IEB 2011

48th Inner Ear Biology Workshop

Symposium on "Improving health and quality of life in children and elderly with hearing impairment"

Torre do Tombo, Lisbon | September 18th - 21st

PROGRAMME AND ABSTRACT BOOK

BioFIG
Center for Biodiversity, Functional and Integrative Genomics

FACULDADE DE CIÊNCIAS
UNIVERSIDADE DE LISBOA

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Torre do Tombo, Lisbon

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"Improving health and quality of life in children and elderly with hearing impairment"

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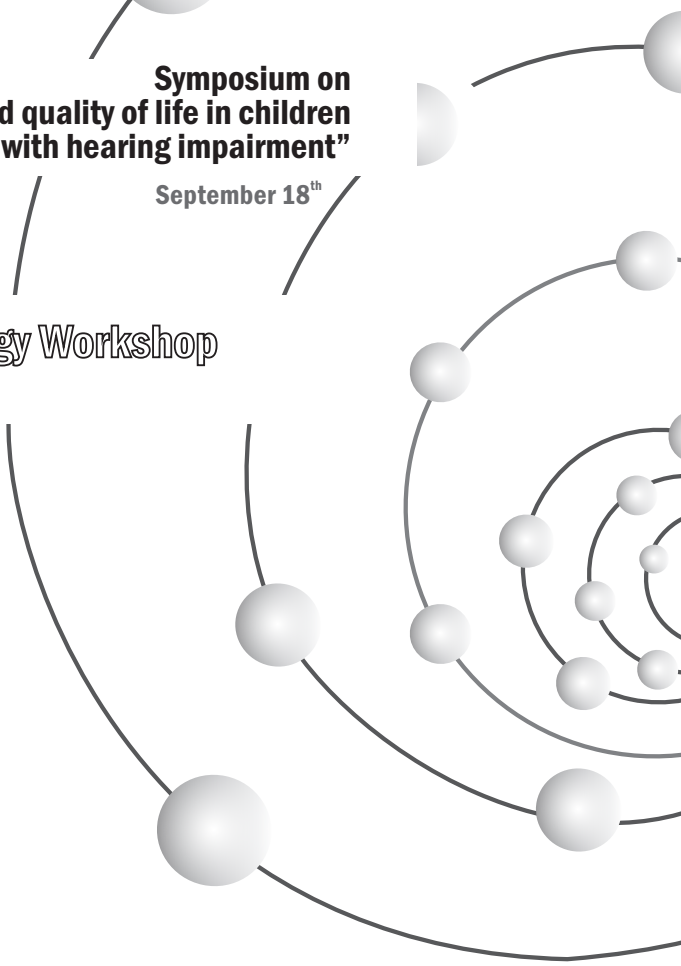

Dear colleagues,

It is with great pleasure that we welcome you to IEB 2011, the 48th Inner Ear Biology Workshop, which is being held for the first time in Lisbon, Europe's Westernmost Capital and one of the oldest cities in the world.

The history of a University in Lisbon goes back to the 13th century, when King Dinis founded the first Portuguese university school in Lisbon. But 250 years later, after moving several times between Lisbon and Coimbra, the university moved definitively to Coimbra. The current University of Lisbon (UL), composed by eight faculties with a total of about 24.000 students, was created in 1911, after the fall of the monarchy, and is now celebrating the 1st Centenary.

We hope that IEB 2011 may provide a stimulating opportunity for an interchange of ideas on most recent advances in the field of inner ear biology that interest both clinicians and research scientists. It can also be the opportunity for students and young scientists to enrich their knowledge on cutting-edge topics of inner ear biology and related areas.

Lively and melancholic, old and trendy, Lisbon is a unique and fascinating city, a place to get lost in, discovering its many distinctive sights and characteristic images.

We wish you all an exciting, enjoyable stay in Lisbon!

Graça Fialho
Chair of the Organizing Committee

Honorary President

Alessandro Martini, MD, PhD (University of Padova)

Organizing Committee

Assunção O'Neill, MD

Carlos Ribeiro, MD

Graça Fialho, PhD, Chair

Helena Caria, PhD, Co-Chair

Helena Rosa, MD

Luísa Monteiro, MD

Óscar Dias, MD, PhD

Scientific Committee

António M. Diogo Paiva, MD, PhD

Graça Fialho, PhD

Helena Caria, PhD

José Carlos Rosmaninho Seabra, MD

José Francisco Madeira da Silva, MD, PhD

Luís Antunes, MD

Manuel Diamantino Bicho, MD, PhD

Mário Andrea, MD, PhD

Nuno Trigueiros, MD, PhD

Óscar Dias, MD, PhD

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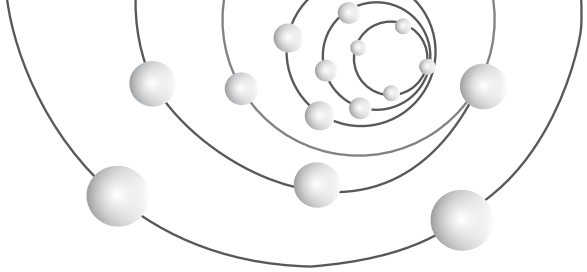


GENERAL PROGRAMME OVERVIEW

Sunday, September 18th

09:00 - 18:00 Symposium on "Improving health and quality of life in children and elderly with hearing impairment"

18:30 - 20:00 Welcome Reception - City Museum

Monday, September 19th

08:30 - 17:45 IEB 2011 Oral Sessions I to IV and Posters

Tuesday, September 20th

08:30 - 17:30 IEB 2011 Oral Sessions V to VIII and Posters

17:30 - 18:00 IEB Business Meeting

20:00 Gala Dinner - Centro Cultural de Belém
'' A Commenda''

Wednesday, September 21st

08:30 - 12:30 IEB 2011 Oral Sessions IX to X

12:30 - 13:00 Closing remarks and End of the Meeting

SYMPOSIUM PROGRAMME

Sunday, September 18th | Morning

09:00 Onwards — **Registration**

09:30 - 10:00 — **Welcome session**
Graça Fialho, Chair of IEB 2011
J. M. Pinto Paixão, Director of the Faculty of Science of Lisbon

SESSION I: DEAFNESS IN CHILDREN
Moderators: *António D. Paiva, Helena Caria*

10:00 - 10:20 — S1 — **Childhood deafness – A multidisciplinary approach**
Óscar Dias, M. Andrea

10:20 - 10:40 — S2 — **Neonatal hearing screening: current situation at national and international level**
Luisa Monteiro

10:40 - 11:00 — Coffee break

SESSION II: STRATEGIES FOR EDUCATION AND REHABILITATION IN CHILDREN AND ELDERLY
Moderators: *Óscar Dias, António Ferreira*

11:00 - 11:20 — S3 — **Models and practices of early intervention with deaf children at the CED Jacob Rodrigues Pereira**
Maria José Cascalho, Conceição Coelho

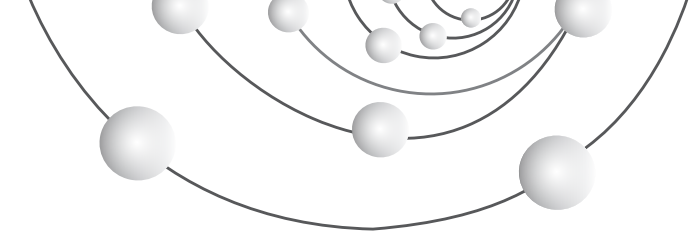
11:20 - 11:40 — S4 — **Speech Therapy in Schools of Reference for the Bilingual Teaching of Deaf Students**
Teresa Neto Carvalho

11:40 - 12:00 — S5 — **Hearing and aural rehabilitation in elderly population**
Jorge Humberto Martins

12:00 - 12:20 — S6 — **Rehabilitation, performance and more: addressing local needs**
Eulalia Juan

12:20 - 12:40 — S7 — **Hearing Rehabilitation in profoundly deaf children: the present situation**
Carlos Ribeiro

12:40 - 13:00 — S8 — **Cochlear implants: The state of art**
Manuel Manrique



Sunday, September 18th | Afternoon

13:00 - 14:30 Lunch

14:30 - 15:15 **S9** **SESSION III: ROUND TABLE ON 'HEARING REHABILITATION IN MULTIDEFICIENCY'**
Chairpersons: *Manuel Manrique, Luis Antunes*
Moderator: *Assunção O'Neill*
Alicia Huarte, Helena Rosa, M. José Lavilla, Pedro Cabral

SESSION IV: FUTURE TRENDS ON DIAGNOSTIC AND THERAPY
Moderators: *Graça Fialho, Nuno Trigueiros*

15:30 - 16:00 **S10** **Genetics for health professionals**
Karen Avraham

16:00 - 16:30 **S11** **Nanotechnology based targeted drug delivery**
Jean Luc Puel

16:30 - 17:00 Coffee break

17:00 - 17:40 **Tribute to Prof. Alessandro Martini**
Mario Andrea

Electrical stimulation of the ear: a long history starting in the eighteenth century
Alessandro Martini

17:40 - 18:00 **Percussion Music by the Group "Ritmo(s)",**
CED Jacob Rodrigues Pereira

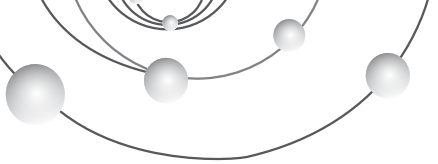
18:30 - 20:00 **Welcome Reception – City Museum**
Mandolin Music by Vicentuna, Tuna of the Faculty of Science of the University of Lisbon

Official languages: English and Portuguese

WORKSHOP PROGRAMME OVERVIEW

Monday, September 19th

08:30 Onwards	**Registration**
09:00 - 09:15	**Welcome and Workshop opening**
09:15 - 09:45	**KEYNOTE LECTURE**
09:45 - 10:30	**ORAL SESSION I** **Developmental Biology (O1 - O3)**
10:30 - 11:00	Coffee Break
11:00 - 12:30	**ORAL SESSION II** **Basic hearing and vestibular research (O4 - O9)**
12:30 - 14:30	Lunch and Poster Viewing
14:30 - 16:00	**ORAL SESSION III** **Cochlear mechanics (O10 - O15)**
16:00 - 16:30	Coffee Break
16:30 - 17:45	**ORAL SESSION IV** **Inner ear function and homeostasis (O16 - O20)**

