubules at the late postnatal stage (P25).
In conclusion, all these data strongly suggest that there is a relationship between the presence of β5-tubulin and 15-protofilament microtubules in the supporting cells of the auditory organ. Further studies are now needed to elucidate their role.

Keywords: inner ear, beta-tubulin, 15-protofilament microtubules, Pillar cells, Deiters cells.

Acknowledgements: This work has been sponsored by a grant from the National funds for Scientific Research, Belgium : FRIA.

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O5. ENDOCYTIC ACTIVITY OF THE OUTER HAIR CELL

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Outer hair cells (OHCs) possess rapid endocytic activity at their apical pole [1–3]. It has been observed that vesicles, endocytosed at the apical pole of the cell, transcytosed to the plasma membrane and towards the subnuclear pole. Electron microscopy and horseradish peroxidase studies have shown endocytic vesicles in the subnuclear region of OHCs [4,5]. However, the significance of the subnuclear endocytic activity in comparison with the apical rapid endocytosis has not been investigated.

In the current study, endocytic activity was investigated using FM1-43 in freshly isolated OHCs from the guinea-pig cochlea. Confocal laser scanning microscopy was used to compare vesicle-accumulation rate at the subnuclear and apical poles.Two different types of dye application were used:(i) to enable the apical and basal membranes to be stained simultaneously, (ii) to stain independent of the opposite pole.

It was found that 5s after application of dye, the relative fluorescence signal intensity changes at about 4 µm beneath the plasma membrane were 0.08 ± 0.03 and 0.09 ± 0.04 in the subnuclear and subcuticular areas, respectively (N=5).These data show no significant difference in the staining between the opposite poles of the OHCs, indicating similar endocyticactivity mechanisms at the opposite poles of the OHC.

Applying the dye to the basal pole of OHCs, time delays to fluorescence onset within the different intracellular areas were normalized to the onset time in the subnuclear pole.The ratio for the supernuclear, the middle and the apical areaswere 1.7 ± 0.4, 5.6 ± 0.8, and 16.5 ± 5, respectively (N=5).The average speed of vesicle traffic along the longitudinal axes, toward the apex of OHCs, was calculated to be 0.23 ± 0.06 µm/s (N=5).

Therefore, it is concluded that vesicles formed at the basal pole of the OHC are not exclusively stored in the subnuclear region, but are also transcytosed towards the apex of the cell.

Keywords: endocytosis, vesicle traffic, outer hair cell.

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O6. EXPRESSION AND LOCALIZATION OF SOMATOSTATIN RECEPTORS SUBTYPE 3, 4, AND 5 IN THE WILD TYPE AND KNOCKOUT MOUSE COCHLEA AND A PROTECTIVE ROLE OF SOMATOSTATIN

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Somatostatin (SST) is a peptide hormone that exerts inhibitory effects mediated through binding to specific cell surface G protein-coupled receptors, of which five distinct subtypes (SSTR1-SSTR5) have been characterized. Our study performed on mouse cochlear hair cells shows the expression and localization of the three receptors (SSTR3-SSTR5) in wild-type (WT), single-knockout (SSTR1 KO), and double-knockout SSTR1/SSTR2 (DKO) mice. Similar SSTRs expression were observed in the inner hair cells (IHC), outer hair cells (OHC) and supporting cells of cultivated P7 mouse organ of Corti (OC) explants as well as in cultivated cochlear neuroepithelial supporting cells (NEsc). We found differences in the expression of SSTR3-5 in WT, SSTR1 KO, and DKO mouse cochlea, which might be explained as a compensatory effect in the cochlea after the loss of SSTR1 and/or SSTR2. Most importantly, we found improved hair cell survival in somatostatin analogue octreotide treated organ of Cori explants that had been exposed to gentamicin compared to those explants exposed to gentamicin alone. These findings propose that the somatostatinergic system within the cochlea may have neuroprotective properties.

Keywords: cochlea, knockout mice, organ of Corti, somatostatin, somatostatin receptor

O7. CALCIUM WAVES IN THE ADULT MOUSE ORGAN OF CORTI.

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Cochlear explants from neonatal rodents exhibit damage–induced increases in intracellular Ca^{2+} (Ca^{2+}_i) that propagate from cell-to-cell. These *inter*cellular Ca^{2+} waves propagate in an extracellular-ATP and purinergic-receptor-dependent manner (1).Spontaneous ATP-dependent Ca^{2+} waves have also been observed in the developing organ of Corti(2).Our aim was to determine if similar Ca^{2+}waves are present in the adult mouse cochlea. For this we use an *ex vivo* auditory bulla preparation combined with Ca^{2+}indicators to visualize changes in Ca^{2+}_i in the organ of Corti.

Selective photo-ablation of single hair cells(HCs) elicited Ca^{2+} waves that propagated away from the ablation site through approximately 10 supporting cells with an average speed of 7±1µm/s(s.e.m, n=6).During these experiments we observed slower Ca^{2+} waves that appeared to be independent of HC ablation. These were primarily observed in Deiters' cells surrounding outer hair cells (OHCs). The slow waves travelled much greater distances (up to 300 µm)at constant speed (0.9 ± 0.1µm/s n=19 measurements, 4 different cochleas). Prolonged imaging for up to 5 hours indicated that there was a general increase in the number of waves and initial analyses indicate a correlation with an overall loss of OHCs occurring with time ex vivo. Slow Ca^{2+} waves persisted in the presence of apyrase, which degrades extracellular ATP, but were strongly and reversibly inhibited by addition of gap junction blockers octanol and carbenoxolone. Slow Ca^{2+} waves were not observed in cochleas from connexin-30 KO mice.

Taken together these data indicate that both fast and slow Ca^{2+} waves, which are respectively ATP dependent and independent, are present in the adult organ of Corti. How the observed ATP independent Ca^{2+} waves affect the supporting cells through which they propagate remains to be determined but it is possible that they alter gene expression linked to repair and regeneration.

Keywords: Ca^{2+} waves, supporting cells, hair cell regeneration

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O8. MATURATION OF MOLECULAR STATE MEMORY WITHIN SLCA26 FOLLOWING MEMBRANE INSERTION.

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The outer hair cell (OHC) possesses molecular motors identified as prestin that drive electromotility and underlie cochlear amplification. We previously showed in OHCs under whole cell voltage clamp that the motor's electrical signature, a **N**on**L**inear**C**apacitance (NLC), is sensitive to initial holding voltage [1]. The electro-mechanical operating voltage range, assessed with the Boltzmann parameter V_h, shifts in the hyperpolarizing direction when held at depolarized levels and vice versa. Here we utilize a tet-inducible, prestin HEK cell line [2] to characterize the development of this effect after prestin insertion into the plasma membrane to test the hypothesis that motor density may influence mechanical feedback among motors. We find that motor charge density (Q_{sp}) increases within a few hours after induction, and the magnitude of ΔV_h (difference between $V_h$obtained following a one minute holding potential either at + 50 or -100 mV) likewise matures with a *tau* of 2.5hours. A simple kinetic model with voltage dependent rate constants influencing the distribution between expanded (X_o) and compact (X_c) states of prestin was used to understand the data. A dependence of the forward rate constant ($X_o \rightarrow X_c$) on $X_c$population can be used to mimic the data. We speculate that membrane tension is developed upon movement into the expanded state which feeds back on adjacent motors to control motor distribution in an apparently voltage-dependent manner.

Keywords: OHC, nonlinear capacitance, prestin

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O9. NEITHER NECESSARY NOR SUFFICIENT: HAIR CELL DEVELOPMENT WITHOUT ATOH1 AND NEURONAL DEVELOPMENT WITH ATOH1.

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Due to its apparently unique ability to differentiate hair cells in the ear, Atoh1 has been proposed to be both necessary and sufficient for hair cell development. While single genes have been in the past associated with specific cellular properties, modern developmental biology has revealed a complex gene regulation underlying such processes [1]. We will first present data showing that the vast majority of neurons in the mammalian brain, the cerebellar granule cells, depend on Atoh1 for development but never develop as hair cells [2]. This demonstrates that Atoh1 mediate differentiation of hair cells depends on the molecular context of the ear. To establish this molecular context, we have generated a new mouse model in which we replaced Atoh1 by a different gene belonging to the same family, Neurog1. Initial data [3] showed no hair cell differentiation due to the apparent inability of Neurog1 to autoregulate the expression of Atoh1 (and thus Neurog1). We have now generated a mouse combining the knockin allele of Neurog1 with the previously reported self-terminating Atoh1 model [4]. This new model provides a transient expression of Atoh1 followed by expression of only Neurog1. Nearly all hair cells will develop but show various alterations in the cellular mosaic consistent with theoretical predictions of effects of internal noise on patterning [5]. We also will present a new mouse that can express at different times of development another transcription factor, Neurod1. Such expression results in hybrid cells that have both neuronal and hair cell properties indicating that Neurod1 is a crucial component of the molecular background that allows normal hair cell differentiation by Atoh1 in the ear. We will present a model of bHLH gene interactions that goes beyond the currently proposed simplistic model of "Atoh1=hair cell" and integrate recent insights into the interactions of transcription factors into such a model.

Keywords: Atoh1, Neurog1, Neurod1, basic Helix-Loop-Helix factors, hair cell.

Acknowledgements: This work has been sponsored by the University of Iowa.

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O10. SMALL-RNA DEEP SEQUENCING ANALYSIS IN HUMAN FETAL AUDITORY STEM CELLS (HFASCS) IDENTIFY NOVEL MIRNAS WITH A POTENTIAL ROLE IN THE DEVELOPMENT OF THE HUMAN INNER EAR

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MicroRNAs are small (20-23 nt) non-coding RNAs that bind to complementary sequences within target messenger RNA transcripts and result in translational repression or target degradation and gene silencing. Several studies have characterized the microRNA expression in both (mouse, zebrafish…) and invertebrate ciliated neurosensory organs. However the microRNA signature which is driving the developmental steps in the human inner ear is still unknown. In these work, we have characterized the microRNA profiling of human fetal auditory progenitor cells expanded in vitro (hFASCs) and after its differentiation in hair cells and neurons by small-RNA deep sequencing. Over 700 different microRNAs have been detected and correlations have been established between specific upregulated/downregulated microRNAs and, respectively, decreased/increased expression of genes which are specific markers of a given developmental stage. For example, the expression of hsa-miR-145, known to repress pluripotency in embryonic stem cells, was significantly increased in hair cell-like cells. Similarly, the entire miR-200 family (miR-200a, miR-200b, miR-200c, miR-141 and miR-429) that are key regulators of the mesenchymal to epithelial transition was upregulated in hair cell-like cells. In differentiated neurons significant increased levels of some microRNAs such as hsa-miR-99a and hsa-miR-18a were confirmed. The cluster formed by miR96-182-183 is detected in both progenitor and differentiated cells but differentially expressed. In hair cells the whole cluster increases 4-5 fold compared with the undifferentiated cells. However, the expression level of miR96 is similar in neurons and progenitor cells, and miR182-183 expression is reduced 40 fold with respect to the undifferentiated cells. In addition, the analysis of the small-RNA molecules in the human fetal auditory progenitor and differentiated cells has also revealed a list of 51 unreported microRNAs for which the qPCR validation process is being performed. Interestingly, *in silico* analysis identify one of the novel validated microRNAs as a putative regulator of several deafness genes (*GJB3, KCNQ4, CDH23, ACTG1, CEACAM16, MYH14, CLDN14, TMPRSS3* and *SMPX*) known to be expressed in different cell types. Therefore, this work provides initial support to the existence of previously unreported microRNA molecular signatures specifically involved in the development and cell fate specification of the human inner ear.

Keywords: MicroRNAs, miR-96, inner ear development, fetal auditory stem cells

O11. PROTECTIVE EFFECT OF METFORMIN AGAINST GENTAMICIN-INDUCED AUDITORY HAIR CELL LOSS *IN VITRO*

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Metformin is a commonly used antidiabetic drug and prevents cells from oxidative stress [1]. Metformin operates by activation of the AMP-activated protein kinase (AMPK) and inhibits downstream at the mammalian target of rapamycin (mTOR) pathway by suppressing the mTOR complex 1 (mTORC1) [2]. It has been demonstrated that AMPK controls cell growth, autophagy, and metabolism [2]. In addition, AMPK activation slows aging in C. elegans and has gerosuppressive effects on mammals [3]. Less is known about the AMPK-mTOR pathway and the effects of metformin in the inner ear.
We evaluated whether metformin is able to protect auditory hair cells from gentamicin-induced apoptotic cell death as well as its effects on spiral ganglion neurons by analyzing the number and lengthof neurites in spiral ganglion explants after metformin treatment in an *in vitro* rat model.
We detected a dose-dependent significant reduction of hair cell loss in the samples treated with metformin in combination with gentamicin, as compared to explants treated with gentamicin alone. Furthermore, neurite number and length of neurites per spiral ganglion explant remained unchanged in all concentrations analyzed. These results suggest a protective effect of Metformin on auditory hair cell survival mediated by the AMPK and mTORC1 pathway.

Keywords: auditory hair cells, AMPK, mTOR, Metformin, spiral ganglion neurons

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O12. INHIBITON OF mTOR BY RAPAMYCIN RESULTS IN AUDITORY HAIR CELL DAMAGE AND DECREASED NUMBER AND LENGTH OF SPIRAL GANGLION NEURITES *IN VITRO*

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Rapamycin is an antifungal agent with immunosuppressive properties [1]. The mechanism of action includes the inhibition of the mammalian target of Rapamycin (mTOR) pathway by blocking the mTOR complex 1 [2]. mTOR is an atypical serine/threonin protein kinase, which controls cell growth, cell proliferation, and metabolism [3]. A dysregulation of the mTOR pathway is found in a variety of human malignant diseases. Less is known about the mTOR pathway in the inner ear and the effects of its inhibition by Rapamycin.

To explore the effects of Rapamycin on auditory hair cells and spiral ganglion neurons, tissue explants of 5-day-old rats were treated with different concentrations (10uM, 50uM and 100 uM) of Rapamycin. Hair cell survival, spiral ganglion neuron number, and length of the neurites were then analyzed.

A dose dependant damage of hair cells was observed. Moreover, neurite number and length of neurites per spiral ganglion explant were significantly decreased in all concentrations used when compared to control samples. Our data indicates that the mTOR pathway may play a role in the survival of the hair cells and modulates spiral ganglion neuronal outgrowth and survival.

Keywords: auditory hair cells, mTOR, Rapamycin, spiral ganglion neurons

Acknowledgements: This work has been supported by the Forschungsfond der Universität Basel, Switzerland.

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O13. TROMBONE – A MODEL OF AGE-RELATED HEARING LOSS SUGGESTS A NOVEL DEAFNESS GENE SLC4A10 IS REQUIRED FOR NORMAL AUDITORY FUNCTION

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Age-related hearing loss (ARHL), or Presbycusis, is the most prevalent sensory impairment observed in the elderly. It is a progressive, symmetrical, age-related sensorineural hearing loss, most pronounced at higher frequencies. ARHL is a multifactorial disease, with contribution from both environmental and genetic factors. To date, little progress has been made in determining the genetic loci involved. Our aim is to elaborate the genetics underlying ARHL through the identification and characterization of ENU-induced mouse models of ARHL.

Our approach has identified *trombone*, a recessive model of ARHL arising from the Harwell Ageing Screen. Recurrent auditory phenotyping at 2, 6, 9 and 12 months of age shows that affected animals display elevated ABR thresholds from 9 months of age, when compared to littermates, and these are further increased at 12 months of age. Genome mapping studies identified a 12.5Mb critical region on chromosome 2 and next generation sequencing identified a T>C mutation in the novel deafness gene *Slc4a10*, causing a leucine to proline substitution in the encoded protein. Immunohistochemical staining of cochlear sections demonstrates that Slc4a10 is expressed in the type II and V fibrocytes of the spiral ligament of wildtype mice, whereas no labeling is observed in $Slc4a10^{trmb/trmb}$ mice. In addition, ultrastructural studies show progressive hair cell loss (inner and outer) in the $Slc4a10^{trmb/trmb}$ mice from >6 months of age. Furthermore, histological assessment of the lateral wall identified strial thinning in the $Slc4a10^{trmb/trmb}$ mice. Given the expression pattern and morphological changes observed, we measured the endocochlear potential in these mice. This identified that $Slc4a10^{trmb/trmb}$ mice have a chronically low endocochlear potential compared to their wildtype and heterozygous littermates.

Our findings establish the presence of Slc4a10 in the inner ear and suggest an important role for this sodium-coupled bicarbonate transporter in normal auditory function. We hypothesize that *trombone* is a model of strial presbycusis and further functional characterization of this model promises to increase our understanding of the pathophysiology associated with age-related hearing loss.

Keywords: Aging, ENU mutagenesis, Mouse, Presbycusis, Slc4a10

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O14. BDNF DELETION IN THE COCHLEA/LOWER BRAINSTEM LEADS TO CENTRAL PLASTICITY CHANGES OVER AGE

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Tissue-specific deletion of brain-derived neurotrophic factor (BDNF) in the whole cochlea, dorsal cochlear nucleus, and inferior colliculus was found to be preventive against loss of auditory brainstem response (ABR) thresholds, ABR wave I amplitudes, and loss of inner hair cell (IHC) synaptic ribbons after exposure to traumatizing sound (Zuccotti et al., 2012). The present study aimed to assess if the deletion of BDNF in the cochlea/lower brainstem alters the vulnerability of hearing, sound processing, and cortical plasticity over age.

We compared the auditory function by using auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE) measurements on young and aged conditional BDNF Pax2 KO mice. We also analyzed the influence of acoustic noise exposure on hearing loss in young and aged animals. We furthermore investigated plasticity dependent genes and patterns of perisomatic disinhibiton in the inferior colliculus and the auditory cortex of the KO mice and the respective aged-matched controls over age.

Analyses of tissue-specific BDNF KO mice over age showed profound differences in auditory function and noise vulnerability between KO mice and the controls. We discuss the results in the context of a differential role of BDNF for bottom-up / top-down circuits that may prevent vulnerability during aging.

Keywords: BDNF; age; plasticity.

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O15. HEARING AND AGEING: A MULTISTEP GENOMIC STRATEGY TO IDENTIFY NEW GENES/VARIANTS IN EUROPEAN AND CENTRAL ASIAN POPULATIONS

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Objectives: To date, very few environmental and genetics factors are known to contribute to Normal Hearing Function (NHF) and Age-related Hearing Loss (ARHL) [1,2,3,4].The lack of knowledge on the molecular basis of these complex traits, prompted us to develop a combined multiphase strategy using approx.3000 individuals from isolated populations of Europe, Caucasus and Central Asia.

Methods: Starting from our past experience on GWAS meta-analysis combined with expression studies [5], the following strategy was designed: PHASE1: GWAS meta-analysis for common tag-SNPs and common functional variants, PHASE2: replication in independent cohorts, PHASE3: SKAT gene-based test for rare functional variants,PHASE4: Whole Exome Sequencing (WES)in a selected subgroup of 250 ARHL cases/controls.

Results: During PHASE1 the following strongly significant associations were identified: *ITFG2* (p=6.92E-11*), PCDH20* (p=4.71E-10), *SLC28A3* (p=2.39E-09*).* The last two belong to gene-families already known as being involved in hearing loss and expressed in the cochlea. During PHASE2 four SNPs within the *PCDH20* gene were replicated at nominal level (p=0.04) in the 1958 British Birth Cohort and one SNP was replicated at nominal level (p=0.03) within the *SLC28A3* gene in the FITSA cohort. Applying PHASE3, the SKAT gene-based test for rare functional variants (0.001<MAF<0.05) revealed highly suggestive associations (ranging from 7.97E-06 to 1.90E-05) for the following genes:*PTPRCAP, FN1, EAF1, METRNL, PDP2, ARRDC1, HDGF, TMOD1*.
Finally, thanks to PHASE4 (carried out in collaboration with CRG, Spain), WES data are now available to identify population disease-specific rare variants.

Conclusions: The strategy planned proved to be very useful and productive. *In vitro* and *in vivo* functional studies are now needed to confirm the role of the whole list of genes/variants identified through the above-described phases. Up-to-date results will be presented and discussed.

Keywords: Age-related Hearing Loss, GWAS, Normal Hearing Function, WES

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O16. UTRICULAR HAIR CELLS AND TRANSITIONAL CELLS RECIPROCALLY REGULATE ENDOLYMPHATICCATION TRANSPORT VIA PURINERGIC STIMULATION

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Little is known about the function of transitional cells of vestibular epithelium in ion transport. This study was performed to identify how the utricular transitional cells and hair cells reciprocally act to regulate inner ear cation movement to protect vestibular hair cells via purinergic regulation. The temporal bones of C57BL/6 mice were dissected and the transitional cell and hair cell area of utricle was exposed. Vibrating probes were used to measure transepithelial current from the area and purinergic agonist, antagonist and various cation absorbing channel inhibitors were used to identify the function of the area. Minimal cation absorption current (5.0 ± 1.5 μA/cm^2) was detected in the transitional cell area and large cation absorption current (20.5 ± 3.4 μA/cm^2) was detected in the hair cell area of the utricle. The cation absorption current of the utricular transitional cell area was transiently increased with the application of ATP (100 μM). However, the cation absorption current was changed to large cation secretion current with the application of ATP (100 μM) in the utricular hair cell area. The current in \ The each EC_{50} value of ATP-induced current from the both area was 15μM and 18μM respectively and the current was inhibited by suramin (100μM), PPADS (10 μM), and 5-BDBD(5μM).This result implies that utricular hair cells secrete cation and transitional cell absorbcation via P2X2 and P2X4 receptor mediated purinergic stimulation. This is likely to protect utricular hair cells in stressful conditions by providing a shunt for cation from hair cells to transitional cells.

Keywords: hair cell, purinergic receptor, transitional cell, utricle.

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O17. UNRAVELLING THE ROLES OF LYSINE ACETYLATION BY ELP3 DURING INNER EAR DEVELOPMENT

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Given the importance of acetylation homeostasis in controlling developmental processes[1-3], we planned to investigate its role in inner ear formation and focused our attention on Elp3 acetyl-transferase, a member of the Elongator complex recently implicated in neurogenesis[4].

To determine the role of Elp3 in the inner ear, we first analysed the spatio-temporal pattern of ELp3 mRNA expression and showed that it was expressed in the entire early otocyst at E11.5 and persisted later in the sensory epithelium of the cochlea (the organ of Corti), in the spiral ganglion, in the striavascularis and in the vestibule.

To unravel *in vivo* functions of Elp3 in the inner ear, we used conditional knock-out mice in which Elp3 gene is deleted from early otocyst (Elp3cKO). We submitted these mice to a battery of vestibular testing (i.e. stereotyped circling ambulation, head bobbing, retropulsion, and absence of reaching response in the tail-hanging test) and found significant abnormalities. Besides, the auditory brain stem response of Elp3cKO indicated that these mice are severely deaf.

At the cellular level, we did not find any structural abnormalities nor cell patterning defects that could explain deafness or balance dysfunction in Elp3cKO mice. However, we detected some defaults in the planar orientation of their auditory hair cell bundle.

We were also able to demonstrate an increased level of apoptosis in the Elp3cKO spiral ganglion at E14.5 leading to a reduced number of fibers innervating the cochlear hair cells as well as a reduced number of their synaptic ribbons at P15.

To find new potential targets for Elp3, transcriptomes from wild-type, heterozygous and Elp3cKO mice were analysed by RNA-Seq at E14.5 and E18.5. Surprisingly, we observed that hair cell markers were upregulated in the Elp3cKO at E14.5, suggesting a premature differentiation in these mice that was confirmed by *in situ* hybridisation.

In conclusion, our results clearly show a role for Elp3 both in hearing and balance. We plan to go deeper in the mechanisms involved through the identification of the proteins that are targeted for acetylation by Elp3.

Keywords: Inner ear; Elongator; acetylation.

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O18. TENASCIN-C AFFECTS THE GROWTH BEHAVIOR AND THE DIFFERENTIATION OF AUDITORY NEURONS

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Introduction: Various growth factors, extracellular matrix (ECM) proteins and signal cascades play an essential role during the development and maturation of the auditory system. The differentiation of auditory neurons and in particular the innervation of the sensory hair cells by the spiral ganglion cells (first neuron of the auditory pathway) are processes in which different activators and inhibitors must interact in order to obtain a normal hearing function. In a microarray analysis of the spiral ganglion and the cochlear nucleus (second neuron of the auditory pathway) and their precursor cells, we have identified tenascin-C (TN-C) as a possible determining factor, which has not yet been sufficiently studied in the auditory system.