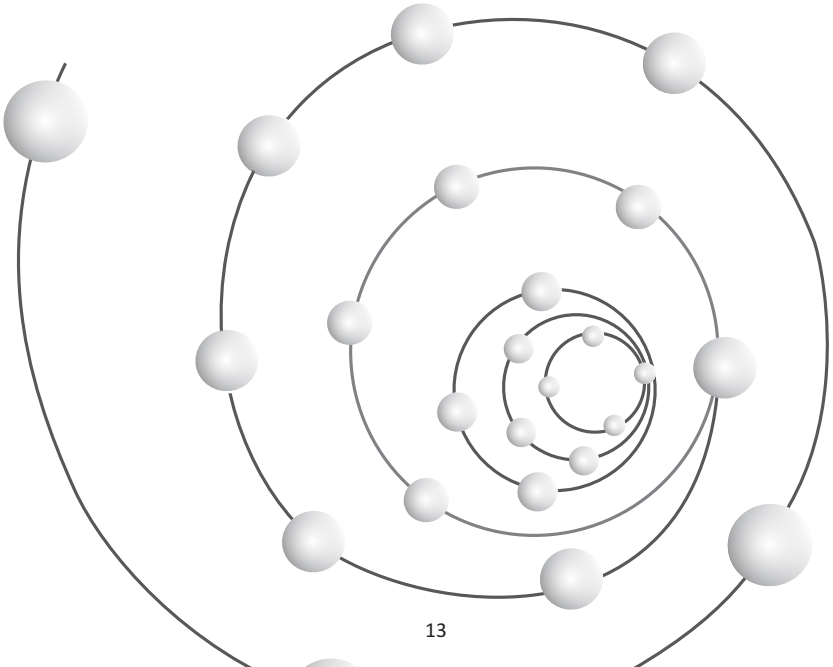


Tuesday, September 20th

08:30 Onwards	**Registration**
09:15 - 09:45	**KEYNOTE LECTURE**
09:45 - 10:30	**ORAL SESSION V** **Ototoxicity, protection and regeneration I (O21 - O23)**
10:30 - 11:00	Coffee Break
11:00 - 12:30	**ORAL SESSION VI** **Ototoxicity, protection and regeneration II (O24 - O29)**
12:30 - 14:30	Lunch and Poster Viewing
14:30- 14:45	**Group Photo**
14:45 - 16:00	**ORAL SESSION VII** **Inner ear (histo)pathologies (O30 - O35)**
16:00 - 16:30	Coffee Break
16:30 - 17:30	**ORAL SESSION VIII** **Hearing genetics I (O36 - O39)**
17:30 - 18:00	**IEB Business Meeting**
20:00	Gala Dinner - Centro Cultural de Belém '' A Commenda''

Wednesday, September 21st

08:30 Onwards	**Registration**
09:30 - 10:30	**ORAL SESSION IX** **Hearing genetics II (O40 - O43)**
10:30 - 11:00	Coffee Break
11:00 - 12:30	**ORAL SESSION X** **Clinical studies (O44 - O49)**
12:30 - 13:00	**Closing remarks and End of the Meeting**
13:00	Lunch



GENERAL INFORMATION

CONFERENCE VENUE

The Symposium and Workshop will be held at Torre do Tombo (Tower of the Tomb), the Portuguese National Archive, located at the Campus of the University of Lisbon, right beside the Faculty of Science.

TRANSPORTS

You can easily access Torre do Tombo by bus (autocarro in Portuguese), underground (Metro in Portuguese) and taxi. Locations of the underground stations (Campo Grande and Cidade Universitária) are represented in the map and you just take about 7 minutes to reach Torre do Tombo.

REGISTRATION

The registration desk will be staffed in the lobby of the "Torre do Tombo" and will be open on:

Sunday, 18th September: 09:00 - 18:00
Monday, 19th September: 08:30 - 17:00
Tuesday, 20th September: 08:30 - 17:00
Wednesday, 21st September: 08:30 - 10:00

OFFICIAL LANGUAGES

Symposium - English and Portuguese
Workshop - English

ORAL PRESENTATIONS

Time allotted to speakers is 12 minutes with an additional 3 minutes for discussion. Speakers should contact the Slide Center to hand in their presentations for uploading at least one hour before the beginning of the session or in the late afternoon of the day before, in case of early morning presentation.

POSTERS

Posters are on display for the duration of the Workshop, from Monday morning 8:30 onwards. Posters should be removed on Wednesday morning not later than 11:00.
Authors should be at their posters during poster presentation time (even numbers on Monday 19, odd numbers on Tuesday 20).

COFFEE AND REFRESHMENTS - LUNCHES

Coffee and refreshments will be provided at the session breaks; lunches will be served in the lobby of the conference auditorium.

WORKSHOP DETAILLED PROGRAMME

Monday, September 19th

08:30 Onwards — **Registration**

09:00 - 09:15 — **Welcoming remarks**

09:15 - 09:45 — **KEYNOTE LECTURE**

GROWTH FACTOR-BASED THERAPY FOR THE PREVENTION AND REPAIR OF HEARING LOSS: THE CASE OF INSULIN-LIKE GROWTH FACTOR I
Isabel Varela-Nieto

ORAL SESSION I: Developmental Biology (O1- O3)
Moderator: Allen Ryan

09:45 **O1** **REGULATION OF NEURITE PATHFINDING IN TYPE II SPIRAL GANGLION NEURONS DIFFERS FROM THAT IN TYPE I NEURONS**
Ryan AF, Pak K, Sung M, Wei E, Parsi A, Jonathan Cheng J, Housley G, Brand Y

10:00 **O2** **TSUKUSHI GENE EXPRESSION IN THE MOUSE COCHLEAE**
Ryosei Minoda, Toru Miwa, Eiji Yumoto

10:15 **O3** **OVEREXPRESSION OF ISL1 PRODUCES CHANGES IN THE AUDITORY AND VESTIBULAR SYSTEMS IN MICE**
Pavlinkova G, Chumak T, Kuthanova L, Bohuslavova R, Buckiova D, Syka J

10:30 - 11:00 — Coffee Break

ORAL SESSION II: Basic hearing and vestibular research (O4 - O9)
Moderators: Sjaak Kliss, Norio Yamamoto

11:00 **O4** **SHORT-TERM SYNAPTIC PLASTICITY DETERMINES THE LEVEL OF OLIVOCOCHLEAR INHIBITION TO AUDITORY HAIR CELLS**
Jimena Ballestero, Javier Zorrilla de San Martín, Juan Goutman, Paul Fuchs, Ana Belén Elgoyhen, Eleonora Katz

11:15 **O5** **PARKIN DEFICIENCY CAUSES PROGRESSIVE HEARING LOSS IN MICE THROUGH OUTER HAIR CELL LOSS**
Norio Yamamoto, Kiyomi Hamaguchi, Takayuki Nakagawa, Juichi Ito

11:30 **O6** **K-ATP CHANNEL KNOCKOUT MICE ARE PROTECTED AGAINST PRESBYACUSIS**
Manuel Groth, Silvi Hoidis, Jean Smolders, Jochen Roeper

11:45 **O7** **CORTICOSTEROID TREATMENT OF IDIOPATHIC SUDDEN SENSORINEURAL HEARING LOSS. TRIAL OR ERROR?**
Elisabeth Hultcrantz, Ramesh Zarenoe

12:00 **O8** **MORPHOMETRICAL DIFFERENCES OF HUMAN SPIRAL GANGLION CELLS: WHAT DOES IT MEAN FOR ELECTRICAL STIMULATION?**
Thomas Potrusil, Cornelia Wenger, Rudolf Glueckert, Anneliese Schrott-Fischer, Frank Rattay

12:15 **O9** **RELATIVE COCHLEAR AND VESTIBULAR OTOTOXICITY PRODUCED BY TRANSTYMPANIC GENTAMICIN UNDER RECOMBINANT HUMAN ERYTHROPOIETIN CYTOPROTECTION IN THE RAT**
Luís L, Rocha J, Castro Caldas, A, Mota Filipe H, Sepodes B

12:30 - 14:30 Lunch and Poster Viewing
(Presentation by authors of even number posters)

ORAL SESSION III: Cochlear mechanics (O10 - O15)
Moderators: Jonathan Ashmore, Glen Martin

14:30 **O10** **THE CHLORIDE-CHANNEL INHIBITOR ANTHRACENE-9-CARBOXYLIC ACID REVERSIBLY BLOCKS THE MOTOR PROTEIN PRESTIN**
Csaba Harasztosi, Anthony W. Gummer

14:45 **O11** **REAL TIME MONITORING OF PRESTIN INSERTION INTO THE PLASMA MEMBRANE**
Bian S, Navaratnam K, Santos-Sacchi J

15:00 **O12** **ORGAN OF CORTI MICROMECHANICS MEASURED WITH LOW COHERENCE INTERFEROMETRY**
Zha D, Chen F, Ramamoorthy S, Choudhury N, Fridberger A, Nuttall AL

15:15 **O13** **A REPORT OF EXTENDED HIGH FREQUENCY AUDIOMETRY THRESHOLDS IN SCHOOL- AGED CHILDREN WITH NO HEARING COMPLAINTS**
Cavalcante JMS, Radael RD, Anastasio ART, Hatzopoulos S

15:30 **O14** **ANALYSIS OF AUDITORY BRAINSTEM RESPONSE BY USE OF C**LICK AND TONEBURST STIMULUS IN TERM AND PRETERM NEONATES.
Cavalcante JMS, Isaac ML

15:45 **O15** **AUDITORY EVALUATION IN PATIENTS TREATED WITH RADIOTHERAPY AND CHEMOTHERAPY EXCLUSIVE OR COMBINED.**
Isaac ML, Dell´aringa AHB, Arruda GV

16:00 - 16:30 Coffee Break

ORAL SESSION IV: Inner ear function and homeostasis (O16 - O20)
Moderators: Joseph Syka, Marlies Kuipper

16:30 **O16** **A NEW ANIMAL MODEL FOR MENIERE´S DISEASE**
Akinobu Kakigi, Naoya Egami, Takashi Sakamoto, Rie Nishioka, Masamitsu Hyodo, Taizo Takeda, Tatsuya Yamosoba

16:45 **O17** **DIRECT ENTRY OF GD-DTPA INTO THE VESTIBULE FOLLOWING INTRATYMPANIC APPLICATION IN GUINEA PIGS**
Elisha King, Alec N. Salt, Hayden Eastwood, Stephen O´Leary

17:00 **O18** **MYOSIN VIIA AND SANS LOCALIZATION AT STEREOCILIA UPPER TIP-LINK DENSITY IMPLICATES THESE USHER SYNDROME PROTEINS IN MECHANOTRANSDUCTION**
M´hamed Grati, Bechara Kachar

17:15 **O19** **ACOUSTIC OSCILLATION OF GUINEA PIG STAPES VISUALIZED THROUGH HIGH-SPEED VIDEO CAMERA ANALYSIS**
Mitsuru Ohashi, Nozomu Matsumoto, Takashi Kimitsuki, Shizuo Komune

17:30 **O20** **OPEN FOR DISSECTION: HEARING IN THE FRUIT FLY DROSOPHILA**
Joerg T. Albert

Tuesday, September 20th

08:30 Onwards — **Registration**

09:15 - 09:45 — **KEYNOTE LECTURE**

NOVEL THERAPIES RELATED TO COCHLEAR IMPLANTS
Astolfi L, Martini A.