Materials and Methods: First, the distribution of TN-C, Laminin and other ECM substrates in the peripheral auditory system of mice were investigated at different time points by histological analysis. Following cell culture experiments with neurons of the spiral ganglion and the cochlear nucleus as well as with neurospheres, the effect of these ECM proteins on the growth behavior and the differentiation into neurons, oligodendrocytes and GFAP-positive astrocytes were analyzed.

Results: TN-C and Laminin show a specific expression pattern in the peripheral hearing pathway. We were able to show a significant impact of various ECM proteins on axonal growth of auditory neurons and on the differentiation of neural stem/progenitor cells into neurons and glial cells.

Conclusion: Our results demonstrate a possible role of TN-C in the development and maturation of the auditory system. The expression of TN-C and the temporal profile suggests a particular importance during the innervation of the sensory hair cells. Further studies need to explore which domain of TN-C mediates the effects in the cochlea and whether the absence of TN-C can be compensated in vivo or causes a functional defect.

Keywords: auditory neurons; extracellular matrix; hearing development; tenascin-C

Acknowledgements: This work has been sponsored by MED-EL Germany GmbH.

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O19. SEPTIN7 CONTRIBUTES TO THE FORMATION OF THE INNER EAR GROSS MORPHOLOGY

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Background and Objectives: Septin proteins are GTP-binding proteins that were originally identified in the budding yeast, which has a mutation causing defective budding. In animals, septin proteins constitute an emerging non-canonical cytoskeleton and their superstructures can associate with lipid membranes, actin filaments, and microtubules to show various biological process including scaffold forming, vesicular trafficking, regulation of cortical rigidity, and cell polarity formation [1]. Thirteen mammalian septin members are categorized into four subgroups depending on their N- and C- terminal structures. Septins are considered to function as complex rather than single protein entities. It is speculated that canonical septin complex is composed of a combination of members of each four subgroups. Since Septin7 constitute one subgroup of septins only by itself, Septin7 is a ubiquitous subunit of the septin complex [2]. In this study, we studied the phenotypes of inner ears using conditional knockout of Septin7 to speculate the function of septin proteins in the inner ear development.

Methods: We crossed Foxg1-Cre or Emx2-Cre mice with Septin7 floxed mice [3] to induce the inner ear specific deletion of *Septin7* gene. We studied the morphological phenotypes of Septin7 conditional knockout mice using paint filling and immunohistochemistry.

Results: Disruption of *Septin7* gene from embryonic day 8.5 (E8.5) caused severe malformation of the inner ear gross morphology starting from E11.5. We could not observe a cochlear duct, semicircular canals and an endolymphatic duct in the mutant mouse and the inner ear was cystic on E18.5, resembling the common cavity anomaly in human inner ears. However, sensory epithelia patches including hair cells were found even in cystic E18.5 inner ears. These sensory epithelia received the innervation from the auditory nerve. In contrast, conditional knockout of *Septin7* gene from E12.5 did not cause any morphological change in the inner ear.

Conclusion: Septin7 is involved in the formation of gross morphology in the early stage of the inner ear development and not involved in the specification of sensory epithelia.

Keywords: Septin; Cochlea; Semicircular canal; endolymphatic duct.

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O20. SEMICIRCULAR CANAL FORMATION IN ZEBRAFISH

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The inner ear has the dual function of detecting sound and motion, enabling us to hear and to maintain balance. During development the inner ear transforms from a simple ball of epithelial cells into a complex labyrinth to detect sound, gravity, linear acceleration and rotational movement. This process of epithelial remodelling to generate form is a fundamental biological process and one that is beautifully demonstrated in the ear. Morphogenesis of the semicircular canal system in the zebrafish embryo begins when projections of tissue, driven by extracellular matrix production, move into the lumen of the otic vesicle. Here, they fuse to form pillars of epithelium that span the vesicle lumen and provide structural support for the developing ear.

We are aiming to understand semicircular canal formation and function at the molecular, cellular and behavioural level. At the molecular level, we have identified genes involved in canal duct formation by mutant analysis, expression pattern and homology to other species. We are studying different mutant lines where specific canal ducts fail to form correctly. In the *gpr126* mutant (*lauscher*), the projections that form the pillars over-express a number of extracellular matrix components, including *versican* genes[1]. The projections become dysmorphic, overgrow, and fail to fuse to make pillars, resulting in defects in formation of all three canals. The *otx1* gene is required for formation of the ventral canal pillar and specification of the lateral semicircular canal, whereas in the *cloudy* mutant, there are specific defects in formation of the dorsal epithelium of the anterior and posterior canal ducts.

To follow cellular movements during formation of the canal system we are using confocal and light sheet microscopy imaging in real time in transgenic zebrafish embryos expressing GFP throughout the otic epithelium. Canal formation is accompanied by dramatic changes in cell shape and adhesion and we are comparing cell dynamics in wild-type and mutant embryos through to adult fish.

Malformation of the semicircular canals leads to balance defects in zebrafish and we are using automated movement tracking in adult homozygous fish to characterize the different movement behaviours associated with different canal defects.

Keywords: seimicircular canal, *gpr126*, vestibular system, zebrafish

Acknowledgements: This work has been sponsored by the BBSRC.

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O21. CONDITIONTING THE COCHLEA TO FACILIATATE SURVIVAL AND INTEGRATION OF EXOGENOUS CELLS INTO THE AUDITORY EPITHELIUM

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Background: Inserting stem cells (SC) into the auditory epithelium (AE) of deaf ears is a complicated task. Cells injected into the perilymph can survive but they do not cross into the auditory epithelium. When injected into the endolymph, cellspromptly die due to the hostile, high potassium environment of the scala media (SM). Even if cells were to survive in SM, integration into the auditory epithelium would be difficult due to the robust junctional complexes between the epithelial cells. To facilitate survival and integration of exogenous injected cells in the AE, it is necessary to "condition" the cochlea: eliminate the high potassium concentration and reversibly open the cell-cell junctions of the AE. As a first step, we evaluated the survival of cells in artificial cochlear fluids in vitro and then conditioned the cochlea in vivo to facilitate survival and integration of exogenous cells into the auditory epithelium.

Methods: Cell survival in cochlear fluids was evaluated by monitoring fluorescence of mCherry labeled HeLa cells after adding artificial fluids (endolymph, perilymph or mixture of endolymph and perilymph) to culture. In vivo experiments used neomycin-deafened guinea pigs. To render SM more hospitable to exogenous cells, we reduced potassium levels by (a) injecting furosemide intravenously, and (b) replacing endolymph with artificial perilymph. To disrupt adherens junctions in the AE, we injected sodium caprate via cochleostomy. We then injected HeLa cells into the SM. Changes of AE junctions were evaluated in whole-mounts stained for ZO-1. Presence of HeLa cells in the cochlea was evaluated by fluorescence stereomicroscopy followed by epi-fluorescence of AE whole-mounts. Effect of sodium caprate on spiral ganglion neuron survival was evaluated by assessing density of spiral ganglion neurons (SGNs) in Rosenthal's canal profiles.

Results: In culture experiments, cells exposed to artificial endolymph detached from the dish, became spherical and died within six hours. Cells cultured in perilymph or in the endolymph-perilymph mixture survived. In vivo, after introducing sodium caprate into the SM, gaps appeared between cells. Some transplanted HeLa cells appeared to adhere to the AE surface, but others appeared at the same focal plane as the native AE cells, suggesting that those exogenous cells integrated into the tissue. HeLa cells survived in the SM of conditioned cochleae for at least 7 days, but in un-conditioned (control) cochleae they died promptly. Transient opening of junctions in the AE with sodium caprate did not induce a drastic SGN degeneration throughout the entire cochlea.

Conclusion: With specially designed measures to condition the cochlea, the recipient tissue can be transiently modified to enhance survival and integration of exogenous cells after injection into SM.

Keywords: Cochlea; Cell transplantation; Scala media; Auditory epithelium

Acknowledgements: This work has been supported by NIH/NIDCD grants R21-DC011631 and P30-DC05188.

O22. LOCALIZATION AND EXPRESSION PROFILES OF ADIPOSE-DERIVED STEM CELLS IN A MODEL OF COCHLEAR INJURY INDUCED BY ACOUSTIC TRAUMA IN THE GUINEA PIG.

Anna R. Fetoni[1], Wanda Lattanzi[2], Sara M.L. Eramo[3], Rolando Rolesi[1], Fabiola Paciello[1], Marta Barba[2], Diana Troiani[3] and *Gaetano Paludetti*[1]

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Sensorineural hearing loss is one of the most common disabilities. Among causes of deafness, noise exposure results in damage of multiple cochlear cell types producing permanent hearing loss with important social consequences. In mammals no regeneration of either damaged hair cells or auditory neurons has been observed and no successful treatment is available to achieve a functional recovery. Adipose-derived stem cells (ASCs) represent a promising tool in diversified regenerative medicine applications, based on their high degree of plasticity and trophic features. This study was aimed at identifying the path of cell migration and *in vivo* expression of trophic growth factors and chemokines, upon ASCs transplantation into the cochlea, following noise-induced trauma. ASCs were isolated in primary culture from the adipose tissue of a guinea pig, transduced using a viral vector to express the green fluorescent protein and implanted into the scala tympani of deafened animals. ASCs' implantation did not increase noise induced hearing loss. The ASCs migrated from the perilymphatic to the endolymphatic compartment within 3-7 after implantation. Upon the acoustic trauma, the expression of chemokines ligands and genes, related to the PDGF, VEGF and TGFbeta pathways, increased in the cochlear tissues, with specific regard to the Organ of Corti, possibly guiding *in vivo* cell migration. Immunofluorescence confirmed the increased expression which appeared to be further strengthened upon ASCs' implantation mainly in the stria vascularis. In conclusion our results indicated that ASCs are able to migrate at the site of tissue damage and express trophic features, upon *in vivo* implantation into the cochlea, providing an original proof of principle, which possibly pave the way for the further development of ASC-based treatments of deafness.

Keywords: Stem cell, acoustic trauma, angiogenesis.

Acknowledgements: This work has been sponsored by PRIN grant (Italian Department of Research) and Fondi d'Ateneo Catholic Univeristy of Rome.

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O23. COCHLEAR HAIR CELL GENERATION FROM LGR5-POSITIVE SUPPORTING CELLS

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The vestibular and auditory organs have a limited ability to replace damaged cells. In contrast to the lack of regeneration in untreated ears, cochlear cells showed a capacity for hair cell replacement when treated with a gamma-secretase inhibitor. Inhibition of Notch signaling within the epithelium by the drug following damage to the cochlea resulting from exposure to noise increased expression of transcription factor, Atoh1. In the neonatal mouse new hair cells were seen following ototoxic damage. Using lineage tracing, we showed that new hair cells, predominantly outer hair cells, arose from supporting cells that expressed Lgr5, a downstream target of the Wnt pathway and a protein that marks intestinal epithelial stem cells, and new hair cell generation was increased by pharmacological inhibition of Notch. Whereas all supporting cells express Sox2, a specific subset expresses Lgr5. Cochlear Lgr5-expressing supporting cells after isolation by flow cytometry gave rise to self-renewing neurospheres that could be induced to differentiate to hair cells. Lgr5-positive cells had distinct phenotypes from other supporting cells and differentiated to hair cells at a higher rate than the total Sox2-positive supporting cells, consistent with these cells playing a role as hair cell progenitors. Hair cells did not differentiate from Lgr5-negative cells. Upregulation of Wnt signaling specifically targeted the Lgr5-expressing cells, leading to proliferation in the postnatal ear, and the cells transdifferentiated to hair cells. These data suggest that manipulation of signaling pathways leads to some regeneration of hair cells and that *Lgr5*-positive cells act as hair cell progenitors in the cochlea.

Keywords: Stem cells, Lgr5, Wnt signaling, transdifferentiation

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O24. HAIR CELL REGENERATION BY ATOH1 GENE THERAPY IN THE COCHLEA OF MATURE DEAFENED GUINEA PIGS

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Background and Objectives: Degeneration of hair cells in the mammalian cochlea results in irreversible hearing loss. Cochlear implants restore hearing to people with profound hearing loss, but have limitations such as the inability to convey music. Hair cell regeneration may provide a more natural hearing solution. The *Atoh1* gene is necessary for hair cell development and recent research has shown that *Atoh1* gene therapy results in new hair cell formation and hearing restoration. The aim of this study is to characterise hair cell formation via *Atoh1*gene therapy in profoundly deaf ototoxically-deafened and noise-deafened mature guinea pigs.

Methods: Guinea pigs were deafened by ototoxic drugs or noise (130 dB, 11-13 kHz, 2 hours). Adenoviral vectors containing control or *Atoh1* genes were injected into the left cochleae. Cells expressing the *Atoh1* gene were detected using a green fluorescent protein (GFP) reporter gene. After 3 weeks, hair cells were assessed for number, maturity and synaptogenesis with auditory neurons. Guinea pig hearing was assessed throughout.

Results: There were significantly more hair cells in cochleae over-expressing *Atoh1* compared to the contralateral cochlea and compared to control guinea pigs ($p<0.05$), however, the number of hair cells in *Atoh1*-treated animals was far below normal. The GFP^{+}*Atoh1* hair cells expressed a number of hair cell markers including myosin VIIa, parvalbumin and calbindin. Expression of the synaptic protein CtBP2 was protected by*Atoh1*expression but not restored to the normal density and location within the cell. There was no evidence of synaptogenesis of auditory neurons with GFP^{+}*Atoh1* hair cells and hearing was not restored.

Conclusions: *Atoh1* gene therapy alone cannot fully convert non-sensory cells into new hair cells after profound or noise-induced hearing loss. This study revealed that the degree of degeneration at the time of gene therapy has a big impact on whether *Atoh1* gene therapy can yield functional hearing improvements.

Key words: Atoh1; Gene therapy; Hair cells; Noise; Ototoxin; Regeneration

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O25. NOVEL LOCI IN CHROMOSOMES 2 AND 6 ASSOCIATED WITH BILATERAL MENIERE DISEASE MAY DEFINE AUTOINMUNE INNER EAR DISEASE.

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Meniere's disease (MD) is an episodic vestibular syndrome associated with sensorineural hearing loss (SNHL) and tinnitus. Patients with MD have an elevated prevalence of several autoimmune diseases suggesting a shared autoimmune background (1). The genotyping of large cohorts of patients with several autoimmune diseases has shown that most of these diseases share susceptibility loci(2). However, no consistent loci or genetic marker have been associated with MD and most of the genetic studies were not replicated in an independent cohort of patients.

The Immunochip, a high density genotyping array containing 195806 single nucleotide polymorphism and 718 small indels, was used to explore the association between sporadic MD and186 autoimmunity loci previously associated with 12 autoimmune disorders. We analyzed 716 cases of MD and 1628 controls. Quality Controls (QC) were applied using the GenomeStudio and PLINK software and single nucleotide variants (SNVs) with: a) visual inspection of cluster,b) cluster separation <0.4, b) Genotype Call scores less than 0.15, c) a genotype success rate of <90%, d) minor allele frequency (MAF) < 5%, e) proportion of alleles share identical by descent (IBD) >0.5, f)heterozygosity outside range (<0.18 and > 0.45) and g) located in chromosome X were excluded. After QC, 99765 markers and 2164 samples (689 cases /1475 controls) remained for further studies. Although nomarker reached a genome-wide significant association ($p<10^{-8}$), two genomic regions showed a high number of non-coding SNVs with $p<10^{-4}$ in the subset of patients with bilateral SNHL. A first locus in chromosome 2 include IL18R1 , IL18RAP and SLC9A4 genes and a second locus in chromosome 6 include DPCR1, MUC21, MUC22, CDSN and PSORS1C1 genes. These regions are candidate loci for future replication and fine mapping to define functional variants associated with autoimmune inner ear disease.

Keywords: Autoimmunity, Immunochip, genotyping.

Acknowledgements: This study was funded by PI13/1242 Grant. Sonia Cabrera is supported by Meniere's Society UK, WES-MD Grant.

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O26. EXOME SEQUENCING IDENTIFIES FAM136A AND DTNA AS CANDIDATE GENES IN FAMILIAL MENIERE'S DISEASE

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Meniere's disease (MD) is a complex disorder defined by cochlear and vestibular symptoms. Familial MD is found in 5-15% of cases in European populations. Although genetic heterogeneity is observed, most of the families have an autosomal dominant (AD) pattern of inheritance with incomplete penetrance (1,2).We have used whole-exome sequencing (WES) in a family with three affected women in consecutive generations to identify rare variants in the family and functional analysis to assess their pathogenicity.

DNA was isolated from peripheral blood and WES of genomic and mitochondrial DNA was carried out in a SOLiD 5500xl platform. Bioinformatic analyses to filter and prioritize were performed obtaining seven candidate variants. We have identified and validated by Sanger sequencing two novel single nucleotide variants, one in the FAM136A gene causing a nonsense mutation and another in the DTNA gene causing a missense mutation. qPCR revealed two mRNA transcripts of FAM136A in lymphoblasts from patients and protein products were confirmed by immunoblotting. Carriers of the FAM136A mutation showed a significant decrease in the expression levels of both transcripts in lymphoblastoid cell lines. Using immunolabeling and confocal microscopy in inner ear rat tissue, we have found that FAM136A co-localizes with the mitochondrial marker COX IV in the basal zone of hair cells in the crista ampullaris. Moreover alpha-dystrobrevin was located in supporting cells in the crista, close to the stromal region.

FAM136A encodes a membrane protein with unknown function associated with mitochondria. DTNA encodes a membrane protein involved in the formation and stability of synapses (3). This protein and dystrophin make up a complex associated with cytoskeletal and structural changes in this protein network could affect hair cell structure.

Localization of FAM136A and alpha-dystrobrevin in the vestibular crista suggest a functional role for both proteins in the clinical phenotype observed in the MD family.

In conclusion, we have identified two potentially damaging novel variants in FAM136A and DTNA genes that were segregated in three patients with definite MD. However, additional functional studies are required using induced pluripotent stem cells from patients with MD and gene-editing technology to rescue the phenotype, before validating these genes as causal for MD.

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

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O27. MOLECULAR DYNAMICS SIMULATIONS HIGHLIGHT STRUCTURAL AND FUNCTIONAL ALTERATIONS IN DEAFNESS-RELATED M34T MUTATION OF CONNEXIN 26.

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Objectives: Mutations of the GJB2 gene encoding the connexin 26 (Cx26) gap junction protein, which is widely expressed in the inner ear, are the primary cause of hereditary non-syndromic hearing loss in several populations. The deafness-associated single amino acid substitution of methionine 34 (M34) in the first transmembrane helix (TM1) with a threonine (T) ensues in the production of mutant Cx26M34T channels that are correctly synthesized and assembled in the plasma membrane. However, mutant channels overexpressed in HeLa cells retain only 11% of the wild type unitary conductance.

Methods: We extend and rationalize those findings by comparing wild type Cx26 (Cx26WT) and Cx26M34T mutant channels *in silico*, using a combination of equilibrium and out of equilibrium molecular dynamics simulations. In particular: (1) we compare the structural stability of the channel by stressing the system with an external force that mimics the presence of the membrane potential; and (2) we compute the free energy profile of the permeation of a potassium ion through both wild type and mutated channels, in order to produce an estimate of the conductance ratio.

Results: Our results indicate that the quaternary structure of the Cx26M34T hemichannel is altered at the level of the pore funnel due to the disruption of the hydrophobic interaction between M34 and tryptophan 3 (W3) in the N-terminal helix (NTH). Our simulations also show that external force stimuli applied to the NTHs can detach them from the inner wall of the pore more readily in the mutant than in the wild type hemichannel. These structural alterations significantly increase the free energy barrier encountered by permeating ions, correspondingly decreasing the unitary conductance of the Cx26M34T hemichannel.

Conclusions: Our results accord with the proposal that the mutant resides most of the time in a low conductance state. However, the small displacement of the NTHs in our Cx26M34T hemichannel model is not compatible with the formation of a pore plug as in the related Cx26M34A mutant.

Keywords: conductance, gap junction channels, genetic deafness, mean first passage time, potential of mean force.

Acknowledgements: Supported by Telethon Italy grant GGP13114 to F.M. Computer simulations were performed at the CINECA supercomputer centers.

O28. NEW HEREDITARY HEARING LOSS (HHL) GENES/MUTATIONS IDENTIFIED BY HIGH THROUGHPUT TECHNOLOGIES IN THE ITALIAN AND QATARI POPULATIONS

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Objectives: To overcome the remarkable genetic heterogeneity of HHL an extremely powerful 2 steps "gene-identification strategy" was designed. STEP1 consists in a screening of 96 HHL genes by targeted re-sequencing (TS). Positive cases contribute to define an accurate molecular epidemiology picture while negative ones undergo a combination of linkage studies and whole exome sequencing (WES) or directly to WES (depending on pedigree size) STEP2.

Methods: Ion Torrent PGM™ (400X of mean coverage and 581.000 Kb of targets size) was used for STEP1 and Illumina genotyping and Ion Proton PGM™(90X of mean coverage and 60Mb of target size) were used for STEP2. Sequencing variants were annotated/filtered according to standard pipelines. This strategy was applied to a first series of 28 Italian and Qatari families and to 10 Italian sporadic cases.

Results: STEP1 characterized36% of Italian and 53% of Qatari families leading to the identification of 20 novel alleles in known HHL genes (*P2RX2, TECTA, TMPRSS3, LOXHD1, MYO15A, TMC1, TRIOBP, WFS1*). Moreover, 50% of Italian sporadic cases were detected in the following genes: *SLC26A4, OTOF, CDH23, MYO3A, POU3F4*. STEP2 had already led to the discovery of 3 new genes (*BDP1, TBLY1, PSIP1*) and it is in progress in the remaining families. Briefly, p*2625Gluext*11 mutation resulting in an elongation of 11 residues of the BDP1 protein was identified in a recessive family and its expression in the inner ear was confirmed by immunohistochemistry[1]. A frameshift deletion (p.518_519del) leading to a premature stop codon 4 residues downstream was found in *PSIP1* gene, recently described as a transcriptional co-activator, regulated by miR-135b in vestibular hair cells of the inner ear [2]. Finally, in a pedigree showing a Y-linked pattern, the predicted pathogenic D69V mutation was identified in *TBLY1* gene, an homologous of a HHL gene, named *TBL1X*[3].The expression of this gene was already demonstrated in the human cDNA and functional studies are in progress to confirm its pathogenicity.

Conclusion: This combined strategy proved to be very successful by explaining several HHL unsolved cases. Updated data will be presented and discussed

Keywords: Next generation sequencing, Hereditary hearing loss, new mutations/genes

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O29. cGMP GENERATORS IN THE INNER EAR: WHICH ONES MAY BE INVOLVED IN OTOPROTECTION AFTER NOISE DAMAGE

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We recently reported that pharmacological PDE5 inhibition reduces HC damage following auditory noise exposure indicating a protective molecular cascade mediated by cGMP signaling [1]. Candidate components of this protective cascade have been described within the last decades (cGMP and GC [2], ANP receptors [3] and ANP receptor coupled GCs [4], nNOS and sGC [5], pGC and sGC [6], cGKI [7], PDE5 and cGKI isoforms [1]). However, the upstream components involved in the protective cascade (cGMP-generators) in the cochlea are still unknown.

In the recent study, we show data on the function and molecular phenotype of mice with deletions in selected genes coding for proteins that are anticipated components of the otoprotective cGMP cascade. Mice deficient for particulate (GC-B / npr2) or soluble guanylate cyclases (NO-GC1, NO-GC2) were examined for their hearing sensitivity, hearing range, and vulnerability to noise exposure (hair cell damage and loss of afferent synaptic contacts). Additionally, we pharmacologically stimulated cGMP production by an sGC-stimulating drug administered in the food of differently aged rats for 25 days. Peripheral and central auditory responses were analyzed and compared to hair cell function (otoacoustic emissions), quantification of CtBP2 positive staining at the IHC synapse, giving an estimate for the number of afferent contacts, and afferent and efferent neuronal projections in the auditory periphery.

The data will be discussed regarding the proposed cGMP generators in the inner ear and their role for an otoprotective molecular cascade after noise induced damage of the ear.

Keywords: cGMP, guanylyl cyclase, sGC, PDE5, npr2, NO-GC, protection, noise exposure.

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O30. STAT3 AND OTOPROTECTION

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Objectives: STAT3 (signal transducer and activator of transcription 3) is a ubiquitous transcription factor residing in cytoplasm. Upon activation, STAT3 translocates into the nucleus to mediate gene transcription or into mitochondria to support complex I of respiratory chain. As a result, following STAT3 activation cells proliferate, differentiate, may produce cytokines, chemokines or neurokines or became resistant against apoptosis. Here, we have investigated the *in vitro* ability of STAT3 to prevent auditory hair cell loss upon exposure to cisplatin. Preventing predictable ototoxicity could be an effective way to avoid hearing loss.

Methods: As an experimental model, we have used explanted cochlear membranes isolated from the inner ear of Wistar rats (post-natal days 3-5). The explants were exposed to cisplatin [15µM] for 24h or pre-treated for one day with STAT3 inducers (interleukin-6 (IL6) [30ng/ml], oncostatin M (OSM) [30ng/ml] or coenzyme Q10 [50µg/ml] and then exposed to cisplatin for another 24h. Activation of STAT3 was controlled for by subcellular fractionation and Western blotting. To better determine the mechanism of otoprotection, STAT3 inhibitor III / WP1066 [5.6µM] was added simultaneously with OSM, IL6 or conezyme Q10. After this, explants were stained with phalloidin to visualize and score the hair cells.

Results: Exposure of cochlear explants to cisplatin resulted in significant hair cell loss. Addition of STAT3 activators to explant cultures prior to exposure to cisplatin significantly reduced hair cell loss. Treatment of explants with IL-6 or OSM induced phosphorylation of STAT-3 on serine- and tyrosine- residues and translocation of STAT-3 from cytoplasm to the nucleus. Treatment of explants with coenzyme Q10 induced induced phosphorylation of STAT3 preferentially on serine residue and STAT3 translocation to mitochondria. WP1066 inhibited the protective effect of IL-6 and OSM but not that induced by coenzyme Q10 indicating that serine STAT3 phosphorylation and translocation to mitochondria could be a key factor in coenzyme Q10-induced otoprotection. Taken together, we have demonstrated that various ways of STAT3 activation in cochlear explants is associated with otoprotective effect.

Keywords: STAT3, cisplatin; interleukin 6; oncostatin M, coenzyme Q10, otoprotection

Acknowledgements: Charité University Hospital Research Funds

O31. CHEMOTHERAPY FOR THE FUTURE: NOVEL AMINOGLYCOSIDES DISSECTING ANTIBACTERIAL ACTIVITY AND OTOTOXICITY

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Ototoxicity is thought to be inherent to aminoglycosides, compromising the clinical use of these important antibacterials. Based on our research into basic mechanisms of ototoxicity, we have shown clinically successful pharmacological mitigation of aminoglycoside-induced hearing loss [1]. Chemotherapy of the future will require aminoglycosides effective against multi-drug resistant bacteria yet devoid of ototoxic potential.

Our mechanistic concept postulates a key role for the mitochondrial ribosome (mitoribosome) in aminoglycoside ototoxicity. We have previously reported [2] that apramycin, a structurally unique aminoglycoside in veterinary oral use for treatment of intestinal infections, shows low activity towards eukaryotic ribosomes, including hybrid ribosomes carrying the aminoglycoside-susceptibility A1555G allele. In cochlear explants, apramycin caused only little hair cell damage. In vivo, and in contrast to gentamicin, ototoxicity developed more gradually and only at higher concentrations. Apramycin's high efficacy against drug-resistant strains combined with low ototoxic potential makes it a drug of immediate clinical interest.

These results have provided proof-of-concept that antibacterial activity can be dissected from aminoglycoside ototoxicity as well as a conceptual framework to develop less toxic aminoglycosides by hypothesis-driven chemical synthesis. With this approach, we have been able to identify aminoglycoside pharmacophores with low ototoxic potential. Several new lead compounds promise a safety margin (antibacterial efficacy vs. ototoxicity) more than an order of magnitude better than gentamicin.

Keywords: Chemical synthesis; in-vitro translation assays; antibacterial activity; reactive oxygen species; ototoxicity.

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O32. ISOLATION AND CHARACTERIZATION OF NASAL AND MIDDLE EAR EPITHELIAL CELLS FOR DEVELOPMENT OF AN OTOPATHOGENIC INFECTION MODEL

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Rationale & Hypothesis: The BPI fold (BPIF) containing/PLUNC family of putative innate defence proteins is amongst the most rapidly evolving mammalian genes. The two most abundant members of this family, BPIFA1 (SPLUNC1) and BPIFB1 (LPLUNC1) are highly expressed in the respiratory tract. Altered expression of both proteins is common in many chronic lung diseases. Genetic studies have associated SNPs in *BPIFs* with *Otitis Media (OM*), a common cause of paediatric deafness. Studies in our lab have shown that BPIFA1 is expressed in middle ear (ME), Eustachian tube and nasal passages of Wt mice and expression of this protein decreases with *OM* development in *Junbo* mice, a model of chronic *OM*. BPIFA1 can also be detected in ear exudates from *Junbo* mice. We hypothesize that BPIFA1 plays a role in protection of the ME and alteration in the levels of this protein is associated with *OM* development.

Objectives: We aim to develop a novel *in vitro* otopathogenic infection model in order to investigate the role BPIFA1 plays in the nasopharynx and the ME.

Methodology: We isolated cells from Wt and BPIFA1 Knockout (Ko) nasal passages and ME and cultured them at Air Liquid Interface (ALI) to facilitate differentiation. We characterised the different epithelial cell populations on a transcriptional level using RT-PCR and on a proteomic level using Immunofluoroscence Confocal Microscopy. BPIFA1 was also detected in the apical secretions from the ALI cultures using Western blotting.

Findings: We have successfully developed an *in vitro* primary nasal and ME epithelial model that can be used effectively for characterising the different cell types present in the ME and nasaopharynx. Both nasal and ME epithelial cell cultures differentiate at ALI.They express intracellular and secreted BPIFA1, replicating the *in vivo* situation. We also found expression of ciliated cells in both cultures using markers such asβ-tubulin and FOXJ1.This model can be used to study to various other epithelial cell markers such as cytokeratin, Mucins, BPIFB1etc. Moreover, it can be used to study the effect of loss of BPIFA1 on infection by otopathogens like NTHiand help us better understand the pathophysiology of *OM*.

Keywords: Air liquid interface; BPIF proteins; Junbo mice; *NTHi*, Otitis media (

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O33. FUTURE TRENDS REGARDING THE BIOELECTRICAL INTERFACE OF COCHLEAR IMPLANTS

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Introduction: In patients with severe to profound hearing loss, cochlear implants (CIs) are currently the only therapeutic option when the amplification with conventional hearing aids does no longer lead to a useful hearing experience. Despite its great success, there are patients in which benefit from these devices is rather limited. One reason may be a poor neuron-device interaction, where the electric fields generated by the electrode array excite a wide range of tonotopically organized spiral ganglion neurons at the cost of spatial resolution. Coating of CI electrodes to provide a welcoming environment combined with suitable surface chemistry (e.g. with neurotrophic factors) has been suggested to create a closer bioelectrical interface between the electrode array and the target tissue, which might lead to better spatial resolution, better frequency discrimination, and ultimately may improve speech perception in patients. In addition, the use of progenitor/stem cells may help to improve this interaction as well in the future.

Materials and Methods: We will present a general concept for future cochlear implant electrode design and show first results of experiments including coating strategies, the use of growth factors and progenitor cells to achieve a better neuron-device interaction.

Conclusion: There have been different approaches suggested to improve the bioelectrical interface of cochlear implants and therefore the hearing performance of these patients. Coating strategies of the CI electrode array to achieve neuroprotection and a directed neurite outgrowth in combination with growth factors or small molecules as well as the use of mini-pumps integrated in the CI device seem to be the most promising approaches right now, whereas the use of stem or progenitor cells may play a role in the future after discovering more insights of neuronal differentiation and neurite outgrowth pathways.

Keywords: auditory neurons; cochlear implants; hearing development; stem-cells

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O34. DEVELOPMENT OF AN *IN VITRO* BIOASSAY TO ANALYZE INTERFACE-DEPENDENT RESPONSE PROFILES OF AUDITORY NEURONS ON MULTI-ELECTRODE ARRAYS

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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 peripheral processes of the auditory neurons grow towards the electrode array to form a gapless interface 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 aim at developing an *in vitro* bioassay based on multi electrode arrays (MEAs) combined with an external stimulation electrode to study auditory neuron activity and to investigate parameters that are relevant in the context of the project. Specifically, how stimulation parameters will need to be adapted in relation to the distance of the CI electrode to fully exploit the theoretical advantages of a gapless interface. Optimum stimulation parameters and distance-related effects are experimentally addressed on a custom-made set up, using murine spiral ganglion neuron cultures on MEAs. The first results confirmed the main hypothesis of the NANOCI project, namely that the smaller the distance between the stimulating electrode and the auditory neurons, the lower the voltage needed to trigger neuronal activity and the larger the dynamic range of responses. Although preliminary, these results are the first of their kind in the auditory field and allow us to address more sophisticated stimulation protocols in the near future.

Keywords: cochlea implants, inner ear, nanotechnology, auditory neurons

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O35. A COMPARISON OF EAR-CANAL DISTORTION PRODUCT OTOACOUSTIC EMISSIONS WITH INFERRED INTRA-COCHLEAR DISTORTION PRODUCTS

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The characteristics of ear-canal distortion product otoacoustic emissions (DPOAEs) have been thoroughly described. In contrast, there is relatively little known concerning intracochlear DPs that are propagated toward the DP frequency place (f_{dp}) on the basilar membrane (BM), i.e., BM-DPs. Detailed comparisons of DPOAEs to BM-DPs permit valuable insights concerning how ear-canal DPOAEs mirror intracochlear BM-DPs. The present study used a technique in alert rabbits whereby the behavior of BM-DPs is inferred by interacting a probe tone with the DP of interest to produce a 'secondary' DPOAE (DPOAE'). In this manner, the DPOAE' method was used to directly compare DPOAE f_2/f_1-ratio functions, level/phase maps, and interference response areas (IRAs) to their BM-DP counterparts. The $2f_1$-f_2 and $2f_2$-f_1 DPOAEs were measured with f_1 and f_2 primary-tone levels varying from 35-75 dB SPL. During collection of the DPOAEs, a 50-dB SPL f_3 was placed at a DP/f_3 ratio of 1.25 to evoke a DPOAE' at $2f_3$-($2f_1$-f_2) or $2f_3$-($2f_2$-f_1). In some experiments, a fixed interference tone (IT) or f_4 was presented, or was alternatively swept in frequency and level to produce an IRA describing the effects of the IT on both standard DPOAEs and BM-DPs. Results showed that low primary-level DPOAE-ratio functions peaked at around f_2/f_1=1.25, while the corresponding BM-DP ratio functions peaked at an f_2/f_1≈1. Also, notches observed in DPOAE-ratio functions were not present in their BM-DP counterparts. Additionally, ITs above f_2 at narrow f_2/f_1 settings often enhanced the DPOAE, while the BM-DP remained unaffected. Finally, level/phase maps showed rapid phase variation with f_{dp} for the narrow ratio $2f_1$-f_2 and all $2f_2$-f_1 DPOAEs, while the corresponding BM-DPs evidenced relatively constant phases. Thus, DPOAEs often provide poor representations of BM-DPs, perhaps because distributed DPOAE components directed basally toward the ear canal are frequently in cancellation, while BM-DP elements directed apically toward f_{dp} add in phase.

Keywords: distortion production otoacoustic emissions, inferred intracochlear distortion products, alert rabbit.

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O36. STATE-DEPENDENT INFORMATION PROCESSING IN AUDITORY THALAMOCORTICAL CIRCUIT

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The brain is always busy. Cortical circuits show coordinated activity even in the absence of auditory inputs. This coordinated spontaneous activity defines ongoing cortical state and its abnormality has long been implicated in tinnitus. Cortical circuits normally exhibit two states: an "inactivated" (or synchronized) state is characterized by slow fluctuations between synchronous and silent population activity, called UP and DOWN states/phases, and appears during slow-wave sleep or quiet wakefulness and under anesthesia. On the other hand, an "activated" (or desynchronized) state is characterized by tonic desynchronized activity and typically appears during attentive state and rapid-eye-movement sleep. One of the central questions in this topic is how cortical states affect auditory processing and perception. Particularly little is known about how cortical states affect temporal processing. To address this issue, we combined *in vivo* large-scale extracellular recording in the auditory thalamocortical circuit of urethane-anesthetized rats with basal forebrain electrical stimulations, which allowed us to manipulate cortical states. We found laminar-specific effects of cortical states on cortical oscillations, population firing and onset responses to single clicks. Rapid auditory processing was improved during the activated state in the primary auditory cortex, but not in the medial geniculate body, suggesting that auditory cortical circuits have a prominent property to integrate internally generated activity with external auditory inputs. We hypothesize that cortical activations improve temporal processing to analyze behaviorally relevant acoustic stimulus in details.

Keywords: auditory cortex, medial geniculate body, basal forebrain, neural oscillations, cell type

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O37. BDNF-DEPENDENT AUDITORY FIBERS SET BRAINS BASELINE FOR SOUND

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Detecting sounds is the elementary task performed by the auditory system, but the physiological basis of the biologically useful 'absolute sound threshold' for mammalian species is still elusive. In the auditory system, perception thresholds and the dynamic range over which sound intensities can be perceived are considered to be determined by cerebral sensitivity. Using mice with deletion of brain-derived neurotrophic factor (BDNF) in peripheral and central auditory neurons ($BDNF^{TrkC}$) versus a deletion comprising in addition the entire cochlea ($BDNF^{Pax2}$), we found that BDNF in the cochlea is essential and sufficient to set low baseline detection thresholds. BDNF from the periphery but not the brain maintains an auditory fibre class in high-frequency cochlear turns that drives short latency, low sound thresholds and thereby expansion of the dynamic range for sound intensities in the ascending pathway as measured through extracellular responses from inferior colliculus neurons. BDNF-dependent afferent activities shape narrowed tuning of inferior colliculus neurons upon generation of high-frequency inhibitory sidebands. As a consequence, baseline levels for detection thresholds are lowered in order to improve amplitudes within frequencies where behavioural auditory thresholds are known to be lowest in mice. When these fibers are lost after injury, as shown for $BDNF^{Pax2}$ wild-type mice after acoustic trauma, thresholds for compensating central gain are lost and central hyperactivity evolves, a maladaptive phenotype observed in various brain disorders, including tinnitus.

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O38. PIP_2 DETERMINES EXCITABILITY OF SPIRAL GANGLION NEURONS VIA REGULATION OF KV1-CONTAINING HETEROMERIC CHANNELS

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Excitability in a sub-population of spiral ganglion neurons (SGNs) is limited by a Dendrotoxin-K-sensitive, low voltage-activating K^+ current (1). Activity of Kv channels can be sensitive to binding of Phosphatidylinositol 4, 5-bisphosphate, known as "PIP_2" (2), and consequently PIP_2 can be an important determinant of neuronal excitability. Here we have assessed the contribution of PIP_2signalling to SGN function. SGN from juvenile C57BL/6 mice (P12-P21) were cultured for 2-3 days in the presence of Brain Derived Neurotrophic Factor. Under control conditions ~70% (8/12) of SGNs fired rapidly-adapting ("phasic") action potentials under current clamp, and the remaining cells were slowly-adapting or non-adapting ("tonic" firing). Pre-incubation for 1 hr at 37^oC with 10 µM Wortmannin, an enzyme inhibitor of PIP_2 production (3), reduced the prevalence of phasic firing to ~40% (7/18). The effects of Wortmannin treatment could be partially rescued by intracellular diC_8-PIP_2, a non-metabolisable PIP_2 analogue (n=8). In separate experiments, SGN were depleted of membrane-bound PIP_2 by transient exposure to a membrane-targeting palmitoylated peptide ("PalPeptide") based on the PIP_2 binding domain of the Kv7.2 channel (4). Bath applied Pal-Peptide (1-3 µM) slowed adaptation in all cases (9/9). Under voltage clamp, PalPeptide inhibited the low voltage-activated K^+ current (77 ± 4%, n=5). This effect could be reduced significantly by intracellular application of diC_8-PIP_2 (41 ± 5%, n=7, $P<0.001$). The PalPeptide-sensitive current was also blocked by Dendrotoxin-K, identifying Kv1.1 as an important contributor. Our observations identify phosphoinositide signalling as a novel therapeutic target for controlling the sensitivity of the auditory nerve.

Keywords: Phosphatidylinositol 4,5-bisphosphate;Spiral Ganglion Neuron

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O39. COMPARISON OF DORSAL COCHLEAR NUCLEI MICROGLIOSIS IN DIFFERENT RAT TINNITUS MODELS

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Objectives: In this work we measured variations in microglia density and morphology in rat dorsal cochlear nuclei (DCN) following several treatments known to induce tinnitus. Both in human patients and animal models, tinnitus can result from several conditions, and it is still unknown how heterogeneous the cellular/circuital imbalances are in the various types of tinnitus. We chose DCN as an observation point because changes in its cerebellar-like interneuronal network have been associated to the earlier stages of tinnitus (Middleton et al 2011). Microgliosis associated to remodeling of the first CNS station is also observed in chronic pain and chorda tympani lesions (Milligan et al. 2008, Bartel 2012), consistently with a major role of microglia regulating circuit excitability in physiological and pathological states (Eyo and Dailey 2013). Therefore, we wanted to observe whether different tinnitus-inducing treatments elicited similar microglial response in DCN.

Methods and Results: In our experiments we induced tinnitus by unilateral cochlear destruction, salicylate injection, and noise trauma, and tested for tinnitus presence by gap-startle paradigm (Turner 2007). Animals testing positive for tinnitus displayed an increase in microglial density, especially for cochlear destruction. In these animals, microgliosis was present both ipsi- and contralaterally. Microglial morphology was clearly ameboid in ipsilateral cochlear destruction DCN and ramified in other conditions, suggesting a different degree of activation. Consistently, systemic treatment with minocycline (Marchand et al. 2009) immediately after cochlear destruction strongly reduced microgliosis in ipsilateral DCN. Microglia density variations were aligned with cochlear nuclei structures, and in most cases density peaks were highly localized (e.g. in the granular cap or the deep layer).

Although still incompletely characterized, microglia actions in regulating excitability include effects on both excitatory and inhibitory synapses (Ferrini and de Koninck, 2013). In order to test the possible effects of microglia on circuit plasticity, we are building a cell-type realistic network model of the DCN from literature data.

Keywords: dorsal cochlear nuclei, microglia, tinnitus

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Poster Presentations

P1. VOLTAGE AND CALCIUM IMAGING IN THE DEVELOPING COCHLEA OF WILD TYPE AND DFNB1 MOUSE MODELS

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Nonsyndromic hearing loss and deafness (DFNB1) is an inherited condition caused by mutations in GJB2 (which encodes connexin26) and GJB6 (which encodes connexin30). Electrical and metabolic coupling mediated by gap junction channels formed by these two protein subunits is fundamental for the development and maintenance of hearing [1-3]. However, precise estimates of the degree of coupling and its alterations under DFNB1 conditions are lacking.

To address these issues, we developed a novel technique based on voltage imaging to map the extent and the degree of electrical coupling in non-sensory cell networks of the developing mouse cochlea. We estimated that cochlear supporting cells are already well coupled in the first postnatal week by as many as 1500 channels per cell pair. In cultures from age-matched connexin30(T5M/T5M) and connexin30(−/−) mice, junctional conductance was reduced respectively by 14% and 91%, and these data account for the increased hearing thresholds exhibited by these animals in the adult stage [1,4]. Besides providing electrical coupling, cochlear gap junction channels and hemichannels have been shown to participate in ATP- and IP_3- dependent intercellular Ca^{2+} signalling [1-3]. We thus performed Ca^{2+} imaging experiments aimed at elucidating the mechanisms underlying the generation and intercellular propagation of these signals. We finally combined the information gathered from the different experimental approaches in a mathematical model that (i) correctly reproduces the range and propagation speed of intercellular Ca^{2+} waves; (ii) reproduces the oscillatory behaviour of intracellular Ca^{2+} signals and (iii) predicts that ATP release through connexin hemichannels is the primary mechanism responsible for the long range propagation of Ca^{2+} signals in the developing cochlea.

Keywords: Connexins; electrical coupling; non-sensory cells; voltage imaging; mathematical modelling.

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P2. HUMAN TECTORIAL MEMBRANE AN IMMUNOHISTOCHEMISTRY ANALYSIS AND ELECTRON MICROSCOPIC STUDY

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Objective: To investigate the structure and macromolecular organization of the human tectorial membrane (TM). The TM is an extracellular matrix that helically advances along the entire cochlea. It causes shearing motions of the stereocilia bundles as sound vibrations enter the cochlea. Its role in frequency tuning and linkage between outer and inner hair cells in the human cochlea is still elusive.

Methods: We studied freshly obtained cochleae from patients undergoing removal of life-threatening petroclival meningioma. Patients had normal audiograms. After fixation, decalcification and cryosectioning, immunohistochemistry was performed using anti-human-antibodies against collagen II, IX and XI, α-tectorin and β-tectorin. Laser-confocal microscopy and high resolution scanning electron microscopy (SEM) were carried out in combination. The radial width and thickness of the TM in the sections at various turns was assessed on the basis of information from the scanning electron micrographs. The gradient morphology of the TM was analyzed using high resolution SEM.

Results and Discussion: The three-dimensional structure of the human TM continuously changed from base to apex. Apically, the TM was wider than at the basal turn. The width of the middle part of TM increased from base to apex. The TM could be separated into three different parts; the marginal, the middle and the limbal part. The overlay surface showed a texture with various sized fibrous bundles and obliquely oriented fibrils (diameter <10nm). The inferior surface disclosed outer hair cell stereocilia imprints from different rows. The inner hair cell stereocilia formed no discernible imprints or surface specializations of the Hensen's stripe (HS). HS formed a hyaline-like semi-circular protrusion whose morphology changed along the frequency range. Immunohistochemistry revealed that the human TM contains collagen II and IX and the proteoglycan α-tectorin. Collagens were strongly expressed in the TM main body, while α-tectorin was expressed in the HS. β-tectorin IHC is so far inconclusive.

Keywords: Collagen II; Human cochlea; SEM; Tectorial membrane; α-tectorin.

P3. DIFFERENTIAL EXPRESSION PATTERNS OF ATP6B1 IN THE INNER EAR LATERAL WALL OF COMMON MARMOSET AND MOUSE

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Mutations in ATP6B1 (also called B1 subunit of H+-ATPase or V-ATPase B1) in humans cause autosomal recessive distal renal tubular acidosis and deafness [1], yet its knock-out mouse does not suffer from hearing loss [2]. The common marmoset (*Callithrix jacchus*) is a new world non-human primate which has recently been used by biomedical researchers since the species is easy to handle and in which transgenics are available[3]. Here, we compared expression patterns of ATP6B1 in the inner ear of mouse and common marmoset for understanding the species differences.

Methods: Temporal bones of young adult common marmoset and mouse were dissected, fixed and decalcified with Decalcifying Solution. B (Wako) for 3d (for mouse) or 1w (for common marmoset) and embedded in Tissue-Tek O.C.T. compound for cryosections. **7**μm sections were used for immunohistochemistry with antibodies for V-ATPase B1 (goat, Santa Cruz).

Results: As reported previously, immunoreactivity for ATP6B was not detected in the mouse inner ear lateral wall [2], while robust signal was observed in the inner ear of the marmoset. The expression was restricted to the outer sulcus cells, which are essential for potassium recycling.

Discussion: Knowledge in the inner ear field is largely dependent on the experiments performed in rodent models (mouse, rat, guinea pig and gerbil etc.) due to their well-established techniques in biotechnologies. However, thehuman phenotypes are not always recapitulated in the rodents, presumably due to the large species differences. Our results suggest that using the common marmoset, an easy handling non-human primate, should be an option for bridging the species gap. Studies using transgenic marmosets would a feasible strategy for elucidating unclarified issues in hereditary hearing loss that cannot be solved by knock-out or transgenic mouse studies.

Keywords: Hereditary hearing loss; ATP6B1, B1 subunit of H+-ATPase; V-ATPase B1; common marmoset (*Callithrix jacchus*).

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P4. MORPHOLOGY, IMAGING AND LOCAL SURGICAL ANATOMY OF THE TEMPORAL BONE OF COMMON MARMOSET *(CALLITHRIX JACCHUS)*

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Model animals are an indispensable tool for biomedical research. We have been using a biotechnologically established small non-human primate, the common marmoset *(Callithrix jacchus)*for the steps right before clinical trials. Here we examined the anatomy of the peripheral auditory system of common marmoset by section, CT, MRI. Surgical anatomy for the route of drug delivery to the inner ear was also investigated.

Methods: For imaging analyses, four temporal bones were evaluated with CT and MRI, followed by histological analyses in paraffin-embedded sections (n=4). For the local surgical anatomy, fixed heads were used (n=3).