ORAL SESSION V: Ototoxicity, protection and regeneration I (O21 - O23)
Moderators: J. Schacht, Óscar Dias

09:45 **O21** **DEVELOPMENT OF A POLYMERIC COATING FOR COCHLEAR IMPLANT ELECTRODES TO DELIVER DEXAMETHASONE INTO THE INNER EAR**
Piera Ceschi, Anne Roock, Katrin Sternberg, Klaus-Peter Schmitz, Thomas Lenarz, Manfred Kietzmann, Timo Stöver, Gerrit Paasche

10:00 **O22** **SIOP PLATINIUM END OF TREATMENT OTOTOXXICITY SCALE**
Dr. Kaukab Rajput

10:15 **O23** **COMBINING CELL-BASED THERAPIES AND A COCHLEAR IMPLANT TO PROMOTE NEURAL SURVIVAL**
Andrew Wise, James Fallon, Alison Neil, Lisa Pettingill, Marilyn Geaney, Robert Shepherd

10:30 - 11:00 — Coffee Break

ORAL SESSION VI: Ototoxicity, protection and regeneration II (O24 - O29)
Moderators: Jean-Luc Puel, Nuno Trigueiros

11:00 **O24** **EFFCTS OF DEXAMETHASONE ELUTING IMPLANTS ON POSTOPERATIVE HEALING - A HISTOLOGICAL STUDY**
Anne Jakob, Katharina Niedermeier, Susanne Braun, Henning Bier, Fred Sinowatz, Thomas Stark

11:15 **O25** **NEURONAL SUPPORTING AND SURVIVAL MECHANISM IN HUMAN COCHLEA**
Wei Liu, Helge Rask-Andersen, Marja Boström, Anders Kinnefors

11:30 **O26** **IN VITRO PROTECTION OF THE AUDITORY HAIR CELLS BY SALICYLATE FROM GENTAMICIN-INDUCED BUT NOT NEOMYCIN-INDUCED-LOSS**
Agnieszka J. Szczepek, Xiangxin Lou, Heidi Olze, Heidemarie Haupt, Birgit Mazurek

11:45 **O27** **HEMATOPOIETIC STEM CELLS PREVENT HAIR CELL DEATH AFTER TRANSIENT COCHLEAR ISCHEMIA THROUGH PARACRINE EFFECTS**
Nobuhiro Hakuba, Tadashi Yoshida, Kiyofumi Gyo

12:00 **O28** **NOVEL AMINOGLYCOSIDE DERIVATIVES WITH REDUCED OTOTOXICITY AND ENHANCED SUPPRESSION OF DISEASE-CAUSING PREMATURE STOP MUTATIONS**
Baasov T, Belakhov V, Kandasamy J, Cherniavsky M, Hainrichson M, Xie J, Schacht J

12:15 **O29** **ADENOSINE AMINE CONGENER AMELIORATES CISPLATIN-INDUCED HEARING LOSS**
Srdjan Vlajkovic, Niliksha Gunewardene, Cindy Guo, Ann Wong, Gary Housley, Peter Thorne

12:30 - 14:30 Lunch and Poster Viewing
(Presentation by authors of odd number posters)

14:30 - 14:45 **Group Photo**

ORAL SESSION VII: Inner ear (histo)pathologies (O30 - O35)
Moderators: Anthony Gummer, Alfred Nuttal

14:45 **O30** **MRI MACROPHAGE DETECTION IN A GUINEA PIG MODEL OF INNER EAR INFLAMMATION**
Le Floc´h J, Tan W, Telang RS, Vlajkovic SM, Pontre B, Thorne PR

15:00 **O32** **MORPHOLOGICAL CHANGES OF SPIRAL GANGLION CELL DENDRITES AFTER INTRACOCHLEAR APPLICATION OF BRAIN-DERIVED NEUROTROPHIC FACTOR IN DEAFENED GUINEA PIGS**
Waaijer L, Klis SFL, Van Deurzen MHW, Hendriksen EGJ, Grolman W

15:15 **O33** **A GUINEA PIG MODEL OF PARTIAL DEAFNESS**
Havenith S, Klis SFL, Versnel H, Grolman W

15:30 **O34** **STRUCTURAL AND MOLECULAR CHANGES IN THE LATERAL WALL OF THE COCHLEA OF MICE WITH AGE-RELATED HEARING LOSS**
Paramanthasivam V, Vlajkovic SM, Housley GD, Donaldson PJ, Thorne PR

15:45 **O35** **TNF-ALPHA COMPROMISES THE INNER EAR MICROCIRCULATION VIA ACTIVATION OF ENDOGENOUS S1P SIGNALLING: A TRANSLATIONAL STUDY FOR THE TREATMENT OF SSHL**
Scherer EQ, Yang J, Reimann K, Canis M, Ivanov K, Diehl CD, Strieth S, Wangemann P, Lidington D, Bolz SS

16:00 - 16:30 Coffee Break

ORAL SESSION VIII: Hearing genetics I (O36 - O39)
Moderators: J. Santos-Saachi, Pilar Levy

16:30 **O36** **CONTRIBUTION OF GJB2 MUTATIONS FOR NON-SYNDROMIC SENSORINEURAL HEARING LOSS IN PORTUGAL**
Matos T.D., Simões-Teixeira H., Caria H., Chora J., Rosa H., Monteiro L., O'Neill A., Dias O., Andrea M., Fialho G.

16:45 **O37** **PREVALENCE OF GJB2 MUTATIONS IN THE PORTUGUESE MAINLAND POPULATION**
Chora J.R., Rodrigues R., Trincão C., Simões-Teixeira H., Matos T. D., Fialho G., Caria H.

17:00 **O38** **GENETIC CHARACTERIZATION OF CHILDREN WITH CONGENITAL HEARING LOSS ATTENDING AN AUDITORY REHABILITATION CLINIC IN LISBON: CASE-REVIEW.**
Araújo-Martins J, Correia I, Ferreira R, Santos PB, Gonçalves R, Nunes L, Monteiro L

17:15 **O39** **MICRORNA REGULATION IN THE INNER EAR: IMPLICATIONS FOR DEAFNESS**
Anya Rudnicki, Lilach Friedman, Tal Elkan-Miller, Karen B. Avraham

17:30 - 18:00 **IEB Business Meeting**

20:00 Gala Dinner - Centro Cultural de Belém
'' A Commenda''

Wednesday, September 21st

08:30 Onwards — **Registration**

09:00 - 09:30 — **KEYNOTE LECTURE**

ORAL SESSION IX: Hearing genetics II (O40 - O43)
Moderators: Isabel Varela-Neto, Laura Astolfi

09:30 **O40** **CHARACTERISATION OF MOUSE MUTANTS WITH NOVEL MUTATIONS IN THE TMC1 GENE.**
Shehnaaz SM Manji, Kerry A Miller, Louise H Williams, Henrik M Dahl

09:45 **O41** **MOLECULAR DISSECTION OF TMPRSS3 COCHLEAR HAIR CELL SIGNALING PATHWAY.**
Lydie Fasquelle, Laurence Molina, Régis Nouvian, Nicolas Salvetat, Michel Guipponi, Jean-Luc Puel, Franck Molina

10:00 **O42** **POLYMORPHISMS IN GENES INVOLVED IN INFLAMMATORY PATHWAYS IN PATIENTS WITH SUDDEN SENSORINEURAL HEARING LOSS**
Masaaki Teranishi, Mariko Hiramatsu; Yasue Uchida; Naoki Nishio; Hidenori Suzuki; Ken Kato; Hironao Otake; Tadao Yoshida; Mitsuhiko Tagaya; Hirokazu Suzuki; Michihiko Sone; Saiko Sugiura; Fujiko Ando; Hiroshi Shimokata; Tsutomu Nakashima

10:15 **O43** **GENERATION OF MICE WITH HEARING IMPAIRMENT INDUCED BY GENE TRANSFER IN THE EMBRYONIC INNER EAR UTILIZING A CONNEXIN30-TARGETED SHRNA EXPRESSION VECTOR**
Toru Miwa, Ryosei Minoda, Eiji Yumoto

10:30 - 11:00 — Coffee Break

ORAL SESSION X: Clinical studies (O44 - O49)
Moderators: Peter Thorne, A. Castro Caldas

11:00 **O44** **CORTICAL REPRESENTATION AT THE PERCEPTION AND PRODUCTION OF SIGN LANGUAGE BY FUNCTIONAL MAGNETIC RESONANCE IMAGING**
Valadão MN

11:15 **O45** **BEST PREDICTIVE FACTOR FOR COCHLEAR IMPLANT PERFORMANCE IN ADULTS: AGE OR DURATION OF DEAFNESS?**
Susana Andrade, José Oliveira, Jorge Humberto Martins, Marisa Alves, Luís Silva, Jorge Quadros, Carlos Ribeiro

11:30 **O46** **IMPACT OF COCHLEAR IMPLANTATION ON QUALITY OF LIFE IN THE ELDERLY: BENEFITS OUTWEIGH RISKS**
Susana Andrade, Conceição Peixoto, Jorge Humberto Martins, Luís Silva, Jorge Quadros, Carlos Ribeiro

11:45 **O47** **CONGENITAL DEAFNESS - DIFFICULTIES IN THE ETIOLOGY**
Rosa MH, MD, Silva MI, MD, Nunes R, Ferreia JC, PhD, Fialho G, PhD, Tavares P, PhD, Antunes L, MD

12:00 **O48** **AUDITORY BRAINSTEM RESPONSE DISTURBANCES IN YOUNGER ADULTS WITH INSULIN DEPENDENT DIABETES MELLITUS TYPE 1**
Cavalcante JMS, Lima MS, Bernardez-Braga GRA, Martins JG, Jorge R, Anastasio ART

12:15 **O49** **BELL'S PALSY – IS SURGICAL TREATMENT AN OPTION?**
Oliveira F., Santos R., Oliveira V., Sousa P., Escada P., Madeira da Silva J.

12:30 - 13:00 **Closing remarks and End of the Meeting**
Graça Fialho, Helena Caria

13:00 Lunch

POSTER PRESENTATION

DEVELOPMENTAL BIOLOGY

P1 **The Role of Actomyosin Contractility in Shaping the Apical Circunference of Hair Cells**
Tomoki FUJITA, Hirofumi Sakaguchi, Toshihiro Suzuki, Shigenobu Yonemura, Yasuo Hisa

BASIC HEARING AND VESTIBULAR RESEARCH

P2 **NEURAL CREST STEM CELLS FROM ADULT HUMAN HAIR FOLLICLES AND THEIR POTENTIAL APPLICATION IN THE DEVELOPMENT OF A THERAPY FOR DEAFNESS**
Huisman MA , Rivolta MN

P3 **OBJECTIVE AUDIOMETRICAL PROFILE OF DIFFERENT ANIMAL MODELS BY COCHLEAR MICROPHONIC AUDIOMETRY**
Francisco Carricondo, Mar Sanjuan, Julio Sanjuan, Pablo Gil-Loyzaga

P4 **DEVELOPMENTAL CHANGES IN SHORT TERM PLASTICITY PROPERTIES AT THE TRANSIENT MEDIAL OLIVOCOCHLEAR-INNER HAIR CELL (MOC-IHC) SYNAPSE**
Javier Zorrilla de San Martín, Jimena Ballestero, Ana Belén Elgoyhen, Eleonora Katz

P5 **GABA REGULATES THE RELEASE OF ACH AT THE TRANSIENT OLIVOCOCHLEAR EFFERENT-INNER HAIR CELL SYNAPSE THROUGH PRESYNAPTIC GABAB RECEPTORS**
Carolina Wedemeyer, Javier Zorrilla de San Martín, Ana Vanesa Torbidoni, Bernhard Bettler, Ana Belén Elgoyhen, Eleonora Katz