Result: The inner ear was relatively large in contrast to its small skull. Similar structures in its attics and tympanic cavity to those in human were observed, yet the mastoid wasoccupied with a single common cavity, which resembles to the bulla structure of the lower mammals such as guinea pig. With fiber tractography using ultra high-resolution diffusion tensor images obtained by a 7T MRI and cryogenic MRI probe, we successfully distinguished apical fibers from basal fibers. Apical-basal tonotopic gradient was preserved in the twisted auditory nerve, as reported in human. Surgical access to semicircular canals was easily achieved via the single mastoid cavity. Access to round window niche was also available via posterior tympanotomy.

Conclusion: Common marmoset would be a useful model animal for translational research. Easy surgical access to the inner ear enables us to use it for pre-clinical phase evaluation of local drug delivery to the inner ear. Repetitive MR neuro imaging would provide information of time-dependent changes in individual samples without sacrifice. Combined with traditional electrophysiology of A1 cortex and recently established genetic modification, studies using this species would be highly-integrated translational researches of auditory diseases/disorders.

Keywords: Common marmoset; CT; MR-histology; translational research; animal model.

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P5. MEASURING GLUTATHIONE CONTENT IN THE ORGAN OF CORTI USING LIVE IMAGING

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Reactive Oxygen Species (ROS) are natural bio-products of mitochondrial metabolism. ROS have been implicated in ototoxicity, noise-induced hearing loss, and age-related hearing loss. One of the key molecules that neutralizes cellular ROS is glutathione (GSH). Here we used a live imaging approach to measure the GSH content of cells in the organ of Corti at different stages of cochlear maturation.

For live imaging, excised auditory bullae from C57BL/6 mice were opened at the apex and incubated with monochlorobimane (MCB, which forms a fluorescent conjugate with GSH) for 40 minutes. Fluorescent MCB-GSH, proportional to the reduced GSH concentration, was detected using multiphotonconfocal microscopy. GSH was evaluated in inner hair cells, inner sulcus cells, outer hair cells, and Deiters' cells in the apical turn from postnatal day (P)4, P15, P30 and P365 mice. Our data show that up to P30 there was a significant increase ofGSH in IHCs ($p<0.05$) whereas other cells did not show a significant increase over this period. Since we were only able to access the apical turn of the acute bullae preparation, we used P5 cochlear cultures to determine whether there was an apex to base gradient in cellular GSH. Initial data suggested no significant difference between the turns in the explant culture.

To test the effect that lowering GSH content has on aminoglycoside toxicity, P5 explants (basal to middle turns) were incubated in either buthioninesulfoximine (BSO, an inhibitor of gamma-glutamylcysteinesynthetase that is critical for GSH synthesis) or control media,for 16-18 hours. Subsequently explants were incubated in 1mM neomycinfor up to 6 hours prior to fixation and immunostained to determine hair-cell survival. BSO-treatment alone did not affect hair survival significantly. Lowering GSH levels did not affect inner hair cell or outer hair cell survival after neomycin-treatment and if anything our data indicate a trend towards increased survival. One interpretation of these findings is that ROS play a lesser role during aminoglycoside-induced hair-cell death than previously thought, although further experiments are required to confirm this.

Keywords: hair cell; ototoxicity; reactive oxygen species (ROS); glutathione (GSH).

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P6. IMMUNOHISTOCHEMISTRY OF METHYL METHACRYLATE EMBEDDED GUINEA PIG AND MOUSE COCHLEAE

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Objective: To establish a resin embedding protocol for high quality conventional staining and immunohistochemistry in guinea pig and mouse cochlea.

Methods: Decalcified cochleae of adult guinea pigs and mice were embedded in Technovit 9100 NEW (Heraeus Kulzer, Wehrheim, Germany), a resin embedding system based on methyl methacrylate. Midmodiolar cochlear sections were obtained at a thickness of 7 µm. Two staining protocols were developed. In the first protocol sections were stained with ETS (Epoxy Tissue Stain, Electron Microscopy Sciences, Philadelphia, USA) for 2 minutes, dehydrated and cleared with xylene. This contrast rich staining technique allowed identification of anatomical structures in the cochlea comparable to EPON embedded sections. On deplaticized neighbouring sections, immunohistology with different antibodies was performed to identify different cell types along the cochlear spiral. In the organ of Corti, immunostaining was performed using, Myosin VIIA and Prestin for hair cells, and SOX-2 for supporting cells. In the Rosenthal canal, GFAP was used for glia cells and Calretinin for spiral ganglion cells, respectively. In the lateral wall, potassium channel Kir4.1 and AQP5 positive cells were investigated.

Results: With this embedding technique, besides a high quality of conventional staining like toluidine blue staining, the specific immunohistochemical identification of the different cell types in different parts of the cochlea was demonstrated.

Conclusions: A combination of high morphological preservation as stained with conventional techniques like Epoxy Tissue Stain and the option to perform immunohistological investigations on the same tissue block is a valuable tool. This resin embedding technique and the established protocol allows precise identification of specific cell types of the inner ear and thus provides a tool to investigate pathological processes like hair cell loss, or to investigate the outcome of a pharmacological treatment.

Keywords: guinea pig; mouse; immunohistochemistry; organ of Corti; spiral ganglion cell; stria vascularis.

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P7. IMAGING OF ANIMAL AND HUMAN INNER EARS WITH X-RAY MICROTOMOGRAPHY

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Non-destructive 3D imaging of animal and human inner ears are important requirements to quantify anatomical variations or survey developmental steps in embryogenesis. X-ray microtomography (micro-CT) is a versatile tool for mineralized tissues but requires contrast enhancement of soft tissue. In ossified temporal bones we face the problem of high contrasting bone with delicate soft tissue structures of the membranous labyrinth and nerve fibres located within the most compact bone in our body.

We present different techniques suitable for human and animal inner ears. Decalcification post-fixation with osmium tetroxide proved to be most suitable for big human temporal bones to visualize especially nervous tissue within bone. Also membranes and certain tissues provide good contrast. We further tested phosphotungstic acid (PTA), iodine potassium iodide (Lugol's formulation-IKI), elementary iodine in ethanol (I2E), the commercial formulation Gastrografin®, tannic acid and osmium tetroxide (OsO4) before and after decalcification.

IKI turned out to give best contrast for soft tissue while decalcified and osmium treated temporal bones were ideal to trace myelinated nerve fibres.

These techniques provide sufficient contrast for soft tissue in the hard bony capsule and enable non-destructive imaging of the inner ear. Data can be used for segmentation of various structures of interest and measured with high accuracy.

Keywords: micro CT imaging, inner ear, 3D non-destructive reconstruction, morphometry

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P8. HUMAN BASILAR MEMBRANE: AN IMMUNOHISTOCHEMISTRY AND ELECTRON MICROSCOPIC STUDY

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Introduction: The molecular composition of the human basilar membrane (BM) is still unclear. Cochlear micromechanics and frequency tuning depend on its macromolecular organization. Cochlear implantation and hybrid hearing techniques motivate further analyses of the BM. Physical interaction between electrode and BM may impair sound-induced vibrations and also provoke inflammation leading to fibrotic reactions.

Materials and Methods: We studied freshly obtained cochleae from patients undergoing removal of life-threatening petroclival meningioma and from a normal patient for decellularization. After fixation, decalcification and cryosectioning, immunohistochemistry was performed using anti-human antibodies against laminin-β2, collagen II, IV and IX, elastin, fibronectin, tenascin and β integrin. Laser-confocal microscopy, high resolution scanning (SEM) and transmission electron microscopy (TEM) were carried out in combination.

Results: The human BM consisted of four separate layers. 1. Epithelial basement membrane positive for laminin-β2 and collagen IV; 2. BM "proper" composed of radial fibers expressing collagen II; 3. Layer of collagen IV; 4.Tympanic covering layer expressing collagen IV, fibronectin and integrin. Elastin was not detected other than in marginal layers at the basal hook region near the round window. Radially oriented fibrils of collagen type-II (10-20nm) were arranged into various sized bundles. Fibers were also arranged spirally. A pars pectinata and arcuata was only seen at the most basal region. BM width (outer pillar region) ranged from 126µm at high frequencies to 418µm in the apex. Thickness varied both radially and along the spiral. BM was thinnest at the OHC region and laterally (mean values respectively 0.55 and 1.16µm) while medially at habenula beneath IHCs it was more robust. TCL thickness increased apically, but disappeared in the apex. Decellularized human temporal bone revealed spirally directed, septa-like structures along the basilar membrane at the region of Boettcher cells.

Conclusions: The human BM consists of acellular, highly organized ECM layers composed of laminin-β2, collagen IV and II and a cellular TCL with fibers expressing collagen IV and blood vessels. The BM width increased linearly 4 times and thickness decreased six times from base to apex partly explaining the reduction in stiffness. Findings suggest that the BM is most vibration-sensitive at the outer pillar feet and Deiter´s cells at the OHCs and less at the inner pillar cells and IHCs. The structural design suggests that IHCs are anchored on a fairly rigid fundament and that their stereocilia bundles undergo oscillatory movements through shearing forces created at the level of Hensen´s stripe. The TCL layer may play a fundamental role for the assembly and maintenance of the ECM strata. We hypothesize that TCL can monitor ECM homeostasis via a fibronectin/trans-membrane β-integrin receptor system. This pathway may be triggered in cochlear implantation leading to foreign body reaction, inflammation and fibrosis.

Keywords: Basilar membrane; human; collagen; laminin; fibronectin; integrin; immunohistochemistry; electron microscopy, decellularization

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P9. DETAILED ANALYSIS OF HISTONE MODIFICATION IN THE SPIRAL GANGLION OF THE MOUSE COCHLEA

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Objectives: 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 Lys9 was detected in the spiral ganglion cells of the young group but not in those of the aged group. Dimethylated histone H3 Lys9 was detected in the spiral ganglion cells of the aged group but not in those of the young group [1]. In this study, we investigated the detailed modification of histones in the aged cochlea of mice using immunohistochemistry.

Methods: Eight mice (C57BL/6(B6)). Animals were divided to young and aged groups. Cochleae were incubated with fixative, decalcified and embedded in paraffin. After removing paraffin, the sections were incubated with the primary antibody to acetyl-histone H2A Lys5, H2B Lys5, H3 Lys9, H4 Lys8, dimethyl-histone H3 Lys4, Lys9, Lys27, Lys36 or Lys79. Confocal scanning microscopy was performed for observation. Hematoxylin-eosin staining was performed for morphological study using a light microscope.

Results: Acetylated histone H3 Lys9 and H4 Lys8 was detected in the spiral ganglion cells of young group. Dimethylated histone H3 Lysd27 was observed in young group and Lys 9 was detected in the aged group. The degeneration of the spiral ganglion cells was severe in aged group by light microscopy.

Conclusion: Acetylation of H3 Lys9 and H4 Lys8 is known to activate transcription and methylation of H3 Lys9 and Lys 27 suppresses transcription. Histone modification has a critical role in neuro-degeneration. Our findings suggest that epigenetic change participates in the process of presbycusis.

Keywords: epigenetics, histone modification, acetylation, dimethylation

Acknowledgements: Authors thank Ms.Sachiko Saito and Ms.Naoko Minematsu

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P10. ULTRASTRUCTURAL LOCALIZATION OF TMHS IN MOUSE COCHLEAR HAIR CELLS

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Hair cells are the mechanosensors in the cochlea where they convert the mechanical vibrations caused by sound into electrical signals to be carried along the auditory nerve to the brain. Depletion of the stereocilia in hair cells is believed to place a strain on these channels which then open, letting through K+ ions which results in cell depolarisation. The molecular components of the mechanotransduction channels of the hair cells are still under investigation. Recently, mechanotransduction has been shown to be impaired in mice lacking a tetraspan membrane protein, TMHS. Immunofluorescence suggests this protein is found near the stereociliary tips [1]. Here we have utilized post-embedding immunogold labelling and stereological analysis to refine and more precisely localize this information in p5 mice. Our data confirm the suggested stereociliary tip localization but it also shows TMHS in the cytoplasm as well as in the membranes suggesting that during development, this protein is being shipped to the membranes from the manufacturing areas of the cell. It would be interesting to know if this shipment continues into adult life as the turnover time of this protein may be relevant to understanding some kinds of hearing loss. As mutations of the gene for TMHS lead to deafness and because it may show the precise anatomical position of the mechanotransduction channels, we believe that ultrastructural localization could provide further clues to its function. Immunogold postembedding labelling gives a way of doing this, particularly if combined with quantitative analysis of precise localization.

Keywords: Cochlea, hair cell, mechanotransduction, TMHS

Acknowledgements: This work has been sponsored by the NIH USA

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P11. MELATONIN'S EFFECT ON THE INNER EAR CELLS' ULTRASTRUCTURE

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Introduction: Sensorineural hearing loss (SNT) is not only a medical but also a social problem. It is ranked third after cardiovascular diseases and hypertension in frequency of adult population and often leads to disability of patients. Etiological factors of SNT include: ototoxic drug supplementation, cardiovascular diseases, stress, mechanical, acoustic and barotrauma, infectious diseases, effects of noise and others.

Objective: To study the changes in the stria vascularis of the inner ear and melatonin's effect in the model of aminoglycoside antibiotic ototoxicity.

Materials and Methods: The study includes 24 healthy guinea pigs, which were divided into 3 groups (8 animals in each). The animals of the first group were injected with gentamicin sulfate in the ototoxic dose. Animals of the second group received gentamicin sulfate in the same dose and melatonin. Animals of the third group didn't receive any drugs. The stria vascularis was isolated from the cochlea of guinea pigs and exposed to the morphological studies using electron microscopy.

Results: Cells' destruction and disorders in mitochondrial structure (reduction, fragmentation of cristae, dilatation of the intermembrane spaces) were detected in stria vascularis of animals in the 1st group. These effects were less prominent in the 2nd group. Numbers of secretory vacuoles in cells in the 2nd and 3rd groups were practically the same. The secretory activity in the first group was significantly lower. The number of cells rough endoplasmic reticulum increased in the 2nd group, and decreased in the 1st group, compared to the control one.

Conclusions: Disorders in the the stria vascularis cells' ultrastructure in case of aminoglycoside ototoxicity were found. Among animals which received melatonin, these changes were less prominent. Increasing of the cells' secretory activity was regarded as a compensatory and adaptive reaction. Our findings show, that melatonin's otoprotective effect can serve as a basis for its application in the treatment and prevention of SNT.

Keywords: melatonin, inner ear, ototoxicity

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P12. RESIDUAL CRE RECOMBINASE EXPRESSION CORRELATES WITH HEARING LOSS AND REDUCED CONNEXIN 26 TRANSCRIPT LEVEL IN THE CX30$^{\Delta/\Delta}$ MOUSE MODEL

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Mutations in Connexin 26 (Cx26) and connexin 30 (Cx30) cause nonsyndromic hearing loss and deafness (DFNB1) [1]. These gap junction proteins display overlapping expression in the inner ear [2] where they are co-ordinately regulated [3]. In a recent study, a Cx30 knock-out model (Cx30$^{\Delta/\Delta}$) was obtained by crossing *PGK*-Cre mice and mice with Cx30 floxed alleles [4]. Cx30$^{\Delta/\Delta}$ animals were reported to exhibit normal hearing despite complete ablation of Cx30 and 50% reduction in the inner ear level Cx26 protein [4].

Here we report that auditory brainstem responses (ABR) recorded from offsprings obtained from two different Cx30$^{\Delta/\Delta}$ progenies exhibit variable levels of hearing loss. Type 1 offsprings (Cx30$^{\Delta1/\Delta1)}$ presented with hearing thresholds as high as ~75dB SPL (average value in the frequency range 4-32kHz), whereas type 2 (Cx30$^{\Delta2/\Delta2}$) were less affected (~40dB SPL). We quantified the transcript levels of Cx30, Cx26 and Cre recombinase in the inner ear of both offsprings by qPCR. Our preliminary data indicate that Cx30 was completely deleted both in Cx30$^{\Delta1/\Delta1}$ and in Cx30$^{\Delta2/\Delta2}$ mice. However, the level of cochlear Cx26 mRNA in Cx30$^{\Delta1/\Delta1}$ mice was reduced to ~30% of Cx30$^{\Delta2/\Delta2}$ mice. The more pronounced reduction of Cx26 in Cx30$^{\Delta1/\Delta1}$ animals correlated with a ~50% higher expression of residual Cre recombinase with respect to Cx30$^{\Delta2/\Delta2}$. We examined the morphology of inner ear explants from both offsprings at the confocal microscopy level by immunohistochemistry, but failed to detect any overt defect.

Keywords: Hearing, connexins, Cre recombinase

Acknowledgements: Supported by Telethon Italy grant GGP13114 to F.M

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P13. EPS8 REGULATES K^+ CURRENT EXPRESSION IN MOUSE COCHLEAR INNER BUT NOT OUTER HAIR CELLS NOR IN VESTIBULAR TYPE I AND TYPE II HAIR CELLS

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Eps8 is involved in modulating cell signaling and receptor trafficking, via its range of protein interactions. We recently showed that cochlear inner and outer hair cells of *Eps8* knockout (KO) mice, which are born deaf, show shorter stereocilia than wild type (WT) mice [1]. Inner, but not outer, hair cells, moreover, showed an altered expression of the mature array of voltage-dependent K^+ channels, despite the fact that they express similar conductances. Vestibular hair cells of *Eps8* KO mice show shorter stereocilia than normal [2], too. However, it is not known if this is accompanied by some functional defect. We therefore patch-clamp whole-cell recorded the voltage-dependent K^+ currents from vestibular Type I and Type II hair cells of *Eps8* KO and WT mice at different postnatal developmental stages. We found that both vestibular hair cell types from KO mice showed a normal pattern of expression of K^+ currents along with maturation up to the adult age. These results indicate that *Eps8* is a specific regulator of K^+ channel expression in mammalian cochlear inner hair cells. This notion appears particularly important in view of the recent discovery that a nonsense mutation in *EPS8* is responsible for a non-syndromic form of human deafness [3].

Keywords: Eps8; inner hair cell; vestibular hair cells.

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P14. CALCIUM ENTRY INTO STEREOCILIA DRIVES ADAPTATION OF THE MECHANOELECTRICAL TRANSDUCER CURRENT IN MAMMALIAN COCHLEAR HAIR CELLS

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Hair cells within the cochlea transduce sound waves into electrical signals which are then propagated to the auditory processing centres within the brain. More specifically, sound waves cause the movement of stereociliary bundles which are found arranged in height order at the apical surface of hair cells. As the bundles are pushed towards the taller stereocilia this stretches the tip links which connect individual stereocilia and initiates the opening of mechanoelectrical transducer channels. These non-selective cation channels allow predominantly potassium but also importantly calcium to move into the hair cell. In response to a constant stimulus the mechanotransducer current declines as the operating range is shifted towards a higher threshold; this phenomenon, known as adaptation, ensures that the hair cells are working at their maximum sensitivity. In non-mammalian vertebrates adaptation has been shown to be calcium dependent [1], however, the calcium dependence of adaptation in mammalian cochlear hair cells has recently been called into question [2].

To investigate this further, we used the piezo-driven fluid jet [3] to mechanically stimulate the hair bundles of both outer and inner hair cells in an *in vitro* organ of Corti preparation. We found that when the calcium influx into stereocilia of cochlea hair cells was abolished/decreased by either depolarising the cells to near the calcium reversal potential or exposing the cells to the *in vivo* endolymphatic calcium concentration (40 μM), the mechanoelectrical transducer current no longer declined in response to a sustained stimulus. The resting open probability, which is dependent on the degree of adaptation, was also increased under these conditions. Decreasing the amount of free calcium within the cell by increasing the concentration of the intracellular calcium buffer BAPTA from 0.1 mM to either 1mM or 5 mM also abolished all manifestations of adaptation. These findings show that mechanoelectrical transducer current adaptation in mouse auditory hair cells is directly modulated by calcium.

Keywords: Mechanotransduction; Calcium; adaptation; hair cells

Acknowledgements: This work has been sponsored by the Cariplo Foundation.

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P15. SENSORY TRANSDUCTION IN OUTER HAIR CELLS DOES NOT ACCOUNT FOR THE HEARING LOSS CAUSED BY HAPLOINSUFFIENCY OF TRANSCRIPTION FACTOR GATA3

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Gata3 regulates the development of mammalian auditory sensory epithelia and spiral ganglion neurons, and haploinsufficiency leads to ~30dB of hearing loss in mice [1]. Haploinsufficiency accounts for hypoparathyroidism, hearing loss and renal anomaly in human HDR syndrome [2]. Hearing loss in heterozygous gata3 mice is thought to originate primarily from functional defects in the outer hair cells (OHCs; 3), although defects in the inner hair cells (IHCs) may be possible. We examined the electrophysiological profile of hair cells to identify potential functional deficits that could explain the observed hearing loss.

Whole cell voltage clamp recordings were used to measure basolateral membrane and mechanoelectrical transducer currents in OHCs and IHCs from wild type (wt) and gata3 heterozygous (het) mice at various stages between postnatal days P6 and P30. Whole cell current clamp recordings were used to measure voltage responses and resting membrane potential.

We found that there is no difference in the amplitude of K^+ currents measured at 0mV between wt and het mice, both at prehearing stages and mature stages. Resting membrane potentials of OHCs at P22 were also comparable. Maximal mechanoelectrical transducer currents at -121mV in het OHCs at P6 were normal. Interestingly, we observed a ~25% reduction in the number of apical OHCs in het cochleae as early as P4.

In immature P6 IHCs, basolateral membrane currents elicited at 0mV were not significantly different between wt and het mice. However, from P16 onwards, the BK current ($I_{k,f}$)measured at -25mV appeared to be reduced in het IHCs, and this was more pronounced at P28-30. Resting membrane potentials of IHCs at this age were comparable. IHC numbers in het animals were unaffected.

We conclude that the physiological differentiation of OHCs and IHCs is not influenced by gata3 haploinsufficiency and that deficits in the sensory function of OHCs do not explain deafness in gata3 heterozygous mice. Functional deficits could, however, be related to electromotility and/or innervation. The premature degeneration of hair cells is accompanied by degeneration of supporting cells and spiral ganglion neurons, which reflect a more general developmental deficit in heterozygous mice.

Keywords: haploinsufficiency; gata3; hair cells; cochlea; physiology

Acknowledgements: This work has been sponsored by Action on Hearing Loss

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P16. ZEBRAFISH: AN EMERGING MODEL FOR INVESTIGATING HAIR CELL PHYSIOLOGY AND FUNCTION.

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Hair cells convert sound information into neuronal activity with remarkable precision, fidelity and reliability in the inner ear and vestibular system. Although they have been studied extensively in the last few decades, we still do not know how they operate *in vivo*. We used the zebrafish (Larvae: 3.0-5.2 dpf and juvenile: 17-37 dpf) to investigate the biophysical properties and the development of the hair cells *in vivo* using electrophysiological techniques (Whole-cell patch-clamp) [1].

Lateral line hair cells expressed different K^+-currents: $I_{K,D}$, I_A, I_h, $I_{K,Ca}$. The expression of those currents differed depending on the position within the neuromast (centre vs. edge) and with age (larvae vs. juvenile). Of all larval hair cells investigated, 80% showed the following current profile: $I_{K,D}$, $I_{K,Ca}$ and a very small I_A. About 34% of hair cells expressed I_h and a large I_A was only seen in 17% of cells (n=41). However, in juvenile zebrafish we found a larger proportion (about 53% compared to 17% in larvae) expressing the large A-type current. Furthermore, the A-type was almost exclusively expressed in hair cells positioned in the centre of the neuromast, which agrees with previous morphological observations indicating that they have a more mature phenotype compared to cells positioned at the edge of the sensory organ [2, 3]. Hair cells in juvenile zebrafish also show measurable Ca^{2+} currents (–10.5 ± 2.7pA, n=6, at -30mV) and neurotransmitter release (6.6 ± 0.6fF (n=5), which reflects about 150 calcium channels and around 178 vesicles.

This study provides crucial information on the development and function of sensory hair cells of the zebrafish *in vivo*. However, it is important to consider that the majority of hair cells only reach maturity from juvenile stages. Consequently, future *in vivo* studies will require a new approach for zebrafish older than 5.2 dpf.

Keywords: Hair cell, zebrafish, lateral line, synaptic transmission

Acknowledgements: This work has been sponsored by the University of Sheffield and Wellcome Trust

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P17. PROTECTIVE EFFECT OF RESVERATROL AND N-ACETYLCYSTEINE COMBINATION ON KANAMYCIN-INDUCED OTOTOXICITY IN RATS

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Aminoglycoside antibiotics are known to have ototoxic effects and may induce sensorineural hearing loss. Oxidative stress and inflammation have been suggested as key mechanisms. This study investigated the protective effect of a combination of Resveratrol and N-acetylcysteine (NAC), which has antioxidant and cytoprotective properties, against kanamycin-furosemide ototoxicity.