P6 **CONGENITAL HEARING LOSS IN PORTUGUESE CHILDREN – HOW MUCH IS CAUSED BY CMV?**
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IEB 2011

Torre do Tombo, Lisbon

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"Improving health and quality of life in children and elderly with hearing impairment"

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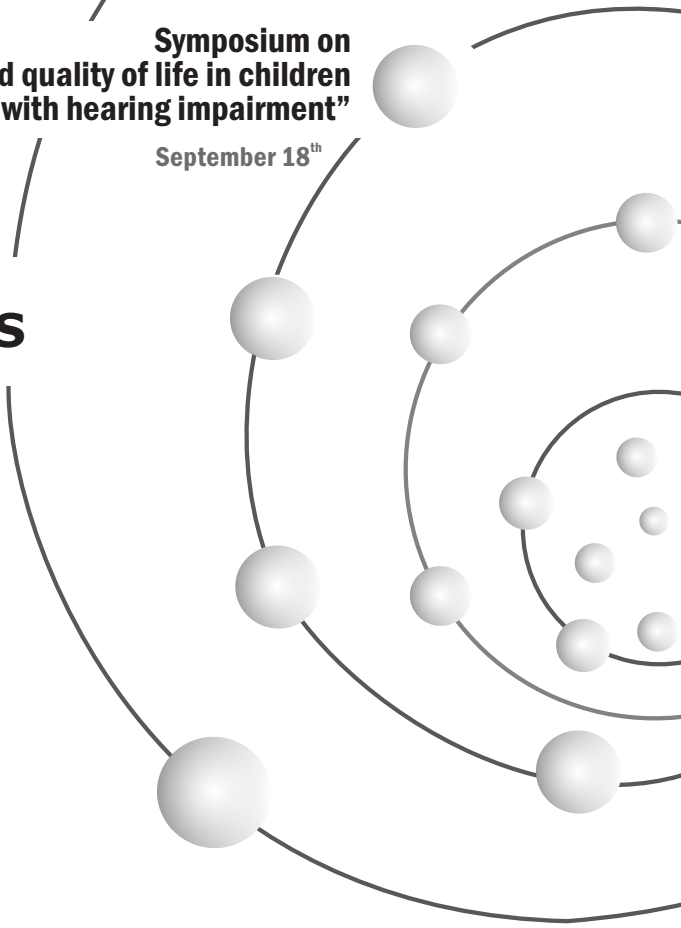
ABSTRACTS



SESSION I: DEAFNESS IN CHILDREN

S01

CHILDHOOD DEAFNESS - MULTIDISCIPLINARY APPROACH

O. Dias, M. Andrea

Department of Otolaryngology, Faculty of Medicine of Lisbon

The benefits of a systematic multidisciplinary approach concerning childhood deafness in Portugal are highlighted based on 30 years of continuous clinical, educational and research work.

Enormous improvements in the age of diagnosis were achieved involving general practitioners, pediatricians and other specialists collaborating with the otolaryngologist and the audiologist.

Epidemiological data has put in evidence the changes in the etiological factors occurring with the health care improvement of the country.

Close relationship between medicine and the education system improved the overall care of the deaf and hearing impaired children and their families.

Cochlear Implants have contributed for the improved results of rehabilitation of the deaf child.

Collaboration between otolaryngology, other specialities and the science community at the national and international level has been nurtured along the years being responsible for the significant improvement of diagnosis observed presently in Portugal.

Based on these results it is expected for the next years a significant reduction of the number of new cases of deafness and the disappearance of deaf muteness. On the other hand the education level reached by the fewer deaf and hearing impaired youngsters will be much higher compared with the previous generations.

Systematic multidisciplinary approach has been the key to the significant improvement concerning the care for the deaf and hearing impaired.

S02

NEONATAL HEARING SCREENING: CURRENT SITUATION AT NATIONAL AND INTERNATIONAL LEVEL

Luísa Monteiro

Hospital Dona Estefânia - CLHC

Early diagnosis of pediatric hearing impairment and timely, appropriate intervention has long been a desire of the professionals involved in medical and academic aspects of childhood development, as hearing impairment has always been recognized as a strong negative factor influencing communication acquisition skills and full social and professional integration.
The development of highly sensitive and highly specific tests using otoacustic emissions (OAE) equipment in the 80's and of automated units of OAE and auditory brainstem response evoked potentials (ABR) in the 90's enabled the screening of large populations of newborns and the early identification of hearing impaired babies. Intervention programs based on early hearing aids adaptation and audioverbal stimulation and therapy are usually implemented alongside the UNHS. Nowadays, Cochlear Implantation is advocated as soon as possible for profoundly hearing impaired babies. Habilitation must start as early as 6 months of age so that the plasticity of the auditory system can be fully harnessed, and that will depend on the efficient and quality of the UNHS.
Wherever these programs where developed, an improvement in audiologic services was noted, new equipments and improved skills could be used in the treatment of children as well as adults. Other professionals involved, like speech-language pathologists, teachers and educators had to adapt, providing services to younger babies and their families. Surgeons and their teams move forward, implanting babies and infants at an earlier age, some of them before twelve months old. Alongside, basic sciences and other specialties involved in diagnosis like genetics, imagiology and virology also noticed a new impulse.
In Europe, some programs are nation based, some are still local, some are mandatory and some are voluntary and this in not always parallel to the wealth and prosperity indicators of the specific country. The first country that claimed 100% coverage of newborn screening was Poland in 2005.
In Portugal, we can describe a positive evolution toward an almost complete coverage of newborn hearing screening. Like in some other countries, this program is still voluntary and no health legislation was ever published on this issue. Nevertheless, with the strong involvement of health professionals, Otolaryngologists and Audiologists ahead, a first recommendation was published in 2003 by GRISI (Grupo de Rastreio e Intervenção ad Surdez Infantil) and was rapidly endorsed by the national professional associations of Otolaryngologists, Audiologists and Pediatricians. Nowadays in almost all the hospitals in the National Health System and private hospitals with a maternity ward there is a hearing screening in place. Usually automated, OAE equipment is used for well-babies clinics and OAE and ABR for targeted screening. GRISI had an important role in the planning and the implementation of earlier screening programs and in the training of the professionals involved. It still provides technical support on demand and also conducts periodic surveys and an annual meeting for sharing experiences and results.
Some important issues are yet to be solved, mainly the need for legislation and health authority support (and funding) of the voluntary locally based EHDI programs. A centrally managed register of all data provided by every program is the only way to assure that quality indicators are achieved and to provide evidence that can support the medical, academic and socioeconomic advantage of this timely, multiprofissional model implemented to manage childhood hearing impairment.

SESSION II: STRATEGIES FOR EDUCATION AND REHABILITATION IN CHILDREN AND ELDERLY

S03

MODELS AND PRACTICES OF EARLY INTERVENTION WITH DEAF CHILDREN AT THE CED JACOB RODRIGUES PEREIRA

Maria José Cascalho, Conceição Coelho

Instituto Jacob Rodrigues Pereira, Casa Pia

Early intervention programs for the deaf and hard-of-hearing were created as an answer to challenges encountered by those who worked daily in this area.

The shift of the concept of early intervention, changes in education paradigms, the evolution of concepts, theories and practices in areas such as developmental and educational psychology and medical intervention have proven the need and benefits of an ecological intervention, specifically regarding the child's development and interactions with the environment.

As well is known, experiences and interactions are of great importance for a child's development therefore, the child's family and peers constitute a privileged context and are part of that growth and development. The situation is more complex when considering a deaf or hard-of-hearing child, as usually there is a struggle with communication and family interactions, putting the child's development at risk.
Hearing loss compromises not only communication but also receptive and expressive language, as well as emotional and social development. As a result the child's future academic performance may be put at risk.

Hence, the deaf or hard-of-hearing child's future depends greatly on an early diagnosis and intervention. The Early Intervention Program at Centro de Educação e Desenvolvimento Jacob Rodrigues Pereira (CED JRP) – Casa Pia de Lisboa, IP, aims to create facilitating conditions for the deaf and hard-of-hearing children and their families by collaborating with the medical team, fortifying family interactions and their competency to create an optimized and protective environment for their child's development.

The Early Intervention Program at CED JRP is comprised of a multidisciplinary team which includes an Audiologist, Kindergarten Teacher, Speech and Language Therapist, Portuguese Sign Language Instructor, Psychologist and Social Worker, as well as other professionals. This program is based on a bilingual model (Portuguese Oral Language and Portuguese Sign Language). The child and family undergo a careful examination to evaluate their shared needs in order to maximize the child's capabilities and improve communication and interaction amongst the family.

S04

SPEECH THERAPY IN SCHOOLS OF REFERENCE FOR THE BILINGUAL TEACHING OF DEAF STUDENTS

Teresa Neto de Carvalho

School of Reference Quinta de Marrocos, Lisboa
Hospital Egas Moniz (Centro Hospitalar de Lisboa Ocidental, EPE)

Looking at Speech Therapy in an overall context, we can see that in Portugal, it has three 'umbrellas' – the Ministry of Education, the Ministry of Health and the Ministry of Solidarity and Social Security. Those Ministries act sometimes in cooperation, sometimes separately, to improve the personal condition of the deaf in what concerns language acquisition and development.
Speech Therapy for people who are deaf or hard of hearing has an educational context in the Ministry of Education. Children can attend regular pre-school or school with or without special educational measures, or they can attend *Schools of reference for the bilingual teaching of students who are deaf,* which are included in public schools. They can also attend Institute Jacob Rodrigues Pereira (integrated in *Casa Pia de Lisboa*)*,* a Special School for students who are deaf, belonging to the Ministry of Solidarity and Social Security. In both schools, this bilingual teaching is the LGP (Portuguese sign language) and the written Portuguese form. LGP is to be taught (by deaf teachers) in the most natural way to children over 3 years old and also to students who don't have enough Portuguese oral skills for oral learning to be socially adapted. These are also taught the written form of Portuguese. Under the age of 3 years, Infants and children who are deaf are mostly attended to by Speech Therapists at the public hospitals depending on the Ministry of Health, promoting in most cases an audio-verbal therapy; *Casa Pia de Lisboa* also has an early intervention project, which includes speech therapy and sign language.
Education in a *School of reference for the bilingual teaching of students who are deaf,* always takes place in the context of Special Education (Dec. Law nº 3/ 2008, January 7) aiming to promote (i) bilingual contexts (LGP; written form of Portuguese and eventually spoken); (ii) necessary adjustments to achieve the curriculum aims; (iii) school and social inclusion; (iv) insertion in a linguistic community of reference; (v) specialized educational response. Its main objective is focused on adequate methodologies and strategies of intervention, flexible and individualized educational responses, and evaluation of the teaching and learning process involving family cooperation.
Speech therapy in the *School of reference* has its own department, integrated in Technical Services. Speech Therapy acts in coordination with teachers, technicians and parents. It integrates a *Multidisciplinary student's observation team*. A Speech therapist works in both regular and special education, with all children and students who are deaf.
All the Speech Therapists' work consists of evaluation, intervention and evaluation based in accurate parameters.
Speech therapy intervention is based on an analysis of the audition skills with devices and taking into account the age when special intervention was started. Depending on this analysis, teaching may be more audio-verbal or use more visual techniques (like speech reading and the written form).

S05

HEARING AND AURAL REHABILITATION IN ELDERLY POPULATION

Jorge Humberto Martins, Aud. Msc

Centro Hospitalar de Coimbra, EPE

Communication plays an essential role in maintaining relationships and the hearing loss deprives not only the individual, but also the family and friends of easy communication. The hearing impairment is a serious problem in the elderly people and can affect their quality of life, personality and the ability to function independently because interferes with their ability to communicate effectively. This kind of hearing loss is called presbyacusis. Two major forms are sensory and strial presbyacusis. The sensory form is characterized by bilateral high-frequency hearing loss; its gradual character determines that many adult patients are not able to perceive that their hearing is diminishing. Presbyacusis is estimated to affect 30-35% of adults between 65 to 75 years old and 40-45% of adults over 75 years old. At the moment, no medical or surgical treatments have been able to overcome this impairment. As a result some elderly people and their families assume that this is not treatable and don´t get help, only about 20% obtain hearing aids. Nowadays, new technologies advances have been applied on various aural rehabilitation devices, such as hearing aids, FM assistive listing devices and telephone amplification devices, providing a better quality of life. Using singly or in combination, this technology can facilitate the daily life of many hearing impaired people. Hearing impairment in elderly people often coexists with other health problems (neurologic, memory, metabolic, vascular) that can complicate treatment and limit the effectiveness of hearing devices.

S06

REHABILITATION, PERFORMANCE AND MORE: ADDRESSING LOCAL NEEDS

Eulalia Juan

Hospital Son Lllátzer, Spain. Cochlear AG, Basel

(*)Aim:

To show the situation of the procedures and habiliation resources for deaf children, families and professionals in Portugal

(*)Conclusions:

The introduction of neonatal hearing screening in many countries, in theory, be widening the pool of very young children, with a confirmed diagnosis of significant hearing impairment.

In Portugal neonatal hearing screening programs are being established throughout the country and every day are making it possible for children to be detected very early. .
Thus habilitation procedures should be adapted to the needs of these babies and their families, with the ultimate goal of establishing communication, different authors agree that if the treatment is established from the first six months of life, there is potential to acquire and develop oral language within the critical period of natural acquisition

(Re) habilitation procedures needs to Focus on the natural bond that exists between parents and their children, the Listen, learn and talk, auditory habilitation tool consists of family centered program with Auditory verbal activities. This program has been developed for both parents and professionals to compliment their child's habilitation program. The program follows the natural development of the child from birth to school age, and contains ideas and strategies for developing spoken language through listening.
This material has been translated and adapted by a team from the Instituto Politécnico de Setúbal, coordinated by the Dra.H.Caria who have not only understood the importance of having an educational resource in Portuguese, but the importance of adaptation to local needs.

S07

HEARING REHABILITATION IN PROFOUNDLY DEAF CHILDREN: THE PRESENT SITUATION

Carlos Ribeiro

Centro Hospitalar de Coimbra, EPE

S08

COCHLEAR IMPLANTS STATE OF THE ART

Prof. Manuel Manrique

Clinical use of cochlear implants (CI) began about 30 years. Since then, there are manyadvances that have undergone this technique for the treatment of sensorineural hearinglossIn this presentation, I will mention the progress especially related these topics:

Extension of audiometric indications.
Audiometric criteria for placement of a cochlear implant have changed to the extent thatthe results have been progressing. It has gone from indications in cases of bilateralprofound hearing loss with minimal residual hearing to moderate-severe hearing loss ,with use of bimodal stimulation strategies (CI + Headset in contralateral ear) or hybrid (CI + Headset in the same ear).

Use of bilateral cochlear implants.
With the use of cochlear implants in both ears is to restore the normal pattern ofstimulation of the auditory system at central level. It discusses the benefits of suchthings as location of sounds, listening in noise, summation effect and auditory plasticity.Implants in children before 12 months of life.It describes the results in this population compared with implants performed in laterstages. It discusses the potential risks of implantation in these early stages of life.

Preservation of hearing in cochlear implant surgery.
The use of atraumatic surgical techniques nowadays allows to preserve largely remainshearing with the use of certain electrode arrays. We analyse the experience gained andthe future prospects that this technique offers.

Use of brainstem auditory implants in children.
It describes the experience in the use of these systems in the treatment of profoundhearing loss in children with congenital malformations that result in a bilateral agenesisof cochlea and / or cochlear nerves.

SESSION III: ROUND TABLE ON 'HEARING REHABILITATION IN MULTIDEFICIENCY'

S09

CYTOMEGALOVIRUS INFECTION AS A CAUSE OF DEAFNESS AND MORE

Maria José Lavilla
Hospital de San Pedro de Alcántara-Cáceres, Espanha

It is well known that congenital cytomegalovirus (CMV) infection may lead not only to deafness but also to additional disorders that may interfere with language or learning processes. This has meant that some authors have questioned the usefulness of these cases .In this presentation we conclude that CMV alone, as a cause of deafness, is not a contraindication for cochlear implantation. Parents should be informed about the wide range of linguistic outcomes after implantation and that these children may need more specific or intensive rehabilitation. Although additional problems are common and outcomes may, on average, be poorer, cochlear implantation can provide useful auditory input to these children. On the other hand, it is believed that congenital CMV infection has a more relevant role in the etiology of sensorineural hearing loss (SNHL) than was previously thought to be the cause of progressive SNHL and to a great degree of unknown hearing losses. The problem is to carry out a diagnosis of the asymptomatic children at birth, but which in the future may have neurological alteration and SNHL. We exposed in this communication the difficulties which exist in carrying out an early diagnosis, and in the appropriateness to dispose of diagnosis test after the first years on life.

MULTIDEFICIENCY - COMPLEMENTARY EXAMS

Maria Helena Rosa (MD), Rosário Mendes (Audiologist), Lisete Santos (Audiologist)
ORL Service, Hospital Garcia de Orta, Almada

The individual with multideficiency, due to motor and communication difficulties, faces limitations as regard the exploration of the surrounding environment, which hinders the ability to access information and, consequently, to access learning. If hearing impairment adds to these limitations, the multidisciplinary rehabilitation may be further complicated.
The otorhinolaryngologist aims to contribute to an early diagnosis and to an adequate therapy, in due course, in order to mitigate the disability, hence facilitating the access to information and learning, and, consequently, the integration into the family life, the school and the society. The otorhinolaryngologist should record the clinical history in detail, and characterize the type (transmission, sensorineural or mixed) and degree of the hearing impairment.
Complementary exams, such as CT and NMRI of the ears, cranium and encephalon are very important in the multideficiency. Laboratorial exams for CMV, rubeola, toxoplasmosis, syphilis, etc., are critical, as well as the genetic study and counseling.
In conclusion, the contribution of the whole diagnostic as regard the hearing function, if made early, may:
-significantly influence the therapeutic attitude;
-allow the child's multidisciplinary rehabilitation to be improved;
-contribute to the modification of the existing relationships between the individual with multideficiency and the surrounding social environment;
-provide a better access to assistance care and allow the social inclusion.

SESSION IV: FUTURE TRENDS ON DIAGNOSTIC AND THERAPY

S10

GENETICS FOR HEALTH PROFESSIONALS

Karen B. Avraham

Tel Aviv University

Genetics is estimated to be responsible for approximately 60% of hearing loss, as a lone phenotype or in association with other clinical abnormalities. Hearing loss is represented by extreme genetic heterogeneity, since mutations in many genes lead to the different genetic forms of deafness (Hereditary Hearing Loss Homepage, http://hereditaryhearingloss.org/). While classic techniques such as linkage analysis and Sanger sequencing have led to the discovery of over 100 genes for hearing loss, it appears that many more genes remain to be discovered. The ability to diagnose the genetic mutation in a child with deafness not only has implications for genetic counseling, but early detection of hearing loss can guide the choice of therapy. Precise genetic characterization enables a greater understanding than in the past of whether a child will, or will not, develop syndromic features that accompany some forms of hearing loss. I will describe the optimal steps for evaluating hearing loss in children, to be performed by audiologists, otolaryngologists, geneticists and other health professionals. Our laboratory is currently using deep sequencing, also known as massively parallel sequencing, to identify more genes in the hearing impaired population. A custom 1.46 MB design of cRNA oligonucleotides was constructed containing 246 genes responsible for either human or mouse deafness. Paired-end libraries were prepared and bar-coded multiplexed samples were sequenced to high depth of coverage. We identified several new mutations responsible for hearing loss in our study. Discovery of new genes and mutations will allow for improved diagnostics, as well as enhance scientific research to allow for a better understanding of the mechanisms of hearing loss, facilitating therapeutic development.

S11

NANOTECHNOLOGY BASED TARGETED DRUG DELIVERY

Jean Luc Puel

Institute of Neurosciences, University Montpellier

ELECTRICAL STIMULATION OF THE EAR: A LONG HISTORY STARTING IN THE EIGHTEENTH CENTURY

Alessandro Martini and Rosario Marchese Ragona

(Neuroscience Dept, Otolaryngology, University Hospital, Padova - Italy)

The cochlear implant (bionic ear) is a device that bypasses a nonfunctional inner ear and stimulates the hearing nerves with patterns of electrical currents so that speech and sounds can be perceived by profoundly deaf people. The bionic ear is the culmination of investigations that started more than two hundred years ago, and it is the first major advance in helping profoundly deaf to communicate since the sign language of the deaf was developed by l"Abbe´ de l"Epe´e at the Paris Deaf School in second half of eighteenth century. The cochlear implant is to date the only direct interface to the central nervous system to restore sensory function. The cochlear implant has been the result of research in many disciplines, including surgical anatomy, surgical pathology, biology, biophysics, neurophysiology, psychophysics, speech science, engineering, surgery, audiology, rehabilitation, and education. The function of cochlear implant is today well known but, its early development through the history of electrical stimulation of the ear is uncertain. The Count Alessandro Volta is generally qualified as the first to stimulate the ear with the electricity. Alessandro Volta, soon after developing the battery, carried out on himself in the late 1790s the first experiment on electrical stimulation of the auditory nerve. His results were read on June 26, 1800, before the Royal Society meeting in London. The report is recorded in the *Philosophical Transactions of the Royal Society of London* for the year 1800, part I, p. 427. Because of the unpleasant sensation experienced by the scientist, any other experiment was carried out over the next half century to study this effect. Investigating exhaustively the available literature of the eighteenth century, we found an electrical stimulation of the ear carried out, half century before Volta. The results of this investigation is reported.

KEYNOTE LECTURES

GROWTH FACTOR-BASED THERAPY FOR THE PREVENTION AND REPAIR OF HEARING LOSS: THE CASE OF INSULIN-LIKE GROWTH FACTOR I

Isabel Varela-Nieto

Institute for Biomedical Research ''Alberto Sols'', IdiPAZ, CIBERER.CSIC-UAM. Madrid, Spain.