Two month-old male Wistar rats (n=44) were divided into Resveratrol+NAC and control saline groups. Resveratrol, 10mg/kg and NAC, 400mg/kg per day, was given for 5 days by intraperitoneal injection. A gelatine sponge soaked in 75µl of a concentrated solution of kanamycin (200mg/ml) and furosemide (50mg/ml) was applied on the right ear round window by bullostomy in the second day of treatment. Hearing was assessed with auditory brainstem response (ABR) tests, before and 5, 14 and 21 days after the beginning of the treatment. Cochlear samples were taken for molecular and morphological evaluation 5 and 21 days after the beginning of the treatment. PCR reactions were performed to evaluate expression of 84 genes related to oxidative stress and antioxidant defense, using RT2 Prolifer™ PCR array PARN-065Z, and also the expression of pro and anti-inflammatory cytokines with Taqman specific probes (*Il1b, Il4, Il6, Il10, Tnfα, Tgfβ1* and *Foxp3*). Paraffin and frozen cochlear section were employed for Nissl staining, TUNEL, Phalloidin, Myosin VIIa and SOX2 immunohistochemistry.

Functional results indicated that local administration of kanamycin and furosemide induced a moderate to severe threshold shift, especially for high frequencies. Resveratrol and NAC partially protected the inner ear from ototoxic damage, but this beneficial effect did not continue after the end of the treatment. Gross histology from right ears 21 days after surgery showed characteristic lesions after ototoxic insult, mainly disruption of Corti structure and loss of hair cells in the basal cochlear region.

Gene expresion results suggested that treatment with Resveratrol and NAC might protect against ototoxicity via mediating expression of genes responsible for regulating ROS metabolism and oxygen transport, and also expression of proinflammatory cytokines. These results suggested that administration of antioxidant drugs like resveratrol and NAC could be useful to protect from ototoxicity during aminoglycoside therapy.

Keywords: Ototoxicity, oxidation, inflammation, resveratrol, cochlea.

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P18. TARGETING TRANSLATION DURING CELLULAR STRESS: PHARMACOLOGICAL MANIPULATION OF STRESS GRANULE FORMATION IN THE OC-2 CELL LINE

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Stress granules (SGs) are cytoplasmic aggregates of proteins and mRNA formed after exposure to cellular stress. SG-sequestered mRNAs are not translated, whilst production of essential proteins continues. Dysregulation of SG-formation has recently been linked to age-related disease in support of the hypothesis that SGs are critical for cell survival during stress.

In the present study we used inner ear-derived OC-2 cells to investigate SG formation and regulation. We evaluated two compounds: pp242, an inhibitor of mTOR shown to prevent SG formation in HeLa cells [1] and (-)-9-hydroxamate, which disrupts the eIF4F translation complex resulting in SG formation [2]. OC-2 cells were assessed using immunofluorescence for two SG-markers, TIA-1 and Caprin-1 and SG-quantification using ImageJ (NIH). Exposing OC-2 cells to heat shock (43^{o}C, for 1 hour) resulted in a significant increase in the numbers of TIA-1/Caprin-1-positive SGs. Pre-incubation with pp242 (2µM, for 24hours) prior to heat shock significantly reduced the number of SGs per cell. SG-size was also reduced after pp242 treatment (~2.5x smaller than vehicle-treated controls). Incubation of unstressed OC-2 cells with (-)-9-hydroxamate for 2, 4 or 8 hours resulted in SG formation. After 8 hours, the number and average size of the SGs was similar to that observed during heat shock stress. In addition to assessing these manipulators of SG-assembly, we have developed an immuno-RNA-FISH protocol allowing us to determine the specific nature of the RNAs sequestered to TIA-1 or Caprin-1-positive SGs. We confirmed co-localization of polyA-mRNA (using Cy3-labelled probes) with TIA-1 and are currently assessing sequence-specific probes to localise specific RNAs to SGs.

Having demonstrated that we can manipulate, quantify and characterise the SGs that form in OC-2 cells we now aim to evaluate the effect of SG manipulation on hair cell survival and to clarify the role of SG in the inner ear.

Keywords: Stress granules, RNA, heat-shock.

Acknowledgements: ACG is funded by an Action on Hearing Loss PhD studentship.

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P19. MECHANISM OF PROTECTIVE EFFECT OF LOW-LEVEL LASER THERAPY ON HEI-OC1 CELLS AFTER GENTAMICIN-INDUCED OTOTOXICITY

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Recently low level laser irradiation has shown its possible role in *in vivo* inner ear damage models. But, how laser irradiation is acting on inner ear hair cells and auditory nerves after various insults is yet to be elucidated. We investigated how LLL irradiation affects hair cell (HEI-OC1 cell line) survival and adenosine triphosphate (ATP) production and reactive oxygen species (ROS) production after aminoglycoside induced ototoxic damage.

Methods: The HEI-OC1 cells used in this study were maintained in DMEM with 10% FBS at 33℃ under 5% CO2 in air. The cells were incubated with two fold diluted gentamicin (GM, Sigma, US) for 24h, and the dose-dependent effects of GM were measured using an MTT assay. Based on MTT assay results as a function of GM concentration, two different concentrations (6.6 and 13.1mM) were chosen for further analysis to see the effects of LLL irradiation on ototoxicity with given concentration. The ATP assay and the reactive oxygen species (ROS) production in HEI-OC1 cells were measured immediately, 1 hour and 2 hours after laser irradiation. The ATP concentration was measured using an ATP assay kit (Colorimetric assay, Abcam, UK) with the OD at 540nm. The ROS was stained using fluorescent dye, H2DCFDA (Invitrogen, US) and observed under confocal microscope (Carl Zeiss, Germany).

Results: Reduced ATP production was observed with 13.1mM compared to control. LLL irradiation (without GM) increased ATP production compared to control. With GM concentration of 13.1mM, LLL augmented ATP production measured immediately, 1hour and 2hours after 15 minutes irradiation. ROS generation was increased with both GM concentrations (6.6, 13.1mM) showing decrease from 5 hours after GM application. Like ATP production, with 13.1mM GM, LLL reduced ROS production measured immediately, 1hour and 2hours after 15 minutes irradiation. But, with 6.6 mM GM, LLL didn't show obvious effects on ROS production.

Conclusion: LLL irradiation facilitates auditory cell line survival increasing ATP production and reducing ROS generation.

Keywords: laser therapy; ATP; gentamicin

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P20. *IN VIVO* PROTECTIVE EFFECT OF QTER AGAINST STYRENE OTOTOXICITY AND ACOUSTIC TRAUMA: A FUNCTIONAL AND MORPHOLOGICAL STUDY IN A MODEL OF NOISE INDUCED HEARING LOSS (NIHL).

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Objectives: 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 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. A progressive increase of reactive oxygen species (ROS) and a redox defence imbalance have been demonstrated to play a significant role in noise induced hearing loss (NIHL) as well as in styrene induced ototoxicity. In this study we evaluated the synergistic effect between exposure to styrene and chronic acoustic trauma on the pattern of cochlear cell damage and the protective effect of the water soluble Coenzyme Q_{10} (Q-ter), molecule known to have antioxidant properties.

Materials and Methods: Wistar rats were exposed to styrene by gavage (400mg/Kg) and to chronic noise exposure (97dB SPL, 10kHZ, 60min/day). The animals were exposed to an acoustic trauma and to styrene for 3 weeks, 5 consecutive days/week. Two groups were simultaneously treated with the antioxidant Q-ter (100mg/kg) over the same period. The induced hearing loss in treated groups was functionally assessed by auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs). We also studied immunostaining for redox imbalance in the outer hair cells, supporting cells, spiral ganglion neurons and stria vascularis.

Results: Our results demonstrate that hearing loss and cochlear damage by styrene exposure are increased by the concomitant exposure to noise and Q-ter treatment can reduce the cochlear damage caused by chronic exposure to noise and styrene.

Conclusion: Based on our preliminary results, we speculate that the association between noise and organic solvent exposure in industrial working represents a risk factor for workers' health and that antioxidant treatment provides a promising preventive approach.

Acknowledgements: This work has been sponsored by PRIN GRANT, Italian Department for Research, "*Fondi di Ateneo*" Catholic University

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P21. THE HARWELL AGING SCREEN IDENTIFIES NOVEL MOUSE MODELS OF EARLY-ONSET AND AGE-RELATED HEARING LOSS

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Age-related hearing loss (ARHL), also known as presbycusis, is a significant health and social burden on the population and is one of the four most common chronic health conditions experienced by the elderly. ARHL is a complex disease that is not fully understood, but the onset and progression is known to be influenced by environmental factors (e.g. noise) and genetic susceptibility. To date there has been little progress in determining the loci involved, although there is considerable effort being undertaken in GWA studies in Europe. Fully elucidating the mechanisms underlying ARHL is of great benefit, as it will allow the development of therapeutic strategies to ameliorate hearing deterioration. However, the study of ARHL in humans is complicated due to genetic heterogeneity within the population and age of onset. At MRC Harwell we are utilizing a large-scale ENU mutagenesis screen to identify mouse models of age-related disease. Large G3 pedigrees are bred and enter a phenotyping pipeline comprising recurrent assessment across a wide range of disease areas including, diabetes, metabolism, neurobehaviour, bone, renal, cardiac, and sensorineural. The Deafness Models and Mechanisms team is taking advantage of this screen to identify models of ARHL, employing recurrent Clickbox and Auditory-Evoked Brainstem Response phenotyping.

As of April 2014, ~130 pedigrees have completed auditory screening, identifying 25 (~19%) with hearing loss phenotypes. Of these, 18 pedigrees display early-onset hearing loss as evidenced by elevated hearing thresholds by 3 months of age. The remaining 7 pedigrees exhibit late-onset progressive hearing loss with elevated hearing thresholds evident from 6 months onwards. For all these pedigrees, genome-wide mapping and whole-genome sequencing (WGS) studies are being undertaken. So far, six of the early-onset and four of the late-onset hearing loss models map to novel deafness loci, and WGS is enabling the identification of non-synonymous lesions within these intervals. Studies to confirm these causative lesions and to relate mutant protein to phenotype are ongoing.

The Harwell Aging Screen is producing pedigrees with interesting early- and late-onset auditory phenotypes. Investigation of these, and as yet unidentified pedigrees, promises to increase our understanding of the genetics underlying hearing and its age-related decline.

Keywords: Aging, ENU mutagenesis, mouse models, presbycusis

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P22. ROLE OF *ACSL4* AND LIPID METABOLISM IN HIGH FREQUENCY HEARING LOSS

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Hearing impairment is a heterogeneous disorder that has both genetic and environmental etiology. In humans, a frequent pattern of hearing loss is high-frequency loss, and in progressive hearing loss it is often the high frequencies that are lost first. This project is focused on *Acsl4* knockout mouse which is one of the first mouse mutants with high frequency hearing impairment. The protein encoded by *Acsl4* is involved in lipid metabolism and has a pivotal role in arachidonic acid metabolism. We aim to elucidate the molecular, cellular and physiological basis of high frequency hearing impairment in *Acsl4* mice; to build up the Acsl4 network of functional and regulatory interactions in order to clarify its role and the role of lipid metabolism in deafness; and finally to determine whether manipulation of lipid metabolism through small molecules, which regulate the Acsl4 pathway, can affect hearing impairment. Performing immunohistochemistry on sections of the inner ear revealed that Acsl4 is expressed in the organ of Corti, spiral ganglion and stria vascularis. We have observed low transmission of the mutated allele in the*Acsl4* mouse colony, suggesting an effect on viability. Therefore, we plan to generate a conditional knockout colony for *Acsl4* in order to delete this gene only in the inner ear. Preliminary data on the Acsl4 network have been obtained initially studying the literature available and later using Ingenuity Pathway Analysis.The investigation of the Acsl4 network can highlight other genes that may be involved in hearing impairment and identify alternative targets for drug manipulation using small molecules.

Keywords: *Acsl4*, high frequency hearing loss, network, conditional knockout mouse

Acknowledgements: This work has been sponsored by Action on Hearing Loss

P23. AGE-RELATED HEARING LOSS IS AFFECTED BY PERIPHERAL IMMUNE SYSTEM

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We provide evidence to support our hypothesis that age-related hearing loss (ARHL) is associated with chronic neurodegenerative changes in the auditory system which respond to systemic inflammation. This is supported by changes in the morphology and priming of microglia observed exclusively in the auditory system of older mice with age-related hearing loss, compared to younger mice with normal hearing.

Ageing leads to changes in the peripheral immune system that manifest as inappropriate immune responses. In some older individuals, this may result in a state of low grade chronic inflammation known as inflammaging. Inflammaging contributes to progression of a number of age-related diseases including frailty and dementia and may play a role in ARHL.

We performed a cross-sectional analysis using 320 subjects aged between 60 and 89 years using a cohort from the National Study of Hearing. We have identified a positive association between the severity of hearing loss and inflammatory level, measured by white blood cell count (WBC). The association between worsening hearing loss and high WBC was strongest for subjects aged 75 years and over. When subjects were stratified into tertiles according to their WBC, subjects with higher WBC had significantly poorer hearing compared to the subjects with lower WBC.

In an ongoing longitudinal study, we are measuring changes in the hearing level of older people and their inflammatory statuses over a 3-year period to identify subjects at the greatest risk of worsening hearing loss.

The findings suggest there is an association between systemic inflammation and age-related hearing loss, which may be potentially amenable to lifestyle and pharmacological interventions.

Keywords: Age-related hearing loss, white blood cell count, inflammaging.

Acknowledgements: This work has been sponsored by Netherlands organization for international cooperation in higher education.

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P24. KINESIN LIGHT CHAIN-2 (*Klc2*) :A NOVEL GENE INVOLVED IN PROGRESSIVE HEARING LOSS

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Kinesin light chain-2 (Klc2) is one of a 4 member family of kinesin light chain proteins and forms part of the kinesin protein complex which is responsible for ATP-dependent movement of cellular organelles and vesicles along microtubule networks and in cell division [1]. Mice carrying a knockout allele of the *Klc2* gene, generated by the Wellcome Trust Sanger Institute Mouse Genetics Project [2] were subject to a standardised battery of phenotypic assessment [3]. When auditory brainstem responses (ABRs) were recorded, mice homozygous for the *Klc2*$^{tm1e(EUCOMM)Wtsi}$ allele were found to have a variable moderate-severe degree of hearing impairment at 14 weeks old. In a separate cohort of mice, we have used repeated ABR recordings of the same mice to estimate thresholds for click stimuli and pure-tones ranging from 3-42kHz, at ages 4, 8, 14 and 26 weeks. Mice were anaesthestised using ketamine and xylazine before recordings and recovered with the help of atipamezole. At each age, wildtype mice and mice heterozygous for the *Klc2*$^{tm1e(EUCOMM)Wtsi}$ allele were found to have comparable hearing sensitivity. However, mice that were homozygous for the knockout allele were found to carry a hearing impairment from as young as 4 weeks old. At this age, thresholds were consistently elevated in the homozygotes especially at low-middle frequencies (6-24kHz). Whilst all homozygote mice were moderately hearing impaired at 4 weeks old, the impairment progressed at different rates, through 8 and 14 weeks old, becoming severe in all mice tested by the age of 26 weeks. We are continuing this study with further physiological investigations alongside structural anatomy and gene expression analyses of this interesting mouse model of progressive hearing loss.

Keywords: Mouse mutant; auditory brainstem responses; progressive hearing loss; kinesin light chain;

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P25. LONG-TERM EFFECT OF OMEGA-3 FATTY ACID SUPPLEMENTATION IN A MODEL OF AGE-RELATED HEARING LOSS

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Omega-3 polyunsaturated fatty acids (PUFAs) are essential dietary nutrients well-known for their beneficial effects on inflammation, oxidative stress and insulin sensitivity, among others [1]. Insufficient levels of docosahexaenoic acid are associated with age-related neurological and vascular disorders, and with human age-related hearing loss (ARHL) [2]. There is an inverse relationship between high plasma levels of PUFAs and ARHL [3], whereas high levels of plasma homocysteine (pHcy) are associated with hearing loss [4]. Here we used C57BL/6J mice and long-term omega-3 supplementation to evaluate the impact on hearing, Hcy levels, oxidative stress and inflammation.
Two-month old mice were fed either control or omega-3 supplemented diets for 10 months. Hearing capacity was assessed monthly by ABR and DPOAE threshold analyses. Blood and tissue samples were taken to measure pHcy, fatty acids and folate concentrations by HPLC. Cochlear morphology was evaluated with Cresyl violet and immunohistochemistry. Biomarkers of inflammation and oxidative stress were analyzed by Western blotting and RT-qPCR.

After 10 months of treatment, the control group showed significantly higher ABR hearing thresholds (25 dB SPL in average) and lower DPOAE amplitude in mid-high frequencies, when compared to the omega-3 group. No histological differences were found. Higher pHcy levels ($p=0.13$), together with decreased serum folate concentrations ($p<0.05$), were detected in control vs. omega-3 supplemented mice. The impact of diet supplementation on inflammatory and redox biomarkers will be discussed.

The results obtained suggest that omega 3 supplementation may have a protective role on ARHL.

Keywords: Presbyacusis; dietary supplementation; homocysteine; inflammation; oxidative stress.

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P26. WHITE MATTER INTEGRITY CHANGES IN AGE-RELATED HEARING LOSS: TRACT BASED SPATIAL STATISTICS STUDY

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Background: Hearing loss disorder is considered as the third common most chronic disease after diabetes and hypertension, affecting more than 250 million people around the world. Age-related hearing loss (presbycusis) is a sensorneural hearing loss that affects the brain structure and function because of the changing of auditory input to some parts of the brain. The aim of this study is to investigate the white matter integrity in age-related hearing loss.

Methods and material: Diffusion tensor imaging (DTI) was applied on 42 adults (mean age 45 (SD 10.48) years, 26 female) recruited to take part in the study. Routine clinical hearing tests were performed for each individual to identify the hearing levels: 21 normal hearers and 21 participants with hearing loss. Tract based spatial statistics (TBSS) was applied to investigate the correlation between white matter voxel differences with high fractional anisotropy (FA) and participants' hearing levels.

Results: TBSS revealed significant changes throughout the white matter in fractional anisotropy (FA). Participants with hearing loss show significant increase in the white matter voxel-wise compared to normal hearers in the right primary auditory cortex, left secondary auditory cortex, right visual cortex (BA18) and right primary motor cortex (BA4a). On the other hand, participants with hearing loss show less white matter voxel-wise concentration in the right pre-motor cortex (BA6), frontal lobe and brain stem.

Conclusion, our results demonstrate that age-related hearing loss can affect auditory and non-auditory brain regions mentioned in the results section.

Keywords: Hearing loss; white matter; diffusion tensor imaging (DTI); fractional anisotropy (FA); tract based spatial statistics (TBSS) and auditory cortex (AC).

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P27. DIFFERENCE OF AUTOANTIBODY BETWEEN MENIERE'S DISEASE AND BILATERAL SUDDEN HEARING LOSS: IS THERE A DIFFERENT AUTOIMMUNITY MECHANISM?

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This study was performed to investigate the difference of autoantibodies and their target tissues between Ménière's disease and bilateral sudden hearing loss.Ten patients with definite Ménière's disease, nine patients with bilateral sudden hearing loss, and ten controls were enrolled in this study. To identify serum circulating autoantibodies, Protoarray® analysis with patients' and controls' sera were performed. To detect Antigen-antibody reaction between circulating autoantibody and each cochlear and vestibular tissue, western blotting using mouse inner ear and patients' sera was performed. In addition, differences in clinical features according to the presence of antigen-antibody reaction were investigated.Eighteen and twelve proteins had more than 2-fold greater signal intensity in the Protoarray® analysis of Ménière's disease and bilateral sudden hearing loss, respectively. The signal intensity of eight and two proteins was more than 10-fold higher in the patients than in the controls in each disease entity (Ménière's disease and sudden hearing loss). None of them were commonly found in both diseases. Western blot analysis showed multiple Ag-Ab reaction between patients' sera and mouse inner ear tissues, and the band distribution was different between Ménière's disease and bilateral sudden hearing loss. Vertigo spells were more severe and treatment response was poorer in the Ménière's disease patients with the presence of Ag-Ab reaction, whereas hearing threshold was not significantly different between the sudden hearing loss patients with and without Ag-Ab reaction. In conclusion, the main pathologic mechanism of Ménière's disease and bilateral sudden hearing loss can be autoimmunity. There were multiple autoantibodies for each disease but their target antigen could be different between Ménière's disease and bilateral sudden hearing loss. The autoimmune reaction was correlated with the severity of vertigo symptoms and represented poor response to conventional medical treatment.

Keywords: autoimmunity; bilateral; Ménière's disease; sudden sensorineural hearing loss.

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P28. VESTIBULAR ANATOMY MODELLING AND SHAPE VARIATION ANALYSIS IN TEN FULLY SEGMENTED HUMAN TEMPORAL BONES USING MICROCT

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The project aims at obtaining detailed models of morphological variations of the inner ear anatomy based on the results of the segmentation of microCT scans. The segmentation of these scans will help in the analysis of principal morphological variations of the vestibular organ to develop an optimized design for the future development of vestibular electrodes for human implants. The analysis of variations of the geometry of bony and membranous labyrinth, location and dimension of sensory structures and course of nerve fibres including circumjacent nerves relevant for possible electrical stimulation sites provides data which shall help in electrode prototype simulation. The segmentation of relevant structures is being performed using a combinatorial approach utilizing manual as well as semi-automatic segmentation procedures using the AMIRA® software to create a 3D mesh representation of the segmented structures in order to calculate the anatomical variations.
Adult human temporal bones (n=10) were excised from fresh cadavers with no known hearing dysfunction. Cadavers had been donated to the department of anatomy. They were first post fixed in Osmium tetroxide in order to enhance the contrast in the µCT and using defined scanning parameters, were then scanned and placed in 10% EDTA to decalcify over a period of 2 months and then rescanned. The final scans both before and after decalcification were then registered and then segmented. The registration of the scans also provides an insight into the amount of shrinkage occurring during the decalcification process.

Keywords: microCT; simulation; human inner ear; variability shape modelling.

Acknowledgements: This project is kindly supported by Amt der Tiroler Landesregierung Technologieförderungsprogramm, Förderung von Wissenschaft, Forschung und Entwicklung (Programm K-Regio).

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P29. A CASE OF A 7-YEAR OLD BOY WITH SENSORINEURAL HEARING LOSS, VERTIGO AND TINNITUS

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Introduction: Menière's disease is a chronic condition of fluctuating sensorineural hearing loss, tinnitus and recurring vertigo. It was first described one hundred and fifty-three years ago and to date there is no definite treatment or guidelines of management. Commonly this condition mainly affects patients in their fifth and sixth decades of life [1]. Menière's disease is a rare cause of vertigo in children [2-4].

Case: A 7 year old male presented with sudden onset deterioration of left sided hearing. Since the age of 4 he was noted to have impaired hearing in the left ear in which he had a hearing aid fitted eighteen months prior to this episode. Also at 4 years of age, the patient was diagnosed with delayed speech and language development and referred for audiological assessment. During this presenting episode, he was also feeling dizzy and was not his usual self. He was playing in a sitting down position with minimal movement. On examination, there was a positive Romberg's with the patient falling backwards. There were no other neurological signs. A repeat audiogram showed significant deterioration in hearing in the left ear. The audiogram for the right ear was within normal. A CT of the brain and an MRI were performed and no abnormalities were noted. He was started on a 40mg prednisolone dose and kept in hospital for observation. The patient improved and the sensation of unsteadiness (vertigo) resolved. Oral steroids were prescribed for fifteen days. His hearing loss stabilised and on review there was no further worsening. During reviews, the patient complained of having a sound in his left ear. This was probably tinnitus.

Conclusion: According to the criteria defined by the American Academy of Otolaryngology – Head and Neck Surgery in 1995, this patient has "Probable Menière's disease" with one definite episode of vertigo, tinnitus and audiometrically defined hearing loss [5]. The patient in this case is still being monitored.

Keywords: children; Menière's disease; oral steroids; unilateral sensorineural hearing loss.

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P30. 3D CULTURE OF HUMAN VESTIBULAR NERVE

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There is currently a great interest to regenerate inner ear spiral ganglion (SG) nerves to reduce or close the nerve/electrode gap in cochlear implantation (CI) to improve frequency and harmonic pitch resolution [1–3]. Although animal studies give relevant information, interspecies differences prevail [4] motivating the use of human models. Research involving human SG is rare due to difficulties in obtaining fresh tissue, situated deep in hard bone.