Insulin like growth factor I (IGF-I) is fundamental for the regulation of cochlear development, growth and differentiation in several species, and its mutations are associated with hearing loss in mice and men. Low levels of IGF-I have been shown to correlate with different human syndromes showing hearing loss and with presbyacusis. Animal models are fundamental to understand the genetic, epigenetic, and environmental factors that contribute to human hearing loss. In the wild type mouse, IGF-I serum levels decrease with ageing in association with progressive hearing loss. Accordingly, Igf1 null mice show early hearing-loss due to neuronal loss and age-related stria vascularis alterations and progressive retinal degeneration. IGF-I actions in the cochlea are mediated by intracellular signaling networks. Activation of the complex IGF1R-IRS2 modulates RAF, AKT and p38 MAPK protein kinases leading to the regulation of the activity of transcription factors AP1, MEF2 and FoxM1, which in turn modulate cell cycle and metabolic responses. Therapy with rhIGF-1 has been approved in humans for the treatment of poor linear growth and certain neurodegenerative diseases. Therefore, IGF-I-based treatments could offer novel opportunities for the protection and repair of hearing loss.

NOVEL THERAPIES RELATED TO COCHLEAR IMPLANTS

Astolfi L, Martini A

Department of Medical and Surgical Specialities, University of Padova, Italy

Intra-cochlear drug delivery may represent a key issue in cochlear implant (CI) success in the future. Its therapeutic goals include the improvement of hearing preservation by reduction of insertion trauma and intra-cochlear tissue growth. Other benefits of intra-cochlear drug delivery include improvements of the auditory nerve status and of the electrode/nerve interface. A number of potential drug delivery devices are currently under development including drug release using electrode arrays.

Among the drugs involved, dexamethasone has been shown to reduce the hearing loss due to mild cochlear implant insertion trauma (Kiefer et al 2010). Based on this evidence we evaluated the effects of intratympanic insertion of 10% dexamethasone-eluting silicone rods in animal models of both minimal and severe insertion trauma.

With the purpose of verifying the hearing protection, the effect on tissue growth and the cochleostomy healing, we used 2 different types of electrode array designed to create either minimal or severe insertion trauma. In one group guinea pigs were gently implanted with soft rods constructed only from silicone, while in the other animals were implanted with a stiffer array containing stiff wire and causing mechanical trauma. In each case, 10% dexamethasone-eluting rods were evaluated for therapeutic benefit and non-eluting rods were implanted as controls.

Implantations were performed through a 0.7mm cochleostomy, followed by 3-mm deep rod insertion. Hearing threshold audiograms were acquired prior to implantation and during the next two/four/eight weeks by recording compound action potentials with electrodes near the round window. After these periods the cochlea was removed, decalcified, embedded in paraffin and longitudinally cut into 5-µm thick sections. For each sample we examined the Scala Tympani occlusion in the cochlear basal turn, and the cochleostomy healing.

Audiological and histological results showed no significant differences in hearing protection between non-eluting or 10% dexamethasone-eluting rods within 60 days. No bacterial contamination was detected in the implant rods. However, in presence of 10% dexamethasone-eluting tubes, the average tissue growth was always lower in comparison to non eluting ones, in particular we observed a significant reduction in the new bone.

This data supports the use of steroid eluting rods with slow-release as an anti-inflammatory additive in cochlear implants. The poor hearing preservation obtained under these conditions is under further histological investigation.

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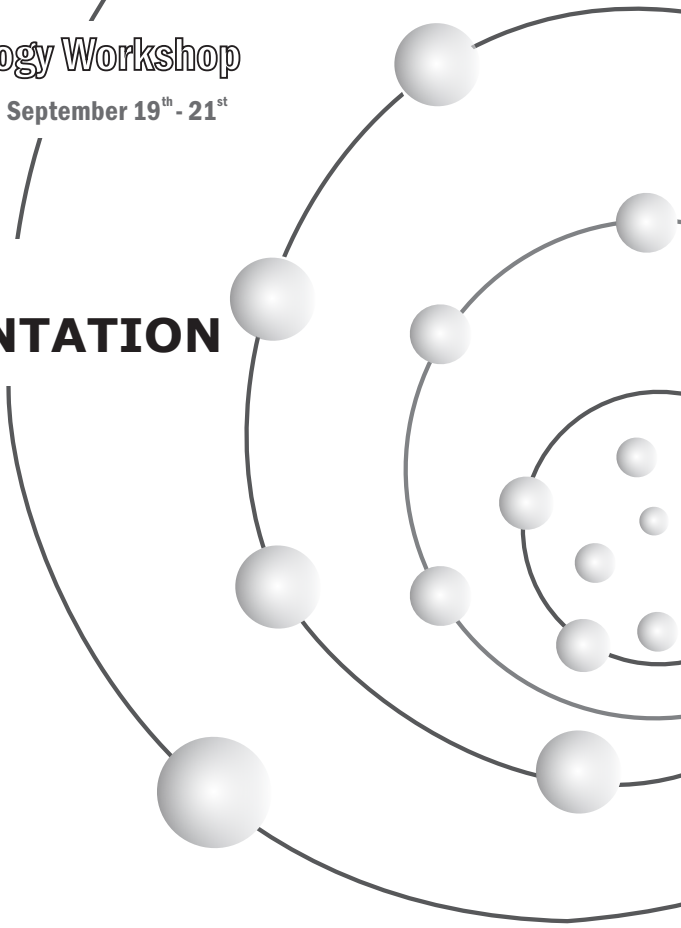
ABSTRACTS
ORAL PRESENTATION



SESSION I: DEVELOPMENTAL BIOLOGY

O1

REGULATION OF NEURITE PATHFINDING IN TYPE II SPIRAL GANGLION NEURONS DIFFERS FROM THAT IN TYPE I NEURONS

Ryan AF[1] , Pak K[1]; Sung M[2]; Wei E[2]; Parsi A[2]; Jonathan Cheng J[2]; Housley G[3]; Brand Y[4]

([1]ENT, UCSD and San Diego VAMC, USA; [2]Biology, UCSD, USA; [3]Physiology, University of New South Wales, Australia; [4]ENT, University of Basel, Switzerland)

Objective: Type I and Type II spiral ganglion neuron (SGN) dendrites follow different paths into the organ of Corti. Factors that determine this difference are poorly understood. We compared directional responses of Type I and Type II SGN processes to guidance factors expressed in the projection path of developing SGN dendrites. Laminin (LN) is strongly expressed in sensory epithelium basement membrane and less strongly beneath inner hair cells (HCs); fibronectin (FN) beneath both inner and outer HCs; L1 cell adhesion molecule (L1) beneath inner HCs, and EphA4 in the walls of the osseous spiral lamina.

Methods: The factors were applied to culture surfaces as 100 µm stripes, on a poly-L-lysine background. Neonatal mouse or rat spiral ganglia were harvested, divided into explants, and cultured on 100 µm guidance factor stripes. Termination and stripe tracking of Type I and II neurites, identified by anti-neurofilament and anti-peripherin immunolabeling, were evaluated.

Results: Type I and Type II neurites avoided FN and EphA4 stripes at all concentrations tested. Type I neurites were attracted to low-concentration LN, but avoided high-concentration stripes. In contrast, Type II neurites avoided LN at all concentrations. Finally, Type I processes preferred L1 stripes, while Type II neurites neither preferred nor avoided L1.

Conclusions: The results suggest that EphA4 may play a role in restricting the growth of Type I and II dendrites to the center of the osseous spiral lamina. FN may induce both types of neurons to terminate beneath HCs. LN may induce Type I neurites to grow away from the basement membrane and toward inner HCs, but Type II processes to avoid the inner HC region. Finally, L1 beneath the inner HCs may induce Type I neurons to preferentially extend into in this area, while Type II dendrites could readily pass through and extend to the outer HCs.

O2

TSUKUSHI GENE EXPRESSION IN THE MOUSE COCHLEAE

Ryosei Minoda[1], Toru Miwa[1]; Eiji Yumoto[1]

([1]Department of Otolaryngology-Head and Neck Surgery, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan)

OBJECTIVE: The Tsukushi (TSK), which is a member of the secreted small leucine rich repeat proteoglycan family, was originally identified as a BMP antagonist (Ohta, 2004). TSK is expressed in the ectoderm, the endoderm, and it is the organizer during the early development stages (Morris, 2007).We examined the TSK expression in mouse cochleae, and surface morphology and cochlear function of TSK knockout mice.

METHODS: TSK knockout mice were provided by Dr. Ohta (Department of Developmental Neurobiology, Kumamoto University). The TSK exons of the knock-mice were replaced with the LacZ gene. Localization of TSK expression was assessed at E9, E11, E14, and P28 mice. Auditory function (ABR: 4k, 12k, 20kHz) and surface morphology were assessed at P35 mice

RESULTS: TSK was detected at the sensory patch region in E9 and E11 mouse cochleae, and at the sensory epithelial progenitor cell region in E11 mice cochleae. At P28, TSK was detected at the inner hair cells, the outer hair cells, and the spiral ganglion cells in the cochleae. Auditory threshold of TSK knockout mice significantly higher than normal mice. SEM analysis revealed that stereocilia of the inner hair cells partly disappeared.

CONCLUSIONS: TSK modulates BMP signaling as a BMP inhibitor during chick gastrulation (Ohta, 2004), and recently it has been reported that TSK plays a role in balancing the retinal stem cell proliferation and differentiation through the inhibition of Wnt signaling in the mouse eye (Ohta, 2008). In the cochleae, it has also been reported that the final size of the sensory patches depends upon the balance between BMP4 and opposing signals (Pujades, 2006). Additionally RT-PCR analysis on postnatal rat cochleae had revealed that Wnt genes were expressed (Daudet, 2002). Considering these findings, TSK probably participates in several aspects of auditory patterning and/or functioning.

O3

OVEREXPRESSION OF ISL1 PRODUCES CHANGES IN THE AUDITORY AND VESTIBULAR SYSTEMS IN MICE

Pavlinkova G[1] , Chumak T[2]; Kuthanova L[1]; Bohuslavova R[1]; Buckiova D[3]; Syka J[4]

([1]Institute of Biotechnology ASCR, Prague, Czech Republic; [2]Institute of Experimental Medicine ASCR, Prague , Czech Republic; [3]Institute of Experimental Medicine ASCR, Prague, Czech Republic; [4]Institue of Experimental Medicine ASCR, Prague, Czech Republic)

Transcription factor ISLET 1 (ISL1) plays a role in the development of the inner ear. Expression studies show that Isl1 is present early in the otocyst in the regions that give rise to both sensory and neuronal lineages in the inner ear. We hypothesize that ISL1 specifies neurosensory precursors. To test our hypothesis, we generated transgenic mice overexpressing Isl1 under Pax2 promoter control. Two founders of *Pax2-Isl1* transgenic mice with different levels of transgene expression (F1-lower and F2-higher) were tested. Some of the mutant mice had evident vestibular abnormality manifested as circling behavior. This finding was accompanied by evident pathological changes in the structure of the vestibular epithelium cells and vestibular ganglion cells. Hearing function was studied with the aid of auditory brainstem responses (ABRs) and distortion product oto-acoustic emissions (DPOAE). No differences in hearing between young (2-6 months) F1 mutant and control mice were detected. However, the hearing thresholds of F1 mutants increased faster starting from the age of 7 months. The thresholds of 14-15-month-old mutant mice were higher over the whole frequency range in comparison with controls. A significant elevation of the hearing thresholds of F2 mutants was detected already at the age of 6 months as compared to controls. Fifteen-month-old F2 mice were completely deaf at 1, 2, 32 and 40 kHz, having an 80 to 92 dB SPL hearing threshold at the middle frequencies, while controls showed only a negligible threshold shift with aging. DPOAE changes were comparable with the ABR changes. In aged mutant mice an evident loss of hair cells, a 50% loss of spiral ganglion cells and a high level of apoptosis in cerebellar neurons were found. Our results show that transgenic *Pax2-Isl1* mice have abnormalities in the central and peripheral parts of the auditory and vestibular pathways.

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SESSION II: BASIC HEARING AND VESTIBULAR RESEARCH

O4

SHORT-TERM SYNAPTIC PLASTICITY DETERMINES THE LEVEL OF OLIVOCOCHLEAR INHIBITION TO AUDITORY HAIR CELLS

Jimena Ballestero[1], Javier Zorrilla de San Martín[1]; Juan Goutman[1]; Paul Fuchs[2]; Ana Belén Elgoyhen[1]; Eleonora Katz[1]

([1]Instituto de Investigaciones en Ingeniería Genética y Biología Molecular (INGEBI-CONICET), Buenos Aires, Argentina; [2]Johns Hopkins University School of Medicine, Baltimore, USA)

In mammals, the gain control of auditory inputs is exerted by medial olivocochlear (MOC) neurons that innervate cochlear outer hair cells (OHCs). OHCs mechanically amplify the incoming sound waves by virtue of their electromotile properties while the MOC system reduces the gain of auditory inputs by inhibiting OHCs function. How this process is orchestrated at the synaptic level remains unknown. In this study, MOC firing was evoked by electrical stimulation while OHCs postsynaptic responses were monitored by whole-cell recordings in excised mouse organs of Corti. We confirmed, by pharmacological methods, that electrically evoked inhibitory postsynaptic currents (eIPSCs) are mediated by alpha9alpha10 nAChRs functionally coupled to calcium-activated SK2 channels. Synaptic release occurred with low probability (Psuccess = 0.25±0.06) when MOC fibers were stimulated at 1Hz. However, upon raising the stimulation frequency, the reliability of release increased due to presynaptic facilitation. Also, the relatively slow decay of eIPSCs gave rise to temporal summation at stimulation frequencies above 10 Hz. This indicates that short-term plasticity (STP) at this synapse has both presynaptic and postsynaptic determinants. The combined effect of facilitation and summation resulted in a frequency-dependent increase in the average amplitude of eIPSCs (Response increments were 4.2±0.3; 7.5±0.8, 12.4±0.7, 21.6±5.9 for 25, 50, 60 and 80 Hz, respectively). Thus, the STP properties of the MOC-OHC synapse determine the level of OHC's inhibition. In preliminary experiments in IHCs from knock-in mice expressing a slow-desensitizing alpha9alpha10 nAChR, synaptic responses to MOC high frequency activity present slower rise times and reduced depression than their wild-type littermates. Interestingly, these changes in synaptic responses closely resemble the alterations in the time course of MOC inhibition measured *in-vivo* in the same animal model (Taranda et al., 2009). We therefore postulate that olivocohlear inhibition of auditory function is finely tuned by the dynamics of the MOC-OHC synapse.

O5

PARKIN DEFICIENCY CAUSES PROGRESSIVE HEARING LOSS IN MICE THROUGH OUTER HAIR CELL LOSS

Norio Yamamoto[1], Kiyomi Hamaguchi[1]; Takayuki Nakagawa[1]; Juichi Ito[1]

([1]Department of Otolaryngology-Head and Neck Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan)

Parkinson´s disease (PD) is the second most common neurodegenerative disorder and is characterized by muscle rigidity, tremor and a slowing of physical movement. Although most PD cases are sporadic, 5-10% of them are hereditary. Recently, some specific genetic mutations causing PD have been identified from familial PD cases. One of these genes includes Park2 (Parkin) whose loss-of-function mutation causes autosomal recessive juvenile parkinsonism in human. Parkin protein is an E3 ubiquitin-protein ligase suggesting that this protein is involved in quality control of other proteins. In Drosophila, *parkin* null mutants show decreased adult lifespan, apoptotic muscle degeneration and male infertility, but no apparent in vivo neuronal phenotypes have been observed in Parkin deficient mice.

Parkin knockout (KO) mice showed progressive hearing loss detected by auditory brain stem response (ABR). Since progressive hearing loss is mostly caused by sensorineurial hearing loss, we performed hisotological and functional analyses on cochlear organs including organs of corti, stria vascularis and spiral ganglion cells in Parkin KO mice. We identified that the cause of progressive hearing loss in Parkin KO mice were loss of outer hair cells from several results we got in this study such as negative distortion product of otoacousitic emission (DPOAE), decreased numbers of outer hair cells, normal endocochlear potentials and normal morphology of spiral ganglion cells and stria vascularis.

O6

K-ATP CHANNEL KNOCKOUT MICE ARE PROTECTED AGAINST PRESBYACUSIS

Manuel Groth[1], Silvi Hoidis[1]; Jean Smolders[1]; Jochen Roeper[1]

([1]Institute for Neurophysiology, Goethe-University Frankfurt, Germany)

Presbyacusis, a major form of sensorineural age-related hearing loss, involves the degeneration and loss of hair cells in the mammalian inner ear. The pathomechanisms of presbyacusis are not well understood but ischemia, oxidative stress and mutations of mitochondrial DNA might contribute to hair cell loss. In mouse models of Parkinson Disease, where aging is also the main risk factor, the genetic inactivation of the ATP-sensitive potassium channel (K-ATP) subunit Kir6.2 completely rescued highly vulnerable dopamine neurons from degeneration (Liss et al. 2005, Nature Neuroscience). Thus, we asked whether the early age of onset and rapid progression of presbyacusis present in the C57Bl6 genetic background were also affected by the absence of Kir6.2-containing K-ATP channels.

ABR responses to tone pips (2-45 kHz) revealed that wildtype mice developed early-onset presbyacusis with increased thresholds in the high frequency range. In contrast, Kir6.2 knockout mice showed a statistically significant slowing and reduction of the age-dependent high-frequency hearing loss by about 50 dB at one year of age. Accordingly, inner and outer hair cells were largely preserved in these aged Kir6.2 knockout mice up to 45 kHz, but lost to a great extent in wildtype mice in the high frequency range. The findings indicate that Kir6.2-containing K-ATP channels might contribute locally or systemically to the vulnerability for age-dependent hair cell loss.

Patch-clamp studies of isolated hair cells from wildtype and Kir6.2 knockout mice (P12 to P50) showed that tolbutamide-sensitive outward currents activated by dialysis of ATP-free pipette solutions were present in wildtype inner hair cells and increased about three-fold with postnatal development. These recordings demonstrated the presence of functional K-ATP channels in mature inner hair cells, which were absent in Kir6.2 knockout mice.

In summary, these findings suggest that functional K-ATP channels in sensory cells of the inner ear might mediate their age-related degeneration.

O7

CORTICOSTEROID TREATMENT OF IDIOPATHIC SUDDEN SENSORINEURAL HEARING LOSS. TRIAL OR ERROR?

Elisabeth Hultcrantz[1], Ramesh Zarenoe[1]

([1]University of Linköping Sweden)

Objective: To analyze the effect of Prednisolone in oral custumary doses on Idiopathic Sudden Sensorineural Hearing Loss (ISSNHL) in a sufficiently large patient group.
Study design: Meta-analysis of data from a RCTon Idiopathic Sudden Sensorineural Hearing Loss (ISSNHL) and a Swedish national database for ISSNHL.on Idiopathic Sudden Sensorineural Hearing Loss (ISSNHL). Multiple regression analyses were used.
Intervention: 45/99 (RCT) and 54/99 (the database) had been treated with Prednisolone in tapering doses from 60 mg daily and 42/93 with placebo (RCT) or 51/93 with no treatment (the database) .Primary outcome was the mean hearing improvement on day 90 for the different groups. A mean difference of >10 dB improvement between the treated and not treated groups was needed to demonstrate treatment effect of Prednisolone compared to placebo/no treatment.
Patients: Data from 192 patients, 18-80 years, with ISSNHL was available. All had a hearing loss of at least 30 dB measured as PTA in the three most affected contiguous frequencies. Patients had been enrolled within one week after onset and evaluated by audiograms after three months

Results
No significant difference was seen between the Prednisolone group and placebo/no treatment (p=0.06). Total recovery was 38% in Prednisolone group, 40% in the placebo and 14% in the no treatment group. Vertigo at the onset of hearing loss and increasing age had a negative prognostic value equally in all groups and signs of inflammation had a positive prognostic value.

Conclusion
Prednisolone, orally, in customary dosage does not influence recovery after ISSNHL.

O8

MORPHOMETRICAL DIFFERENCES OF HUMAN SPIRAL GANGLION CELLS: WHAT DOES IT MEAN FOR ELECTRICAL STIMULATION?

Thomas Potrusil[1], Cornelia Wenger[2], Rudolf Glueckert[3], Anneliese Schrott-Fischer[3], Frank Rattay[2]

([1]Department of Otorhinolaryngology, Innsbruck Medical University, Austria; Faculty of Informatics, Vienna University of Technology, Austria; [2]Institute for Analysis and Scientific Computing, Vienna University of Technology, Austria; [3]Department of Otorhinolaryngology, Innsbruck Medical University, Austria)

Objective: The sizes of unmyelinated spiral ganglion cells (SGC) in the human cochlea have a powerful impact on the action potential (AP) travelling from the hair cell to the auditory cortex. The aim of the study was to analyze these spike-barriers morphometrically and mimic different stimulation strategies. Furthermore, new information about the distribution of SGC in the different regions of the inner ear was acquired and their impact concerning electrical stimulation was studied.

Methods: Two human cryoembedded cochleae from different subjects have been serially sectioned perpendicular and radial respectively to the modiolus with a section thickness of 35 µm providing to have bipolar SGC in their full dimensions on each cryosection. Immunohistochemistry including confocal microscopy has been performed for detecting perikarya and their appropriate nuclei. These structures were segmented manually for calculating the volumes. For determining different cell populations hierarchical cluster analysis was performed. A compartment model of the human cochlea neuron was used to determine intra- and extracellular excitation thresholds, the point of spike generation and the action potential delay over the soma.

Results: Hierarchical clustering of perikarya volumes revealed four distinct populations of SGC within the human cochlea. The somatic volume (n=146) varies enormously between 532 and 8258 µm³ resulting in delay differences of the generated AP passing the soma in extracellular stimulation. The excitation thresholds for an AP passing a soma are ranging from -171 µA to -480 µA respectively using monophasic stimulation.

Conclusion: Systematic morphometric measurements on two human inner ears indicate four different sub-populations of spiral ganglion cells. In extracellular stimulation the variation in perikarya size results in different excitation thresholds as well as different points of spike initiation. These resulting spike delays over the unmyelinated cell soma lead to variations of the temporal pattern which might be an important component for speech understanding.