Here, we used the vestibular ganglion (Scarpas ganglion, VG) as a source for human studies. The regenerative capacity of the vestibular organ has been demonstrated in both rodents and humans [5, 6]. Human VG can be collected during vestibular schwannoma surgery using the translabyrinthine approach. We cultured the VG in a 3D laminin-based matrix (Matrigel™)and analyzed the re-sprouting occurring within 6 days using time-lapse video microscopy. After culture, the neural explants were cryosectioned for immuno/histological verification. Surgically removed human vestibular ganglia may prove useful as an additional source for the study of the eighth cranial nerve in man.

Keywords: Neural regeneration; inner ear; vestibular neurons.

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P31. AUTHENTIC BIOPHYSICAL PROPERTIES OF $I_{K,L}$ IN MAMMALIAN VESTIBULAR TYPE I HAIR CELLS REVEALED AFTER CALYX REMOVAL

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Mammalian vestibular epithelia are characterized by the expression of two different sensory cells named Type I and Type II hair cells. Different from Type II cells, the basolateral membrane of Type I cells shows two distinguishing properties: it is entirely wrapped by a single nerve terminal, called the calyx, and it expresses a low-voltage-activated outward rectifying K^+ current, $I_{K,L}$ which is responsible for the much lower input resistance at rest as compared to Type II hair cells. Strikingly, the principal biophysical features of $I_{K,L}$ have not been unequivocally described so far. In fact, its voltage- and time-dependent properties have been reported to vary widely not only among Type I hair cells, but also in the same cell over time. On the basis of our electrophysiological recordings from in situ and dissociated mouse crista Type I cells, we showed that the large variability in $I_{K,L}$ properties is attributable to different degrees of K^+ accumulation in the narrow space of the synaptic cleft between the hair cell and the residual calyx. Hence, in order to obtain a genuine description of $I_{K,L}$, we developed a procedure to refine the removal of the calyx prior to patching. Only once the calyx had been effectively removed could we show that the biophysical properties of $I_{K,L}$ are in fact consistent among cells, and quite constant during the recordings. In particular, $I_{K,L}$ showed significantly slower deactivation kinetics (time constant: ~1s at -80mV), a less negative voltage activation (half-activation voltage: -69 mV) and a steeper voltage dependence (S: 3.72mV) than previously reported. In conclusion, our data provide for the first time a complete description of the authentic biophysical properties of $I_{K,L}$. As a corollary, we demonstrate that the calyx represents a strong barrier to K^+ diffusion out of the synaptic cleft, which provides a direct way to depolarize either the hair cells and their calyx.

Keywords: $I_{K,L}$; nerve calyx; type I hair cells; vestibular;

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P32. AUTOIMMUNITY AS A CANDIDATE FOR THE ETIOPATHOGENESIS OF MENIERE'S DISEASE: DETECTION OF AUTOIMMUNE REACTIONS AND ITS CLINICAL CORRELATION WITH SYMPTOMS.

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Ménière's disease is an inner ear disorder that can manifest as fluctuating vertigo, sensorineural hearing loss, tinnitus, and aural fullness. However, the pathologic mechanism of Ménière's disease is still unclear. In this study, we evaluated autoimmunity as a potential cause of Ménière's disease. In addition we tried to find useful biomarker candidates for diagnosis and clinical correlation between autoimmune reaction and symptoms. We investigated the protein composition of human inner ear fluid using liquid column mass spectrometry, the autoimmune reaction between circulating autoantibodies in patient serum and multiple antigens using the Protoarray® system, the immune reaction between patient serum and mouse inner ear tissues using western blot analysis, and the association between the occurrence of the immune reaction and clinical features of the patients. Nine proteins, including immunoglobulin and its variants and interferon regulatory factor 7, were found only in the inner ear fluid of patients with Ménière's disease. Enhanced immune reactions with 18 candidate antigens were detected in patients with Ménière's disease in Protoarray® analysis; levels of 8 of these antigens were more than 10-fold higher in patients than in controls. Antigen-antibody reactions between mouse inner ear proteins with molecular weights of 23-48kDa and 63-75kDa and patient sera were detected in 8 patients. The number of vertigo spells experienced by these 8 patients was higher and their response to conventional medical treatment was poorer. These findings suggest that autoimmunity could be one of the pathologic mechanisms behind Ménière's disease. Multiple autoantibodies and antigens may be involved in the autoimmune reaction and their antigen-antibody reactions were related with the severity of vertigo symptom in patients. Specific antigens that caused immune reactions with patient's serum in Protoarray® analysis can be candidates for the diagnostic biomarkers of Ménière's disease.

Keywords: autoantibody; autoimmunity; endolymphatic sac; Ménière's disease,

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P33. GENE EXPRESSION ANALYSIS OF THE VESTIBULAR ORGAN OF *IGF1*-/- AND *IGF1*+/+ MICE USING AFFYMETRIX WHOLE TRANSCRIPT ARRAYS.

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Insulin-like growth factor 1 (IGF-1) has a central role in mammalian hearing and hearing loss. The auditory and vestibular systems form the inner ear and have a common developmental origin. During chicken early development, IGF-1 modulates neurogenesis of the cochleovestibular ganglion but no further studies have been conducted to explore the potential role of IGF-1 in the vestibular system. In this study we have compared the whole transcriptome of the vestibular organ from wild type and Igf1-/- null mice at different developmental times. RNA was prepared from E18.5, P15 and P90 vestibular organs of Igf1-/- and Igf1+/+ mice and the transcriptome analyzed in triplicates using Affymetrix® Mouse Gene 1.1 ST Array Plates. These plates are whole-transcript arrays that include probes to measure both messenger (mRNA) and long intergenic non-coding RNA transcripts (lincRNA), with a coverage of over 28 thousand coding transcripts and over seven thousand non-coding transcripts. To describe the number of false positives and quantify uncertainty, the data were analysed and then compared using two different methods VSN-RMA [1] and mmBGX [2]. The morphology of the vestibular organ of both genotypes did not show striking cellular differences. No evident alterations were observed in the vestibular sensory areas of the null mice. Accordingly, these mice did not show a dramatic vestibular phenotype. Finally, functional analysis was carried out on genes that were differentially expressed between genotypes and across time to define new pathways that are involved in the development of the vestibular organ as well as pathways that maybe affected by the lack of IGF-1.

Keywords: Insulin like growth factor 1; vestibular system; transcriptomics; Affymetrix mouse gene 1.1. ST arrays.

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P34. A REPORTER MOUSE AND CELL LINE FOR THE TRANSCRIPTION FACTOR GATA3 IN THE INNER EAR

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The zinc finger transcription factor gata3 is essential for inner ear development and it is expressed in most embryonic components of the auditory system [1]. Haploinsufficiency causes hypoparathyroidism, deafness and renal anomaly (HDR) Syndrome [2]. Understanding the function of early regulators of sensory development provides clues to targets that are important for regenerative therapy. Gata3 is relevant because it not only regulates critical signaling pathways in sensory cells and neurons but also augments the ability of the bHLH transcription factor Atoh1 to stimulate hair cell differentiation [3]. We sought a method of generating gata3-eGFP reporter cell lines to enable high throughput screens for genetic and extrinsic regulators of gata3. We obtained a gata3-eGFP BAC transgenic mouse line from Dr Stavros Malas at the Cyprus Institute of Neurology and Genetics and characterized expression in the inner ear from embryonic day E10.5. We then crossed it with heterozygous Immortomice, which carry a temperature-sensitive variant of the immortalising T-antigen under the control of a gamma-interferon-sensitive promoter [4]. Otocysts were dissected from E10.5 embryos, teased apart mechanically and cultured under 'permissive' conditions for T-antigen expression, namely in Minimal Essential Medium with 10% fetal calf serum and gamma-interferon at 33°C. Cells were immunolabeled for the T-antigen and positive cultures were passaged twice, dissociated and either cloned by limiting dilution or analysed by Fluorescence-Activated Cell Sorting prior to plating the highest expressing cells into 96-well culture plates. eGFP was observed in expected tissues in the nervous system, eye, kidney, gut and hair follicles. In E10.5 embryos it recapitulated expression in the otic epithelium and in the efferent innervation from rhombomere 4. It was also present in the supporting cells and spiral ganglion neurons of organs of Corti from 3-week old animals. Cell lines expressed T-antigen and relatively low levels of eGFP, compared to those seen *in vivo*. Expression may be lower due to the fact that cells were heterozygous or it may reflect changes related to lack of appropriate environmental cues *in vitro*. In future work the lines will be characterized with respect to tissue and cell type and subjected to an RNAi screen.

Keywords: cell line; development; eGFP; gata3, inner ear; mouse; otocyst.

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P35. OTOLITH TETHERING IN THE ZEBRAFISH INNER EAR

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Otoliths ('ear stones') are biomineralised structures important for both hearing and balance in fish. Their counterparts in the mammalian ear, otoconia, have a primarily vestibular function. Otoliths and otoconia form over sensory maculae in the inner ear, and are held in place by the otolithic membrane, a gelatinous matrix that provides a physical coupling between the otolith and its associated sensory epithelium. The composition of this membrane and the functions of its constituents have not previously been described in zebrafish. We have identified two components of the zebrafish otolithic membrane, and propose that there are at least two stages of otolith tethering in the zebrafish ear: initiation and maintenance. At the very earliest stages, otolith precursor particles adhere directly to the tips of hair cell kinocilia, a process that fails to occur in the *einstein* (*eis*) mutant. We have identified the gene disrupted in *eis* as *otogelin* by whole-genome sequencing analysis; mutations in the human *OTOG* gene have recently been identified as causative for deafness and vestibular dysfunction [1]. Otogelin, a glycoprotein related to mucins, is thus a candidate for the 'otolith precursor-binding factor' postulated in previous studies [2,3,4]. During later larval stages, otolith tethering to the saccular macula is dependent on *tectorin a* (*tecta*) function, which is disrupted in the *rolling stones* (*rst*) mutant. The *tecta* gene codes for Alpha-Tectorin, a glycoprotein that is a known component of the mammalian otoconial membrane, and a major constituent of the tectorial membrane in the mammalian cochlea [5]. Mutations in the human *TECTA* gene can cause either dominant (DFNA8/12) or recessive (DFNB21) forms of deafness [6]. Both the *eis* and *rst* mutant lines are homozygous adult viable, and show hyperactivity consistent with vestibular defects in behavioural testing. Our findings indicate that the composition of extracellular otic membranes is highly conserved between mammals and fish, reinforcing the view that the zebrafish is an excellent model system for the study of deafness and vestibular disease.

Keywords: otolith; otolithic membrane; vestibular system; zebrafish

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P36. THE QUANTITATIVE ANALYSIS OF AQUAPORIN EXPRESSION LEVELS IN THE INNER EAR

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Objectives: Aquaporins (AQPs) are water channel proteins, which are considered to be major candidates for water regulation in the inner ear. The exact function of AQP in the inner ear remains unclear although localizations of AQPs are being reported. To elucidate functions of AQPs in the inner ear, we measured expression levels of AQP subtypes in the developing and maturating inner ear using quantitative reverse transcription polymerase chain reaction (qRT-PCR). We also evaluated localization of several subtypes by immunohistochemistry (IHC) to confirm the results of the qRT-PCR.

Methods: Whole inner ears were dissected from C57BL6/J mice at embryonic days (E) 10, 13 and 18, and at postnatal days (P) 3, 10 and 21. Total RNA was extracted and the expression level of each AQP subtype was determined by qRT-PCR. Frozen sections of inner ears from E10 to P21 were subjected to IHC using anti-AQP2, 4 and 5 antibodies (Millipore) and anti-AQP6 and 9 antibodies (Abcam).

Results: The expression level of each AQP subtype changed in 4 patterns from E10 to P21; constant (AQP4 and 11), increasing (AQP0, 1 and 9), peaking at E18 (AQP2, 3, 5 and 7), and decreasing (AQP6, 8 and 12). Notably, AQP9 expression levels increased about 70-fold from P3 to P21. IHC revealed that expression sites of AQP2, 4, 6 and 9 accumulate subtype-specific sites as development and maturation proceeds. The expression of AQP5 expanded from the basal turn to the apical turn during development (E13 – E18) as well as accumulating from lateral wall to basilar membrane and outer sulcus cells.

Conclusions: Subtypes with increasing expression levels (e.g. AQP9) are considered to be involved in the establishment of inner ear functions. Subtypes with decreasing expression levels (e.g. AQP6) suggest important roles at developmental stages. From the results of IHC, subtypes harboring a peak at E18 may represent increase during development and decrease by accumulation after birth. Expansion of AQP5 localization during development is compatible with the establishment of the potassium recycling mechanism considering that outer sulcus cells are contained in the pathway of recycling.

Keywords: Aquaporin; immunohistochemistry; inner ear, RT-PCR

P37. MODELLING AXIAL POLARITY IN THE DEVELOPING ZEBRAFISH EAR

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The developing zebrafish ear, along with the inner ear of all jawed vertebrates, must undergo a symmetry-breaking event as it progresses from an ectodermal placode to a highly asymmetric otic vesicle. In zebrafish, modulation of Fgf and Hh signalling between 14 and 18 hours post-fertilisation (hpf) has been shown to result in the duplication of anterior or posterior domains of the otic vesicle [1,2]. This makes the asymmetric localisation of morphogen sources across the anterior-posterior axis an attractive model to explain this symmetry-breaking event. We are studying the genetic regulatory networks that are responsible for the spatial patterning of the otic anterior-posterior (AP) axis and the extent to which various morphogen sources localised in the surrounding tissue influence these networks. To achieve this, we are using a transgenic line driving expression of the photo-convertible protein Kaede within the otic placode. We hope to utilise this to isolate and sequence the transcriptome of different regions of the otic placode at various developmental time points. This should give new insight into the genes that regulate otic AP axis patterning and their temporal dynamics.

We have also begun characterising a number of genes that show expression within the posterior otic domain before 18 hpf, to identify potential gene networks that may specify the posterior domain, which is currently poorly understood. We aim to bring these data together with a mathematical modelling approach to develop a greater overall understanding of the events that pattern early otic tissue.

Keywords: zebrafish; anterior-posterior; development; photo-conversion; gene networks.

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P38. REGULATION OF THE ATOH1 ENHANCER IN THE INNER EAR: A LINK BETWEEN DEVELOPMENT AND REGENERATION OF HAIR CELLS

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The proneural gene Atoh1 is crucial for the development and regeneration of hair cells (HCs) [1, 4]. Atoh1 expression is recapitulated by an enhancer situated 3.5kb downstream of the coding region [1]. Notch signaling plays an important role in ear development and in the regulation of Atoh1 expression. Notch ligands and targets are expressed during ear development: Jag1 and Hey1 are involved in sensory patch specification, whereas Dl1 and Hes5 are related to HC determination. Sox2 promotes sensory competence and self-renewal of otic progenitors, and it activates Atoh1 through direct binding to the 3'Atoh1-enhancer. In parallel, Sox2 induces also the expression of several bHLH factors like Notch target genes Hes5 and Hey1, neurogenic genes Neurog1, NeuroD, and BMP targets like Ids. Therefore, Sox2 triggers an incoherent response that both promotes and counteracts Atoh1 expression [3]. We have studied the regulation of Atoh1 during inner ear development by analyzing the interactions between Sox2, Notch and other factors with the 3'Atoh1-enhancer. One specific aim of this project is to understand how repressors interact with Atoh1 and prevent the onset of HC differentiation during development. This question has a direct link with HC regeneration that relies on the reactivation of Atoh1 and is facilitated by Notch blockade [4].

The 3'Atoh1-enhancer contains putative Ebox binding sites for bHLH factors. We are currently studying the behaviour of the 3'Atoh1-enhancer in the presence of the Sox2 bHLH target genes during early chick inner ear development. The results show that all the bHLH tested are able to repress the activity of the 3'Atoh1-enhancer *in vivo*, while Atoh1 and Sox2 activate it. However, the mutation of multiple putative Ebox binding sites does not affect the activity of the enhancer. This suggests that the repression of Atoh1 is rather robust and tightly regulated by complex interactions among different repressor factors.

Keywords: Atoh1; hair cell; regeneration; Notch; sensory development.

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P39. MONITORING OTIC DIFFERENTIATION USING AN OTIC PLACODE-SPECIFIC HUMAN EMBRYONIC STEM CELL REPORTER LINE

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Previously we developed a protocol to direct the differentiation of human embryonic stem (hES) cells into progenitors of the otic lineage, based on signals involved in the initial specification of the otic placode *in vivo* [1]. Two types of otic progenitors are induced with distinct morphologies (epithelial and neural) and both types co-express characteristic otic markers such as PAX2, PAX8, FOXG1 and SOX2. With the aim to create a system where we could monitor the generation of otic progenitors in real time, we started by exploring *in vitro* the activity of two enhancers that restrict SOX2 expression to the nasal and otic placodes (NOP-1 and NOP-2) [2]. Transfection of human fetal auditory stem cells (hFASCs) [3] with constructs where NOP-1 and NOP-2 drove EGFP has shown that the enhancers were active in almost 100% of the transfectable cells. Similar results were obtained when using stem cells derived from the mouse fetal cochlea. A NOP1/2-SOX2-hES cell reporter line has now been created using a tetrameric form of the enhancers in the pEGFP-1 vector (Clontech). When the NOP1/2-SOX2-hES cells are subjected to our otic differentiation protocol, approximately 20-30% of the differentiated cells co-express high levels of EGFP with PAX2, PAX8 and FOXG1. This reporter system will allow us to monitor otic differentiation in real time, further investigate the dynamics of otic progenitor induction from hES cells, and visualise the effects that manipulating various other signaling pathways could have on the efficiency of the protocols for the generation of otic lineages.

Keywords: Human embryonic stem cells; otic placode; SOX2

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P40. HETEROGENEITY OF THE STEM CELL COMPARTMENT AND ITS IMPACT ON THE GENERATION OF OTIC PROGENITORS FROM HUMAN EMBRYONIC STEM CELLS

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Previous work from our laboratory has shown that otic progenitors could be derived from human embryonic stem (hES) cells using the ligands FGF3 and FGF10 in a serum-free, monolayer culture system. These otic progenitors are defined by the concerted expression of PAX8, PAX2, FOXG1 and SOX2 as occurs during inner ear development *in vivo* [1]. A variable degree of heterogeneity has been described within the pluripotent hESC compartment. Cell fate decisions in stem cell populations can be substantially affected by these variations, generating lineage bias and becoming an important factor to consider when fine tuning protocols with potential clinical application. In this study we have aimed to address whether fluctuations within the pluripotent state affect the differentiation outcome. More specifically, if these fluctuations generate subsets of cells that are primed to favourably differentiate into otic lineages. The surface antigen SSEA3 (Stage Specific Embryonic Antigen 3) has been described as a good marker of undifferentiated hES cells and it is one of the earliest to be lost upon differentiation [2]. Interestingly, heterogeneous expression of SSEA3 has been systematically observed in undifferentiated hES cultures. Initially, we aimed to address whether such fluctuations within the starting culture affected the differentiation outcome. hES cells were separated by fluorescence-activated cell sorting based on the presence or absence of SSEA3 and subjected to our differentiation protocol. Moreover, we sorted cells using a SOX2, otic-specific reporter. Gene expression analysis by QPCR and immunolabelling showed that the cells from the two SSEA3 fractions, and from fractions combining SSEA3 and otic-SOX2 displayed distinct differentiation propensities, suggesting that defined substates exists in the stem cell population that could differentially influence otic differentiation. Capturing cells in these states could improve our methods to derive otic progenitors *in vitro*.

Keywords: hESCs; otic progenitors; stem cell heterogeneity.

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P41. GENERATION OF OTIC PROGENITORS AND SENSORY LINEAGES FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Otic progenitors derived from human embryonic stem cells (hESCs) have been proposed as potential therapeutics for deafness [1]. The discovery that adult somatic cells can be reprogrammed into a pluripotent state [2] expanded the potential application of stem cells by conceptually allowing the generation of patient-specific, induced pluripotent stem cells (hiPSCs). We sought to verify the applicability of hESC otic differentiation protocols to hiPSCs. Human iPSC lines were subjected to the same two-stage protocols developed with hESCs, first to generate otic progenitor cells and then to further differentiate along either a sensory hair cell or neuronal lineage. Expression of proteins associated with the early otic and later neural/hair cell phenotypes were assessed via antibody probing and immunofluorescence detection. RNA transcript levels were compared between samples by qPCR. Recording of electrophysiological properties was undertaken on differentiated cells, to explore the presence of currents typical of the lineages under study. Human iPSCs were induced to differentiate into cells expressing otic progenitor markers such as PAX8, PAX2, SOX2 and FOXG1. As for hESCs, two types of otic progenitors were obtained, otic neuroprogenitors (ONPs) and otic epithelial progenitors (OEPs). ONPs were further differentiated towards sensory neuronal fates as evidenced both by the expression of β-tubulinIII, neurofilament and POU4F1 proteins, and the detection of sodium and potassium currents. Human iPSC-derived OEPs were used to generate cells co-expressing POU4F3 with MYO7A and ATOH1, and which presented electrophysiological profiles similar to immature sensory hair cells. In contrast to hESCs, hiPSCs appear less dependent on exogenous FGF3 and FGF10 for induction during the first phase of differentiation. Functional analysis supports protein expression data indicating that hiPSC-derived otic progenitors can be used to generate immature sensory hair cells and neurons. Together, these data validate for this new platform the protocols originally established with hESCs and support the potential application of hiPSCs in future regenerative therapies to treat deafness.

Keywords: iPSCs; otic progenitors; stem cells.

Acknowledgements: This work was supported by Action on Hearing Loss, Deafness Research UK and the EUFP7 Consortium Otostem.

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P42. GROWTH FACTOR-INDUCED DIFFERENTIATION OF HUMAN ADIPOSE-DERIVED STEM CELLS TOWARDS OTIC FATE

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Inner ear hair cells are vital for our ability to hear and balance. These cells are challenging to study as they are few in number, very fragile and difficult to maintain *in vitro*. Furthermore, human inner ear tissue is particularly difficult to source. The aim of the project is to develop a stem cell-derived *in vitro* hair cell epithelium, containing both hair cells and supporting cells, that can be used as a model for study and drug screening. For this purpose, we are investigating the potential of using human adipose-derived stem cells (ADSCs). ADSCs were chosen because of their stem cell characteristics, their capability to differentiate into neural-like cells, and clinical potential. They are an abundant and accessible autologous source of cells and lack the ethical, technical and regulatory obstructions posed by embryonic or induced pluripotent stem cells.

We adapted two different procedures that have been used to differentiate mouse and human embryonic stem cells into hair cell-like cells [1, 2]. Both are stepwise induction protocols devised to mimic embryonic otic development via growth factor-induced differentiation. Morphological were monitored using brightfield microscopy, and neural and otic differentiation assayed by PCR and immunocytochemistry.

Both differentiation protocols induce substantial morphological changes in the ADSCs. Cells gain neural-and/or glial-like morphology. PCR analyses indicate that the untreated ADSCs already express a number of differentiation markers, including certain ion channels (KCND3 and SCN9a) and otic developmental genes (Six1 and Gata3).One differentiation paradigm induces cells to express Pax8, a developmental gene that functions in otic vesicle formation and otic specification. We are currently assaying functional changes using calcium imaging and electrophysiology and protein expression using immunocytochemistry.

Keywords: Human adipose-derived stem cells; *in vitro* differentiation; hair cell model; stem cells.

Acknowledgements: Sponsored by a UCL/AstraZeneca Impact Studentship.

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P43. MULTIMODAL VISUALIZATION OF MOUSE HAIR-FOLLICLE-BULGE-DERIVED STEM CELLS

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Cell-based inner ear therapy may restore sensorineural hearing loss. Multipotent hair-follicle-bulge-derived stem cells (HFBSCs) are promising candidates for evoking cochlear nerve regeneration. However, cell-based inner ear therapy could be limited by the problem of ascertaining the viability of grafted cells and their location. An approach combining bioluminescence and magnetic resonance imaging (MRI) may be advantageous to monitor grafted cells within the cochlea. The bioluminescent signal from cells expressing ATP-dependent luciferase is a proxy of their viability, whereas cells loaded with magnetic nanoparticles can be localized by means of MRI.

The aim of this study was to investigate the suitability of HFBSCs for lentiviral transduction with reporter genes in combination with nanoparticle loading.

To this end, HFBSCs were transduced with the Luc2-GFP reporter gene construct containing genes encoding the copepod green fluorescent protein (copGFP) and a codon-optimized firefly luciferase (Luc2).We studied transduction efficiency, proliferation rate, cell viability and persistence of reporter protein expression during long-term culture (till P15) using fluorescence and bioluminescence microscopy. Transduced HFBSCs were loaded with TMSR50 nanoparticles (containing a magnetic core and a red-fluorescent dye) and cultured for 7 days. The loading efficiency was established using fluorescence microscopy. The possible cytotoxic effect of TMSR50 nanoparticles on proliferation and viability was investigated using MTS assay and bioluminescence microscopy.

Transduction with the Luc2-GFP reporter gene construct did not influence proliferation of the HFBSCs. Green fluorescence of copGFP was immediately visible after transduction and persisted for at least 15 passages. Luc2 expression was concomitant with copGFP expression. Additional loading with red-fluorescent TMSR50 nanoparticles did not affect cell proliferation and viability, as compared to non-loaded cells. Nanoparticle-loaded HFBSCs could be detected by means of MRI in agarose-embedded cell layers containing no less than $1x10^4$ cells.

We conclude that HFBSCs can efficiently be transduced with the Luc2-GFP reporter gene construct and subsequently be loaded with nanoparticles without affecting cell proliferation and viability. This implies that combined MRI and bioluminescence imaging may enable *in vivo* localization of grafted HFBSCs within the cochlea of deafened animals, allowing long-term monitoring of cochlear nerve regeneration.

Keywords: stem cells; bioluminescence; fluorescence; magnetic resonance imaging; nanoparticles.

Acknowledgements: This project is financially supported by funding from MED-EL (Innsbruck, Austria).

P44. EAR IN A DISH: DEVELOPMENT OF AN *IN VITRO* ASSAY FOR OTOTOXIC AND OTOPROTECTIVE DRUG SCREENING

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Ototoxic compounds cause irreversible hearing loss because of the limited capacity of cell regeneration in the mammalian organ of Corti. Towards a rational therapy for the cure of sensorineural hearing loss, an *in vitro* culture model of ototoxic hair cell loss that can be utilized to screen for otoprotective drugs is needed. Such models using cell lines or zebrafish larvae have been introduced. However, because inner ear cell lines are likely to behave differently to primary cells in response to drug treatment due to their immortalization state, and because the zebrafish larva is a non-mammalian model, we have developed 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- (HC) and supporting (SC) cell-like cells *in vitro* [1]. Chemical inhibition of the Notch pathway with a gamma-secretase inhibitor (1.5 µM L-685,458; at 1 div for 24 h) on differentiated otospheres from the postnatal (p0)mouse organ of Corti increased the relative number of hair cell-like cells (MyosinVIIa) by a factor of about 2-3.This was supported by down-regulation of expression in downstream genes in the Notch signaling pathway, and up-regulation of HC and SC specific genes was seen. Furthermore, towards an "ototoxic hearing loss-in-a-dish" model, neomycin (1 mM, for 24h) was added to the cell culture. Almost complete loss of newly differentiated HC-like cells was achieved, while the number of SC-like cells (SOX2) remained stable.

Our results show that the mammalian *in vitro* assay based on cochlear stem- or progenitor cells is an outstanding tool for screening of potential ototoxic and otoprotective drugs.

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

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P45. CONTROLLED DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS TO AN OTIC PROGENITOR CELL FATE

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Human induced pluripotent stem cell (hiPSC) technology holds great expectations for drug discovery and clinical applications such as cell transplantation. Along this line, hopeful progress has been made in applying hiPSC technology to a variety of organ systems such as the retina, the cardiovascular system as well as the peripheral and central nervous system. Notably, the inner ear represents another system of interest for translational studies relying on the use of hiPSC. Accordingly, one of the challenges associated to the inner ear is to be able to faithfully recapitulate *in vitro* the developmental steps leading to otic progenitors and their derivatives. Up to date, the *in vitro* generation of otic progenitors from iPSCs was reported in systems using mouse pluripotent stem cells, either iPSCs or embryonic stem cells (ES). Moreover, the robustness and efficiency associated with available protocols remain unsatisfactory.

In order to address current issues associated with iPSC technology applied to the inner ear, we sought to establish a new differentiation protocol allowing for the robust and highly efficient derivation of otic progenitor cells from hiPSCs, which may represent the first necessary step for the development of human cell-based therapy for inner ear disorders. Thus, using lessons learnt from developmental studies, we applied a combination of small molecules to modulate major signaling pathways (FGF, BMP, WNT) involved in otic placode induction, and established stepwise, serum-free, and feeder-free differentiating culture conditions. Interestingly, our defined protocol leads to the upregulation of otic cell markerssuch as Pax2, Pax8, Six1, Eya1 and Dlx5 upon differentiation. Using our newly established protocol, we differentiated two fully characterized hiPSC lines (transgene and integration-free) and compared the results between these two pluripotent cell lines. To this end, using immunocytochemistry and qPCR, we analyzed the dynamic expression of a panel of comprehensive markers specific for otic cell fate determination.

We will present preliminary findings of our ongoing works aiming at generating a highly suitable differentiation protocol allowing the derivation of otic progenitors from hiPSCs. Ultimately, our study may provide a new and powerful tool for disease modeling studies as well as transplantation-based studies.

Keywords: human induced pluripotent stem cells; inner ear; otic progenitors.

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P46. EFFECT OF EXTRACELLULAR MATRIX ELASTICITY UPON STEM CELL DIFFERENTIATION

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Hair-follicle-bulge-derived stem cells (HFBSCs) provide a potential source of cells to treat sensorineural hearing loss, but are new in the field of inner ear regenerative therapy. To induce differentiation of stem cells into appropriate cell types after intracochlear implantation, insight into factors dictating their fate is essential. Stem cell fate is regulated by complex interaction between soluble factors and extracellular matrix. Reports show that high-elasticity matrices (mimicking soft brain matrix) induce neurogenic lineage commitment, whereas low-elasticity matrices (mimicking rigid bone matrix) lead to osteogenic differentiation. The aim of this research is to understand how cues from soluble factors and extracellular matrix regulate differentiation of HFBSCs.

HFBSCs from mouse whisker pads were cultured in poly-D-lysine-coated wells containing basic growth medium (BGM): DMEM/Ham's F-12 (1:1) supplemented with 1% GlutaMax™, 1% antibiotic/antimycotic solution, 10% fetal bovine serum, 2% B-27 supplement, 1% N-2 supplement, recombinant human ßFGF and EGF (20 ng/ml each). Cells were used at low passage numbers, subconfluently cultured and plated at $\sim 10^3$ cells/cm^2 for differentiation. Differentiation was done in a high-elasticity matrix (0.15% Puramatix™, a peptide hydrogel) and the commonly-used low-elasticity matrix (0.01% collagen I). Four differentiation media were used: BGM with and without growth factors, αMEM with chicken embryo extract and αMEM with vitamin C. After 8-14 days of culture, the cells were fixed and immunostained for nestin, SOX9, S100b, GFAP and class III ß-tubulin. Osteogenesis was established by measurement of alkaline phosphatase activity, and alizarin red S staining was used to detect calcium deposits.

HFBSCs cultured in a high-elasticity matrix demonstrated a bipolar appearance and preferentially formed networks. These glia-like cells showed immunoreactivity to nestin, GFAP and S100b. HFBSCs in a low-elasticity matrix differentiated into nestin-positive, osteoblast-like cells. These cells, when cultured in αMEM with vitamin C expressed phosphatase activity and produced calcium, suggesting osteogenic differentiation. Differences in culture media minimally affected cell morphology and immunophenotype. The results show that lineage-specific differentiation of HFBSCs is more effectively induced by extracellular matrix elasticity than by the composition of the culture medium.
We recommend encapsulation of stem cells within high-elasticity hydrogels prior to grafting to support neural repair in the cochlea.

Keywords: extracellular matrix; elasticity; hair-follicle-bulge stem cells; lineage-specific differentiation

P47. LGR5+ COCHLEAR STEM/PROGENITOR CELLS EXPAND *IN VITRO* UPON WNT SIGNALING ACTIVATION USING A SMALL MOLECULE INHIBITOR.

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The adult mammalian auditory epithelium or organ of Corti (OC) lacks intrinsic regenerative capacity. Nevertheless, a population of tissue-resident dormant stem/progenitor cells expressing the R-Spondin receptor Lgr5 has been recently identified.

Much effort is put into identifying ways to trigger endogenous stem cell activity within this organ to induce tissue regeneration and hopefully harness the potential of stem cells within clinical settings.
Small molecule inhibitors able to induce stem cell activity by re-expression of positive cell cycle regulators or by triggering developmental genes represent an interesting pharmaceutical approach, as they could be rapidly screened with *in vitro* bioassays and locally applied to the inner ear to reach their cellular target.

Lgr5 expressing cells in the OC have been previously shown to be responsive to Wnt pathway agonists such as recombinant Wnt3a or R-Spondin1. In order to explore the possibility that small molecule compounds activating Wnt signaling could be used to stimulate stem cell activity, a specific GSK3 inhibitor (GSK3i) has been tested for its potency in expanding the stem cell pool in dissociated cultures and in organotypic OC cultures.

We show here that treatment with 10μM GSK3i strongly increases the proliferative potential of Lgr5-GFP+ stem/progenitor cells. This is evaluated as sphere size, cell number, GFP expression/Edu incorporation by flow cytometry and fluorescence microscopy. Moreover, cells maintain their capacity to differentiate towards hair cells.

Using organotypic cultures we have been able to show that treatment with the described small molecule compound induces proliferation in intact organs. We detect 15% Sox2+ cells positive for the proliferative marker Ki67, in GSK3i treated samples.

These data show for the first time the possibility to enhance proliferation of stem/progenitor cells from undissociated early postnatal Organ of Corti using small molecule compounds. *In vivo* validation of these findings is currently ongoing.

Keywords: Lgr5; proliferation; small molecule inhibitors;Wnt.

Acknowledgements: This work was sponsored by the Johanna Dürmüller-Bol foundation

P48. POLYMORPHISMS IN GENES INVOLVED IN THE IMMUNO-INFLAMMATORY PROCESS IN PATIENTS WITH MÉNIÈRE'S DISEASE

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

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

Results: The *GPIa* polymorphism was significantly associated with a risk of MD; in addition, the OR for the *GPIa* polymorphism and MD risk was 1.435 (CI: 1.035–1.990) with adjustment for age and sex. The remaining polymorphisms failed to show any associations with the risk of MD.

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

Keywords: Ménière's disease; case-control study; polymorphism.

Acknowledgements: This study was supported by research grants for Longevity Sciences (25-2) from the Ministry of Health, Labour and Welfare and research grants (25462634) from the Ministry of Education,Culture, Sports, Science and Technology of Japan.

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P49. EXOME SEQUENCING AND BIOINFORMATIC ANALYSIS IN IDENTICAL TWINS WITH MÉNIÈRE'S DISEASE

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Ménière's disease (MD) has a strong familial aggregation. Previous analysis in a wide cohort of MD patients has shown a prevalence of 8-10% of familial cases [1, 2]. Moreover, the presence of the disease in monozygotic twins increases the evidence that MD could have a genetic component. Genetic heterogeneity is observed, but most families have an autosomal dominant inheritance with anticipation [3]. We performed whole-exome sequencing (WES) analysis in a family with monozygotic twins with MD and two healthy individuals to identify candidate variants affecting this family.

After processing the data from SOLiD 5500xl platform, we obtained different types of files containing ~50000 single nucleotide variants (SNV) per exome. To prioritize pathogenic variants we annotated a score according to: a) the effect in protein structure and phylogenetic conservation by using a seven point scoring system (SIFT (Sort Intolerant from Tolerant), PolyPhen2 (Polymorphism Phenotyping v2), Graham's Matrix, GERP+ (Genomic Evolutionary Rate Profiling), Mutation taster, PhastCons and PhyloP); b) cross species phenotype comparison according to the inheritance pattern and mouse as model organism phenotype by the Exomizer software [4]; c) minor allelic frequency (MAF) <0.01.

Since a homozygous recessive variant may affect both twins, we also performed homozygosity mapping to discard the possibility of a recessive inheritance pattern [5]. Copy number variants (CNVs) were analyzed by using Conifer software, but we did not find any CNV with significant Z-scores associated with MD phenotype. Five variants remained after the prioritization process and filtering. Four of them were discarded because of their low level of expression in the inner ear and by their biological function. THAP1, a gene associated with dystonia 6, encoding a THAP domain-containing protein considered to be involved in endothelial cell proliferation and proapoptotic processes, and assumed to act as a transcription factor, was considered the best candidate gene in these twins. It has normal level of expression in the inner ear and mutations in this gene have cause loss of DNA binding and transcriptional dysregulation of downstream targets.

Further studies are required to evaluate the functional impact of this novel variant as well as its role in MD development.

Keywords: Familial Ménière's disease; monozygotic twins; single nucleotide variants; whole-exome sequencing.

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P50. FUNCTIONAL STUDIES OF KCNQ4 MUTATIONS REVEAL THREE DIFFERENT PATHOGENIC MECHANIMS

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Mutations in the potassium channel gene *KCNQ4* underlie DFNA2A a subtype of autosomal dominant progressive, high-frequency hearing loss. In a previous study we identified a novel mutation, p.G296S, in the pore region of the KCNQ4 channel that impaired its activity in two manners: it greatly reduced surface expression and abolished channel function. Moreover, the p.G296S mutant exerted a strong dominant-negative effect on potassium currents by reducing the wild type *KCNQ4* channel expression at the cell surface. In the present study we have extended the genetic analysis of *KCNQ4* gene in 300 hearing-impaired Spanish families compatible with a dominant inheritance and identified two novel pathogenic mutations, p.Y270_A271del and p.G287R. For these two mutations, and for most of those previously described in different domains of the channel protein (p.Q71Sfs*68, q.Q71Pfs*64, p.F182L, p.W242*, p.E260K, p.D262V, p.L274H, p.W276S, p.L281S, p.G285C, p.G285S and p.G321S) we have investigated the underlying pathogenic mechanism. Functional studies carried out in *Xenopus* oocytes reveal a lack of function for all the mutant channels except p.F182L, that behaved as the wild type. In co-injection experiments with the wt allele at a 1:1 ratio we observed a dominant-negative inhibition of the current amplitude for all mutations assayed except for the three truncating mutations, for which a mechanism of haploinsufficiency was expected. The latter results were also observed for artificially generated mutants lacking the Ct region of the protein. Additional studies performed in NIH3T3 indicated a reduced surface expression of the mutants. Altogether these findings showed that the dominant negative mechanism is mediated by the interaction through the Ct region of KCNQ4 and it is associated to trafficking defects. However, the p.W242* mutant was detected in cell surface of NIH3T3 and, interestingly, this mutant when co-injected with the wt, produced an alteration in the activation curve of the wt currents, likely occurring by means of a non-canonical interaction with the wt KCNQ4 monomers. These findings may suggest a novel way of action for some of the truncating mutations in *KCNQ4* causing hearing loss that is different to that of haploinsuficiency or dominant-negative mechanisms.

Keywords: *KCNQ4*; deafness; humans; oocytes; flow cytometry.

P51. MITOCHONDRIAL MUTATIONS M.A3243G AND M.A1555G IN THE SLOVAK HEARING IMPAIRED POPULATION

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Introduction: Sensorineural hearing loss (SNHL) associated with mitochondrial (mtDNA) mutations represents a specific group of hearing disorders characterized by matrilineal inheritance and highly variable phenotype. The clinical importance is highlighted by several syndromes (e.g. MIDD – Maternally Inherited Diabetes and Deafness or MELAS – Mitochondrial Encephalomyopathies, Lactic Acidosis, Stroke-like episodes) developing in many mtDNA mutation carriers.

Objectives: In this study we focused on prevalence and clinical phenotype of two mtDNA mutations (m.A3243G, m.A1555G) among Slovak patients with bilateral SNHL.

Methods: We selected 177 unrelated individuals to be tested for these mutations according to the following inclusion criteria: m.A3243G – matrilineal inheritance and/or progressive SNHL with or without diabetes (96 subjects); A1555G - matrilineal inheritance with or without aminoglycosides before hearing loss onset (81 subjects). The m.A3243G mutation was analyzed from peripheral blood and/or buccal mucosa using real-time PCR. For determination of the m.A1555G mutation, DNA from peripheral blood was analyzed by bidirectional sequencing. The phenotypes were also evaluated in 12 additional m.A3243G positive subjects (6 probands and 6 family members) identified in our parallel study on monogenic diabetes.

Results: The m.A3243G mutation was detected in 3 of 96 tested hearing impaired probands, with the prevalence achieving 3.1%. The phenotype was assessed in 9 families (19 subjects). The mutation carriers presented with a broad phenotype spectrum ranging from yet asymptomatic, through hearing loss or diabetes as a sole symptom, to fully developed MELAS syndrome. The m.A1555G mutation was identified in 1 of 81 tested families (1.2%). Progressive SNHL was present in all but the youngest mutation carrier, who had so far normal hearing thresholds.

Conclusions: Mitochondrial mutations m.A3243G and m.A1555G are another genetic causes of SNHL recently identified in the Slovak hearing impaired population. Their prevalence corresponds to previous data from other countries in the Central European region [1,2]. It is also reasonable to test for the m.A3243G mutation in selected patients with hearing impairment even without diabetes as an early diagnostics tool for prevention of possible complication.

Keywords: deafness; DNA analysis; MIDD and MELAS syndrome; matrilineal inheritance.

Acknowledgements: This work has been sponsored by APVV 0148-10 and KCMM (ITMS 26240220071)

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P52. DFNA15 CAUSED BY POU4F3 MUTATIONS IS ONE OF THE MOST FREQUENT FORMS OF HEREDITARY DEAFNESS IN SPAIN WITH DIFFERENT UNDERLYING PATHOGENIC MECHANISMS

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POU4F3 encodes a POU-domain transcription factor that is required for hearing. It is expressed in hair cells in the inner ear and is essential for hair cell functional maturation. Mutations in this gene are responsible for the autosomal dominant nonsyndromic hearing loss subtype DFNA15, whose prevalence in the Spanish population is largely unknown. dHPLC-based mutation screening of *POU4F3* in 80 Spanish patients suffering from dominant inherited hearing impairment has revealed five novel missense mutations: p.R230S, p.W251Cfs*65, p.W251X, p.F293L and p.F322L. In addition, a novel nonsense mutation, p.G225X, was identified in a large Spanish family by genome-wide SNP genotyping using the Illumina linkage panel with 6090 SNPs. To test the potential effects of the identified POU4F3 mutants we performed a series of functional analyses in NIH3T3 cell cultures. Whereas the wild type POU4F3 is found exclusively in the nucleus, our studies demonstrate that the mutant proteins exhibited four different distribution patterns: 1) p.R230S, p.F293L and p.F322L mutants were localized in both the nucleus and the cytoplasm; 2) p.W251Cfs*65 exhibited a wild type-like pattern, likely as a consequence of a *de novo* nuclear localization signal generated in the novel 65 amino-acid mutated tail; 3) p.W251X mutant is distributed in cytoplasmic clumps; and 4) p.G225X was uniformly dispersed in the cytoplasm. In addition, mRNA and Western blot analysis showed that whereas the mutant transcript levels are similar to that of the wild type, the POU4F3 protein levels were reduced in all mutants, indicating that the synthesis or stability of the protein is impaired. This is the first report of genetic epidemiology of POU4F3 in the Spanish population in which 6 novel mutations have been identified and functionally analysed. On the basis of our results, DFNA15 may be one of the most prevalent deafness forms accounting for roughly 7% of ADNSHL in Spain.

Keywords: POU4F3; DFNA15; hearing loss.

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P53. MOLECULAR STUDY OF BRAZILIAN PATIENTS WITH AUDITORY NEUROPATHY

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Auditory neuropathy (AN) is a type of sensorineural hearing loss characterized by absent or abnormal auditory brainstem responses and preserved otoacoustic emissions and/or cochlear microphonics. The disorder can be caused by a variety of environmental and genetic factors. In the last decade, the identification of genes responsible for AN has greatly contributed to the diagnosis and better understanding of the mechanisms involved in the disorder. To date, four loci associated with nonsyndromic AN have been mapped: DFNB9 (*OTOF* gene) and DFNB59 (*PJVK* gene), responsible for autosomal recessive pattern; AUNA1 (*DIAPH3* gene) for autosomal dominant; and AUNX1 for X-linked. Connexin 26 mutations were also reported in subjects with AN. AN is a challenging condition, as many factors concerned with its etiology and pathogenesis remain unclear. Additionally studies are needed to provide a better understanding of the disorder. The main goal of the study was to investigate genetic mutations in patients with a clinical diagnosis of AN, to verify their importance and involvement in the etiology of AN in the Brazilian population. Clinical information and genetic evaluation of 40 patients were analyzed. We investigated the most common causes of genetic hearing loss, including pathogenic variants in the *GJB2* gene, deletions in the *GJB6* gene and m.1555A>G mutation in the *MTRNR1* gene. Additionally, direct sequencing was performed for mutation screening of the *OTOF* gene. The common Spanish p.Q829X mutation in the otoferlin gene was not detected in our cohort. The c.35delG mutation in the *GJB2* gene was found in three patients in homozygous genotypes. However, it is not established if pathogenic variants in connexin 26 could be involved with AN or if the otoacoustic emissions that were recorded from these subjects only represent the residual activity of few outer hair cells that are still alive. Further investigation is needed to clarify the link between *GJB2* mutations and AN. The study of AN's genetic basis is extremely important to improve the diagnosis, management, therapy and genetic counseling of the affected subjects.

Keywords: Auditory neuropathy; connexin 26; genetics; hearing loss; otoferlin.

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P54. JAK2/STAT3 IS INVOLVED IN THE NOISE-INDUCED STRESS RESPONSE IN THE INNER EAR

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Background: Signal transducers and activators of transcription 3 (STAT3) is a stress responsive transcription factor. The primary mechanism of STAT3 activation is through phosphorylation leading to dimer formation, nuclear translocation and transcriptional activation. STAT3 also possesses transcription-independent activities including direct regulation of mitochondrial respiration and complex I activity as well as inhibition of autophagy under normal physiological conditions.

Objective: To determine the role of STAT3 in the noise-induced stress response in the inner ear.

Methods and Results: Exposure of male CBA/CaJ mice (9-10 weeks old, n=5/group) to a moderately damaging level of sound (110 dB SPL, 4-48 kHz, 3 hrs) resulted in phosphorylation and nuclear translocation of STAT3 in many cell types in the inner ear including auditory hair cells. Pharmacological inhibition of the JAK2/STAT3 pathway with JSI-124 prior to noise exposure revealed improved recovery of outer hair cell (OHC) function following noise exposure as measured by auditory brain stem response and distortion product otoacoustic emission measurements. In mice possessing an OHC-specific STAT3 deletion, the OHCs appeared morphologically normal and alterations in markers of autophagy were observed.

Conclusions: Exposure to moderately damaging levels of loud sound induces phosphorylation and nuclear translocation of STAT3 in many different cell types in the cochlea. Chemical inhibition of STAT3 phosphorylation proved protective of OHC function following noise exposure. These findings suggest a role for the JAK2/STAT3 pathway in noise-induced ROS production and inflammatory responses that lead to a reduction in OHC function and survival. Additionally, the observed increase in a key marker of autophagy in the OHC-specific STAT3 deletion mouse suggests that autophagic processes were involved in OHC recovery following noise exposure in the JSI-124 treated mice.

Keywords: STAT3; cochlea; acoustic trauma; outer hair cell.

Acknowledgements: This work has been supported by NIH NIDCD DC R01-000105 and P30 005983

P55. ROSMARINIC ACID PROTECTS AGAINST NOISE INDUCED HEARING LOSS ENHANCING ENDOGENOUS ANTIOXIDANT RESPONSES

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Objectives: It has been demonstrated that the increase of ROS generation and of lipid peroxidation, together with a concurrent decrease of antioxidant defenses, play a significant role in noise-induced hearing loss (NIHL). This redox imbalance is largely the responsibility of cellular mechanisms that underlie hair cell death after noise exposure. Several molecules with antioxidant and scavenging properties have been proved to restore redox balance and to prevent oxidative stress-induced hair cell death. In this study, we focused on rosmarinic acid (RA) a natural antioxidant found in many herbs of the *Lamiaceae* family with antioxidant, anti-inflammatory and antiviral properties. Furthermore, recent studies have shown that RA is able to enter spontaneously into membranes, and it is efficiently able to prevent lipid peroxidation. Namely, the aim of this study was to evaluate the protective effect of RA against NIHL.

Materials and Methods: Wistar rats (200-250g) were used in this study. Animals were exposed for 60 minutes to a pure tone of 120dB SPL, 10kHz. Of these, a group was treated with RA (i.p. 10mg/Kg 1h pre trauma and for 3 consecutive days). We evaluated auditory function by auditory brainstem response (ABR) recording, the extent of damage with rhodamine-phalloidin staining, the magnitude of lipid peroxidation by 4-hydroxynonenal (4-HNE) expression, the superoxide amount with DHE assay, apoptosis with caspase-3 immunostaining and the level of endogenous antioxidant responses with superoxide dismutase (SOD) and glutathione (GSH) detection.

Results: Our results demonstrate that RA can: (a) prevent hearing loss by reducing ABR threshold shift at 1, 3, 7 and 21 days after acoustic trauma, (b) decrease hair cell loss as shown by cochleogram, (c) decrease cell damage in the cochlea by reducing superoxide amount, lipid peroxidation and caspase-3 activation, (d) upgrade endogenous antioxidant responses as shown by the increase of SOD and GSH expression.

Conclusion: Our results demonstrate that RA treatment can reduce the oxidative cochlear damage caused by noise through the enhancement of endogenous antioxidant defenses. Thus, RA provides a promising approach against NIHL.

Keywords: Acoustic trauma; rosmarinic acid; cochlea.

Acknowledgements: This work has been sponsored by PRIN GRANT, Italian Department for Research, "*Fondi di Ateneo*" Catholic University

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P56. THE EFFECTS OF ENDOTHELIN RECEPTOR BLOCKER ON NOISE-INDUCED COCHLEAR INJURY IN MICE

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Abstract
Noise-induced hearing loss is one of the most common forms of sensorineural hearing loss and is attributed to various mechanisms including damage by reactive oxidative species. Endothelin-1, predominantly via endothelinA receptor (ETAR), is a potent vasoconstrictor which promotes thrombosis and hypoxic damage. In this study, we evaluated whether administration of inhibitor of ETAR prevents against noise-inducedcochlear damage in the mouse. BALB/C mice (4-5 weeks, male) were exposed to broad band noise (250-8 kHz, 120dB SPL) for 8 hours. ETAR blocker, BQ-123,was injected intravenously for 6 days in mice before noise exposure. Change of hearing threshold and hair cell were analyzed. Expressions of reactive oxidative species were evaluated using immunohistochemistry and quantitative polymerase chain reaction. ETAR pretreatment group showed less hearing threshold shift and reduced hair cell death after noise exposure compared to no treatment group. Expression of HO-1 and 4-HNE are significantly decreased in pretreatment group. These findings suggest that ETAR blocker may be beneficial in reducing susceptibility to cochlear acoustic damage.

P57. RECRUITMENT STUDY OF WORKERS IN FURNITURE COMPANIES

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Introduction: The impact of the complex factors in the industrial environment is a feature of working conditions on modern furniture companies. The most important are intense noise, elevated concentrations of chemicals in combination with heavy physical exertion.
Objective: To assess the state of auditory function and recruitment for persons working in furniture companies.

Materials and Methods: We were surveyed 50 employees of a furniture company (1st group) and 15 healthy persons (2nd group). Exceeding the permissible noise level in a workplace was 1,2-8 dB. The tone threshold audiometry and Luscher test frequencies 0,5, 1, 2, 4 kHz at an intensity of 40 dB above the threshold of audibility were also conducted.

Results: Sensorineural hearing loss of different degrees was revealed in the first group. Recruitment was seen in 38% of patients with early signs of noise exposure, in 68% of patients with the first degree of hearing loss, in 80% of patients with 1-2 - degree of hearing loss by the test Lusher results. Frequently recruitment was fixing at frequencies 2 and 4 kHz, at least - at a frequency of 0.5 kHz. Unilateral recruitment was recorded in the early stages of hearing loss, while bilateral at the 1 – 2 stages.

Conclusions: Workers in a furniture company have recruitment at various stages of hearing loss. The frequencies 2 and 4 kHz are the most informative frequency during the test Lusher. The frequency of recruitment from both sides increases to 60 % with the progression of hearing disorders.

Keywords: hearing, furniture companies, recruitment

P58. ROLE OF P66SHC IN NOISE INDUCED HEARING LOSS AND PRESBYACUSIS

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Objectives: The p66Shc, a member of the ShcA protein family, harbours a unique N-terminal proline-rich domain with a serine phosphorylation site (Ser36) whose phosphorylation is essential in the cellular response to oxidative stress. Accordingly, p66Shc protein has been recognized as an important element of the free radical theory of aging. Namely, p66Shc-induced formation of Reactive Oxygen Species (ROS) in mitochondria promotes apoptotic cell death in response to a variety of pro-oxidant noxious stimuli, and mice lacking the p66 isoform display increased resistance to oxidative stress and delayed aging. However, no study of p66Shc in noise-induced hearing loss (NIHL), a phenomenon linked to oxidative stress, has been previously reported. Thus, the aim of this study was to investigate the role of p66Shc in noise-induced oxidative damage to inner ear and in and in age-related hearing loss.

Material and Methods: Mice 126 SvEv wild type (WT) or knock-out (KO) for p66Shc at 2, 7, 12 and 24 months old were used. Auditory function was measured by recording the auditory brainstem responses (ABR) at 6-32 kHz in all age group animals. Acoustic trauma was also induced in animals of 2 months by a continuous pure tone of 120 dB SPL, 10 kHz for 1 hour and 24h after the acoustic trauma auditory function was retested. These NIHL animals were sacrificed, the cochleae were quickly removed and processed for immunofluorescence labelling and western blot analyses to evaluate p66Shc, Ser36-P-p66Shc, SOD2 and superoxide amount.

Results: The functional evaluation illustrated that: (1) At 2 and 7 months old there was no differences in threshold value between KO and WT groups; (2) At 12 months WT animals showed a threshold value of about 25-30 dB higher compared to KO; (3) Two months old WT mice were more vulnerable to acoustic trauma with respect to KO mice. Moreover, the morphological analyses revealed an increase of p66, Ser36-P-p66Shc, SOD2 and superoxide amount in mouse WT cochleae after noise exposure, in particular, in the stria vascularis.

Conclusion: In conclusion, we established that the p66Shc plays a role in both oxidative damage induced by noise and in the presbycusis process.

Keywords: Noise, p66, aging, cochlea.

Acknowledgements: This work has been sponsored by PRIN GRANT, Italian Department for Research, "*Fondi di Ateneo*" Catholic University

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P59. AGE-RELATED EFFECTIVENESS OF 7,8,3'-TRIHYDROXYFLAVONE ON NEURITE OUTGROWTH FROM SPIRAL GANGLION EXPLANTS

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Cochlear implants (CIs) are used to restore auditory function by stimulating spiral ganglion neurons (SGNs) electrically. The benefit of CIs is limited by the spatial distance between the electrode array and the auditory nerve. Therefore, hearing function might be improved by stimulating neurite outgrowth towards the CI. Small-molecule compounds like 7,8,3'-trihydroxyflavone (THF) are promising candidates for the protection of SGNs and currently also described to stimulate neurite outgrowth [1,2].

We applied 1 µM THF to mouse spiral ganglion explants to stimulateneurite outgrowth. For SG explants from postnatal day 3 to day 5 mice (P3 to P5) no stimulating effect on neurite outgrowth was detected.Effective stimulation of neurite outgrowth was observed only when explants from mice at postnatal day 7were used, thus THF treatment showed age-related effectiveness. Using brain-derived neurotrophic factor (BDNF) applied at 25 ng/ml BDNF resulted in similarly stimulated neurite outgrowth from explantstaken from P3 to P6mice, whereas in comparison explants from P7 miceshowed an increased neurite outgrowth. To analyze whether there is an age-related difference in spontaneousneurite outgrowthSG explants were cultured without neurotrophic support. As observed for THF treatment,neurite outgrowth from explants at P3 to P5 was not observed. Spontaneous neurite outgrowth emergedfirst at P6 and became more pronouncedat P7 showing that there is also an age-related difference in spontaneous neurite outgrowth from SG explants.

Although THF acts through the same receptor (TrkB) as BDNF the effectiveness in the spiral ganglion explant culture is different. BDNF stimulates neurite outgrowth at all ages analyzed, whereas THF possesses an age-related effectiveness on neurite outgrowth. This indicates that different signal processingmust occur resulting in differentialimpact on neurite outgrowth from SG explants.

Keywords: cochlear implant; spiral ganglion neurons; explant culture; neurite outgrowth; 7,8,3'-trihydroxyflavone; age-related effectiveness

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P60. THERMOSENSITIVE DRUG DELIVERY SYSTEMS: A PREFERRABLE SHUTTLE FOR NANOPARTICLES TO THE INNER EAR?

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Drug delivery to the inner ear by local intratympanic administration of thermosensitive hydrogels directly onto the round window membrane (RWM) emerged as a beneficial approach to reach therapeutic relevant drug levels in the inner ear [1]. Hydrogels made from the FDA-approved Poloxamer 407 (POX 407) are liquid at room temperature but form a hydrogel at 37°C [2] and can be easily applied intratympanal. The gelation on the RWM prolongs the contact time of the drug with the diffusion barrier and higher drug levels in the perilymph are expected. As the upper size limit for permeation through the RWM is 150 nm [3], incorporation of biodegradable and biocompatible Poly-lactic-co-glycolic-acid nanoparticles (PLGA-NP) into the thermosensitive transport system might prolong the release and efficacy of glucocorticoids.

To approach this aim, the glucocorticoid *Triamcinolone acetonide* (TAAc) was encapsulated into PLGA-NP and the manufacturing parameters were optimized to downsize the diameter as well as to increase the drug loading. In-vitro release studies were performed with TAAc-PLGA NP loaded thermoreversible micellar hydrogels based on POX 407 using an established in-vitro model consisting of a cellulose filter with 0.4 µm pore diameter mimicking the RWM and artificial perilymph fluid as acceptor medium. The volume ratio between hydrogel and perilymph fluid was set close to in-vivo conditions.

The optimized TAAc-PLGA NP possess a hydrodynamic diameter of 100-120 nm with a polydispersity index of 0.21±0.03 and a remarkable drug loading of 31-35.0%. In preliminary in-vitro release studies the highest amount of 4.46% TAAc was released from hydrogels containing TAAc-PLGA-NP of 109 nm after 24 hours as compared to 2.43% TAAc from 121.8 nm PLGA-NP or 3.53% TAAc from drug particles with 180 µm in diameter. The initial release rate was similar in all formulations.

All in all, smaller particles diffuse more rapidly through the membrane and prolongation of the release of TAAc increases with the PLGA-load of the NP. Thus, injectable micellar thermoreversible drug delivery systems containing TAAc-loaded PLGA-NP seem to be beneficial for intratympanic therapy of inner ear diseases and traumata after cochlear implantation.

Keywords: micellar thermosensitive hydrogel, Triamcinolone acetonide, PLGA-nanoparticle, drug delivery, in-vitro release

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P61. THE NEUEAR PROJECT: DEVELOPING A NEUROTROPHIC ENCAPSULATED CELL IMPLANT FOR SEVERE HEARING LOSS

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Sensori-neural hearing loss (SNHL) results from a severe damage or complete loss of the hair cells and/or spiral ganglion neurons (SGN) in the hearing organ caused by aging, noise, ototoxic drugs, genetic disorders. For severe SNHL, a cochlear implant is the only clinical treatment available today. It provides auditory cues by electrically stimulating the residual auditory neurons [1]. However, the loss of hair cells causes an ongoing degeneration of SGNsandthe efficiency of the cochlear implant may decline over the years[2-4]due to a loss of trophic support otherwise provided by the hair cells. Neurotrophins (NTs), in particular neutrophin-3 (NT3), brain derived neurotrophic factor (BDNF), glial-cell derived neurotrophic factor (GDNF) have been shown to play key roles in auditory neurons development and survival [5-7]. Administration of NTs to the deafened inner ear has been shown to promote auditory neuronal survival and peripheral fiber regrowth[5,6,8].A long-term NT source is needed for prolonged neural protection. The encapsulated cell devices (ECD) is a novel and innovative devicethat uses live genetically engineeredcells producing a a therapeutic proteini.g. NTs (Figure 1).Cell are encapsulated in semipermeable polymer capsulesthat allows the influx of nutrients and the outflow of the NTs. The ECDcan then be implanted into the target sites. Here, we tested the potency of different neurotrophins (NT) produced by genetically modified ARPE-19 cell clones that are used in ECDs later. Effects of BDNF, GDNF, and CDNF produced by ARPE-19 clones were tested *in vitro* onratauditory neurons. Thedissociated cells or explants of spiral ganglion were incubated with different concentrations of the NT. An increase in neuron survival was observed when treated with BDNF, and an increased neurite outgrowth as well as survival was seen in the SGN treated with high concentrations of GDNF secreted from the ARPE-19-NT cell clones. Furthermore, SG explants were incubated with a NT-producing ECD to study the directional effect of the ECD on neurite outgrowth. Intra-cochlear delivery of neurotrophic factors with beneficial protective and/or regenerative effects on SGN could significantly improve the CI/patient interface, leading to a better treatment of profoundly deaf patients.

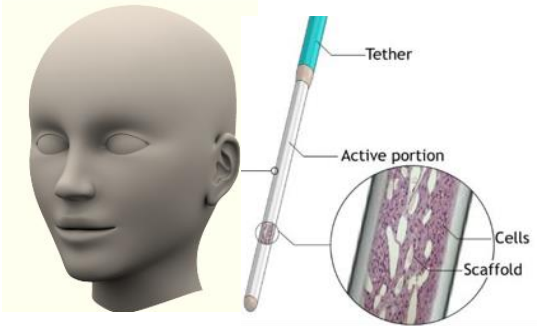

Figure 1: The encapsulated cell implant. A few millimeter-long tube consist two parts a tether (inactive part for holding) and the active portion containing genetically modified human cells, encapsulated by the membrane. The cells secrete NT that diffuses in to the surrounding tissue i.e. inner ear.

Keywords: Spiral ganglion neurons, neurotrophic factors, encapsulated cell device, cochlear implant, BDNF, GDNF.

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P62. EVALUATION OF HEARING AID'S SUCSESS PROBABILITY BASED ON AIDED AUDITORY STEADY STATE RESPONSE THRESHOLDS

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Background and Aim: Some authors believe that aided thresholds can be used to predict hearing aid success and to decide between hearing aid and coclear implant [1-6]. The aim of this study was to evaluate if the ASSR could anticipate the hearing aid sucsss; and to compare characteristics of aided and unaided responses in the unifrequensy versus simultaneous presentation modes.

Method: 10 normal hearing and 26 severe to profound hearing impared subjects participated in this study. The subjects underwent acoustic immitance, sound field behavioral audiometery and auditory steady state response, at first without hearing aid and then with it.

Results: Despite observing any response withot hearing aid, aided thresholds,however, could be recorded in some frequencies. Aided responses were recorded in fewer frequencies in subjects with poorer speech clarity and speech reading. Multifrequency stimulation did not get the ASSR response affected in the moderate intensities (about 60 dB HL) or in the normal subjects, but it did elevated the thresholds in the highier presentation levels, especially for the highier frequencies. This elevation was more prominent in the aided condition.

Conclusion: The probability of hearing aid sucsess seems to be very poor if auditory steady state responses (especially aided responses) could not be recorded [7-8]. Simultaneous presentation can make the response of the highier frequncy regions of the basilar membrane to be interfered with the lower frequenccy parts (probably due to unnatural tuning curves in the damaged cochlea); and so, threshold overestimation [9-10]. In conclusion, special care should be taken about haring aid's settings and its fitting, testing environment and response repeatability for the aided evaluations.
Key words: Auditory Steady State Response, hearing aid, hearing loss

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P63. METAMORPHOSIS OF WAVE PROCESSES IN INNER EAR: Part 1. FROM VIBRATIONS TO WAVES AND BACK

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Objectives: The sound in the inner ear arises by the fluctuations of round window membrane under the action of the stapes in the form of a wave, running on the perilymph. The further transformation of it is a big problem of the otology, the main issue of which is the possibility of converting the energy of the sound into the nerve impulses. The mechanisms of such a transformation still remain unclear.

Methods: The study is based on analysis of real physical laws and mathematical modeling.

Results: In general [1], the longitudinal sound wave of pressure, spreading across the perilymphatic channel as through the waveguide, passes from the vestibular duct into the tympanic and, performing there the role of the running wave, in fact, becomes reflected, because it is directed against the axis of the cochlea. Reflected from the membrane of the round window it actually becomes direct because it spreads towards the cochlear axis. When the wave returns in the vestibular duct, it is reflected both in form and in substance. As a result of the interference of the direct and reflected waves of the same frequency are formed the standing waves of pressure inside the perilymphatic channel. However, the standing wave is not a wave process.

Due to the elasticity of the membranes of the middle duct and the constancy of the fluid volume, the changes of pressure in the perilymph leads to transverse deformation of the vestibular and basilar membranes and their oscillatory motion. The basilar membrane through the structures, existing on it, directly causes displacements of the auditory receptors. Because of the remoteness from the receptors, the vestibular membrane at first have to create a longitudinal running wave of the pressure in the endolymphatic duct, and only after that – fluctuations of the tectorial membrane with consequent impact on the receptor cells.

Conclusion: Mathematical modeling suggests [2] that such processes are typical for waves of any frequency sound range.

Keywords: acoustic-wave hearing model, cochlear membrane, inner ear, sound wave, vibration.

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P64. METAMORPHOSIS OF WAVE PROCESS IN INNER EAR: PART 2. SOUND ENERGY WAYS AT PRE-RECEPTOR LEVEL

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Objectives: The sound energy enters the cochlea as a kinetic energy of the running longitudinal wave, created by the vibrations of the stapes in the round window of the vestibular membrane of perilymphatic canal. The wave energy flux of this direction is not capable to transmit the energy to the auditory receptors for their activation. This explains the complexity of the structure of the cochlear duct and the presence of the middle duct [1].

Methods: The study is based on analysis of real physical laws and mathematical modeling.

Results: The kinetic energy of the running waves, due to the reflection from the membrane of the round window and interference with the emerged standing waves, is converted into potential energy of deformation of the basilar membrane and the Reissner's membrane and is divided between them equally with changing in direction of flow distribution. The first part of the energy from the basilar membrane is directly converted through the structures of the inner ear, lying on the membrane, into the kinetic energy of the oscillating movements of auditory receptors.

The multimodal perception of the volume at each frequency in each coordinates of the cochlear duct requires the complication of the organ. The asymmetry of the middle duct in comparison with the auditory receptors causes the primary transform of the potential deformation energy of the Reissner's membrane into the kinetic energy of the wave motion of the particles of the endolymph. Thereafter it is completely converted into the potential energy of oscillations of the tectorial membrane and then divided into two parts for interaction of tectorial membrane with the outer and the inner hair cells.

Conclusion: Mathematical modeling suggests [2] that such processes are typical for waves of any frequency sound range.

Keywords: acoustic-wave hearing model, cochlear membrane, inner ear, sound energy, transmission of the energy.

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P65. A FRAMEWORK FOR MULTIDISCIPLINARY PROJECT WORK WITH COCHLEAR IMPLANT USERS: INVOLVING CI USERS IN DEVELOPING REHABILITATION TOOLS TO IMPROVE PERFORMANCE IN MULTI-SPEAKER CONVERSATION

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Simultaneous or overlapping talk by two or more speakers (OT) is surprisingly frequent. In casual conversations among young British adults recorded for a recent project it occupies 16% of total talking time, while 41% of speaker turns are overlapped by another speaker. A recent survey as part of this interdisciplinary project has shown OT is a common problem for Cochlear Implant (CI) users. As in neuropsychological modelling, understanding both typical (normal hearing) and atypical (CI) performance can contribute to modelling task-based performance. In this case we have a detailed corpus comprising audio-visual recordings of real time multi-speaker conversation and associated interactional analyses of these materials related to overlapping talk management. This corpus allows an understanding of the acoustic complexity of the task in more detail and can be correlated with known performance limits of CI users across listening tasks. In the current project this information is used to develop rehabilitative to assist CI users to improve performance of multi-speaker overlapping talk, a task that has high level acoustic and multi-modal cognitive demands. The improvements in CI performance over the last decade through surgical technique and signal processing and the in-depth understanding of CI performance in real-time acoustic environments now place us in a position to explore more complex listening skills with CI users in relation to clinical needs for rehabilitation. The framework for this project provides clinical access to research developments by involving users in the project and in addition by involving clinicians and rehabilitation specialists it allows us to consider how we integratenew research findings into everyday NHS practice in a timely fashion, an important aspect of multidisciplinary CI research as translational research gathers pace.

Keywords: Cochlear Implant, Conversational Analysis, Simultaneous Talk.

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P66. IMPACT OF HYPERBARIC OXYGEN THERAPY ON PROLIFERATION AND BDNF-RELEASE OF GENETICALLY MODIFIED NIH3T3 FIBROBLASTS

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Hyperbaric oxygen therapy (HBOT) is a noninvasive modality that increases the oxygen tension of tissues and is used in different areas of medical practice: for an enhancement of wound healing or to cure decompression sickness. HBOT leads to an increased proliferation of fibroblasts. In cochlear implant (CI) research, delivery of neurotrophic factors from CI electrodes improves survival of spiral ganglion neurons (SGN). These factors such as brain-derived neurotrophic factor (BDNF) can be delivered by genetically modified murine fibroblasts (NIH3T3) from the surface of the CI electrode materials. We hypothesize that HBOT enhances the release of BDNF from these cells.

The cells were grown in 96-well plates at different seeding densities and incubated in a compression chamber. Cells were repeatedly exposed to 100% oxygen at pressures of 1.0, 1.5 and 2.0 bar for 90 minutes. The effects of HBOT on cell proliferation and BDNF production of recombinant and native NIH3T3 cells were investigated in relation to the applied pressure and the number of treatments.
Both, the recombinant and native NIH3T3, showed an increased proliferation after 5 days of HBOT in comparison to the control. Further *in vitro* data indicate that survival of SGN is enhanced when these cells are cultivated in the supernatant of the fibroblasts treated with HBOT.

Our data demonstrated that HBOT has positive effects on the secretion of neurotrophic factors from BDNF producing fibroblasts.

Keywords: Hyperbaric oxygen therapy (HBOT), fibroblasts, BDNF secretion, neuroprotection, spiral ganglion neurons (SGN)

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P67. DELAYED DEVELOPMENT OF CEREBRAL WHITE MATTER IN CHILDREN WITH CONGENITAL DEAFNESS

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Objectives: Removal of sensory receptors or changes in afferent activity have profound effects on the maturation of neuronal structure. Also, in congenital deaf children, it is supposed that a similar phenomenon would take place and that development delay of the white matter tract would be induced. However, the development of white matter has not yet been evaluated in the prelingually deaf children. The purpose of this study was to investigate the differences of white matter development in the congenitally deaf children as compared to normal hearing ones using tract-based spatial statistics (TBSS) method.

Methods: Diffusion tensor imaging (DTI) was performed in 21 congenitally deaf (DEAF group) and 20 normal hearing subjects (HEAR group) from 1.7 to 7.7 years old. Using TBSS, we evaluated the regions of significant difference in fractional anisotropy (FA) values between groups. To evaluate the correlations between FA values and age in each group, we examined voxel-wise correlation analyses on the TBSS skeleton.

Results: Lower FA values at the white-matter tracts to Heschl's gyrus, inferior fronto-occipital fasciculus, uncinate fasciculus, superior longitudinal fasciculus, forceps major were found in DEAF group than in HEAR group less than 4 years old, but significant difference was not found between groups in subjects older than 4 years old. We also found the age-related development of the white-matter tract may continue until 8 years old in deaf children.

Conclusions: These results suggest the delayed development of cerebral white matter tracts in the congenitally deaf children.

Key words: Congenital deafness, diffusion tensor imaging, fractional anisotropy, white matter

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P68. DIFFERENTIAL REGULATION OF RESPONSIVENESS IN CENTRAL CIRCUITRIES TO AUDITORY DEPRIVATION UNDERLIE THE GENERATION OF HYPERACUSIS AND TINNITUS

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Noise overexposure has been linked to various auditory perceptual abnormalities including tinnitus and hyperacusis. Tinnitus, the persistent perception of phantom sound, has been observed to be related to a failure of the central auditory pathway to adapt to a critical loss of afferent fibers (Rüttiger et al. 2013). The present study aimed to assess differences in central responsiveness, deafferentation in the periphery and cortical plasticity of equally noise exposed animals either with preferential hyperacusis, tinnitus or none of both.

We recorded the auditory brainstem response (ABR) and distortion products of the otoacoustic emissions of rats before and after intense noise exposure and compared the data obtained from animals that showed signs for behaviorally tested hyperacusis percept, data from rats exhibiting preferentially tinnitus-like-behavior and data from rats did not show evident signs of altered auditory perception. We investigated the degree of deafferentation of inner hair cells by analyzing the number of CTBP2/RIBEYE positive presynaptic ribbons and analyzed the expression of activity dependent genes in the inferior colliculus and the auditory cortex. Expression data were correlated to the altered ABR wave size for differentially behavioral animal groups. The results are discussed in the context of the role of different responsiveness in central circuitries and the degrees of deafferentation underlying the generation of hyperacusis and tinnitus.

Keywords: hyperacusis; tinnitus; deafferentation; central responsiveness

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