O9

RELATIVE COCHLEAR AND VESTIBULAR OTOTOXICITY PRODUCED BY TRANSTYMPANIC GENTAMICIN UNDER RECOMBINANT HUMAN ERYTHROPOIETIN CYTOPROTECTION IN THE RAT

Luís L[1], Rocha J[2], Castro Caldas A[3], Mota Filipe H[2], Sepodes B[2]

([1]Instituto de Ciências da Saúde, Universidade Católica Portuguesa, Lisbon, Portugal, biMED.UL-Pharmacology & Translational Research, Lisbon, Portugal; [2]biMED.UL-Pharmacology & Translational Research, Lisbon, Portugal; [3] Instituto de Ciências da Saúde, Universidade Católica Portuguesa, Lisbon, Portugal)

Gentamycin, an aminoglycoside used transtympanically for Meniere´s disease, is thought to be selectively vestibulotoxic. Depending on the protocol, various rates of cochlear and vestibular toxicity occur. Little experimental data exists on the concomitant use of citroprotectors as the human recombinant erythropoietin (Epo) or its derivates on the relative cochlear and vestibular ototoxicity of aminoglicosides.

High-frequency (4 to 35 KHz) DPOAE's (Distortion product otoacoustic emissions) and cVEMP (cervical vestibular-evoked myogenic potential) were performed on rats to study cochlear and vestibular damage. Average DP-grams and P1 and N1 amplitudes and latencies were recorded before, two and four weeks after injection. Comparisons were made between animals receiving single dosage transtympanic gentamicine, and similarly injected gentamicine and peritoneal non-erytropoietic Epo as well as non-injected controls. Given the rat resistance to amynoglicoside cochlear lesion comparisons were also made with the concomitant administration of the peritoneal amynoglicoside kanamycin and the loop diuretic bumetanide with and without the Epo administration. Preliminary results are presented.

O10

THE CHLORIDE-CHANNEL INHIBITOR ANTHRACENE-9-CARBOXYLIC ACID REVERSIBLY BLOCKS THE MOTOR PROTEIN PRESTIN

Csaba Harasztosi[1], Anthony W. Gummer[1]

([1]University Tübingen)

The voltage-dependent, chloride-channel blocker anthracene-9-carboxylic acid (9AC) influences the axial impedance of isolated outer hair cells (OHCs; Eckrich et al., 2008 ARO, #517) and reduces the electrically induced motion of the organ of Corti (Nowotny and Gummer, 2006; Scherer and Gummer, 2004).

To reveal whether 9AC might be directly interacting with the motor protein, we measured the nonlinear capacitance (NLC) as a signature of prestin in OHCs isolated from guinea pig and in prestin-transfected human embryonic kidney 293 (HEK293) cells. Measurements were made using the patch-clamp technique in whole-cell configuration. The absolute electrical admittance parameters of OHCs were determined using the Lindau-Neher algorithm. The voltage-dependent membrane capacitance data were fitted with the sum of a linear component and a nonlinear component, given as the sum of the first derivative of the two-state Boltzmann function and a sigmoidal function (Santos-Sacchi and Navarrete, 2002).

Extracellular application of 9AC significantly and reversibly reduced the NLC in both OHCs and HEK293 cells. The presence of 9AC in the intracellular solution did not influence the blocking effect of the extracellularly applied drug. Reduction of the intracellular chloride concentration by the replacement of chloride ions with sulfate did not change the effectivity of the extracellularly applied 9AC. However, reduction of the chloride concentration in the extracellular and intracellular solutions to 5 mM by replacing chloride with gluconate caused a negative shift of the dose-dependence curve.
Reduction of the NLC of both OHCs and HEK293 cells by the lipophilic 9AC suggests that 9AC directly interacts with the motor complex. The chloride sensitivity of the effect of 9AC implies that the binding sites of 9AC and chloride ions are at least overlapping. The ineffectiveness of intracellularly applied chloride and the observed relative fast extracellular effect suggests that 9AC has probably more extracellular accessibility to prestin.

O11

REAL TIME MONITORING OF PRESTIN INSERTION INTO THE PLASMA MEMBRANE

Bian S

(Otolaryngology, Neurobiology, Neurology, and Cellular and Molecular Physiology - Yale University School of Medicine)

We recently developed a tetracycline-inducible HEK cell line that expresses prestin at levels far above that obtained with transient transfection. Because of the greater signal to noise ratio we were able to observe recapitulation of events occurring during the development OHC electromotility, showing an early rise in prestin's nonlinear charge density (Qmax) and a shift in prestin's voltage operating point (Vh). The cell line also enabled us to observe developmental characteristics that were impossible to measure in developing OHCs, including an abrupt change in the valance (z) of nonlinear charge, and the development of a prestin associated leakage current. We have begun to analyze in real-time the insertion of prestin into the membrane by synchronizing bolus delivery of prestin from the Golgi apparatus, using real time monitoring of membrane capacitance coupled with temperature jump techniques. Additionally, we monitor prestin delivery to the membrane using fluorescence observation of YFP tagged prestin. This approach provides a powerful means to understand not only membrane trafficking of prestin, but also subsequent events following its insertion. (Supported by NIH NIDCD DC000273, DC008130)

O12

ORGAN OF CORTI MICROMECHANICS MEASURED WITH LOW COHERENCE INTERFEROMETRY

Zha D[1], Chen F[1], Ramamoorthy S[1], Choudhury N[1], Fridberger A[2], Nuttall AL[1]

([1]Oregon Health & Science University; [2]Karolinska Insitutet)

The outer hair cells (OHC) can generate internal force by one or more molecular mechanisms and change the shape of the organ. To learn that shape, the motion of the reticular lamina (RL) must be measured in the high frequency region of the cochlea and has been difficult to measure *in vivo*. That motion directly influences the deflection of stereocilia bundles and for power to be added to the vibration of the organ of Corti, it is required that the OHC dynamically change its length, in addition to generating the force. The OHC length change induced by its somatic electro-motility, originating from the voltage activation of the molecule Prestin, has been studied extensively *in vitro* and *in situ.*The OHC dynamic length has not been studied *in vivo*. Albino guinea pigs (250 - 350 g) with normal hearing were used. After anesthesia and surgery to gain access to the organ of Corti, using low coherence interferometry method, the vibration of the organ of Corti was measured at two locations: the reticular lamina (RL) and the basilar membrane (BM). These locations can be used to effectively define the OHC length change and to show the motion of the RL compared to the BM. We demonstrate *in vivo* that the OHC change length and move the RL in such a way to cause it to have a larger motion than the BM and a relative phase lead. These phenomena are sound level dependent in the sensitive cochlea. The phase and magnitude of the RL and the level-dependent OHC length change are direct manifestations of the cochlear amplification process. Combined with the assumed OHC produced force, the OHC length change is a necessary condition for directly validating that power is expended by the active cochlear amplification process. Supported by NIDCD DC 00141, NIDCD DC 010399 and NIDCD DC 005983.

O13

A REPORT OF EXTENDED HIGH FREQUENCY AUDIOMETRY THRESHOLDS IN SCHOOL- AGED CHILDREN WITH NO HEARING COMPLAINTS

Cavalcante JMS[1], Radael RD[1], Anastasio ART[1], Hatzopoulos S[2]

([1]School of Medicine of University of São Paulo; [2]University of Ferrara, Italy)

OBJECTIVE: to establish hearing thresholds of extended high frequency (EHF) of school-aged children with no hearing complaints.

METHODS**:** The study was conducted on 50 children aged 8 to 12 years with pure tone thresholds (0.5, 1 and 2 kHz) of 15 dB HL or less, with normal speech discrimination, tympanometry and with the presence of contralateral acoustic reflexes of 0.5, 1, 2 and 4 kHz. EHF thresholds were obtained with at frequencies of 9, 10, 11.2, 12.5, 14 and 16 kHz. Repeated Measures ANOVA with post-test (Bonferroni Multiple Comparisons Test) and Kruskal-Wallis test with post test (Dunn´s Multiple Comparisons Test) were used to compare the EHF thresholds.

RESULTS*:* No statistical differences were found among the right and left ears for female and male groups. A significant difference was observed at 16kHz for comparation between females and males, with high average thresholds for the boys. The difference at 16kHz was not valued in this study, since the standard deviation did not exceed 10dBNA, according to clinical criteria that can occur due to intra-subject variability, so this difference was not considered clinically relevant. The results allowed the group of the children into a single sample with mean thresholds (dB) of 8.6 (9 kHz); 6.2 (10 kHz); 8.2 (11.2 kHz); 7.1 (12.5 kHz); 0.4 (14 kHz) and -3.6 (16 kHz). The thresholds improved with increasing frequency (14 and 16kHz). The mean thresholds added to the value of standard deviation of the population studied was less than or equal to 16.1dBNA. The values of standard deviation to be expected for a population similar to this study are up 10.2dBNA.

CONCLUSIONS**:** For school-aged children, the extended high frequency hearing thresholds below 20dBHL could be used as indicative of normal hearing sensitivity.

O14

ANALYSIS OF AUDITORY BRAINSTEM RESPONSE BY USE OF CLICK AND TONEBURST STIMULUS IN TERM AND PRETERM NEONATES

Cavalcante JMS , Isaac MI[1]

(School of Medicine of Sao Paulo)

OBJECTIVE: to characterize the response of the ABR with click and tone bursts of 500, 2000 and 4000Hz stimulus, in neonates born at term and preterm period, with normal peripheral auditory function.

METHODS: Sixty-one healthy neonates has been examinated, 31 at term and 30 preterm neonates until 28 days old without any risks factors of hearing loss and all passed on transient evoked otoacoustic emissions (TOAEs). The ABR by click and tone burst were recorded at 80, 60, 40 and 30dB HL, bilaterally and in an ipsilateral way. The responses were processed by the equipment SMART-EP, with insertion earphones and electrodes fixed after cleaning the skin.

RESULTS: We observed that the latencies of waves I, III and V and interpeak latencies I-III, III-V and I-V at 80dB HL were significantly longer in preterm than in term neonates. The latencies of wave V in the intensities of 60, 40 and 30dB HL were also longer in preterm infants. The analysis of ABR obtained with tone burst stimulus, showed wave V longer latencies in preterm neonates at all intensities and frequencies. It was observed shorter latencies for the click stimulus than to tone burst, and tone burst showed a reduction in latency with increasing frequency. The difference between genders was not considered relevant.

CONCLUSIONS: There is great necessity to investigate the hearing of neonates to register the audiometric configuration in a shorter period of time. Therefore, it suggested to use click to determine the sensitivity of hearing at high frequencies and tone bursts to low frequencies.

O15

AUDITORY EVALUATION IN PATIENTS TREATED WITH RADIOTHERAPY AND CHEMOTHERAPY EXCLUSIVE OR COMBINED

Isaac ML, Dell´aringa AHB; Arruda GV

(School of Medicine of Sao Paulo)

Objective: To evaluate the functionality of the auditory system in patients who underwent radiotherapy to treat head and neck tumors and chemotherapy based on cisplatin, exclusive or combined.

Methods: From June 2006 to January 2008 audiological evaluation (Pure Tone Audiometry (air and bone conduction), Speech Audiometry, Tympanometry, Acoustic Reflex testing and Distortion Product Otoacoustic Emissions) was performed in 70 patients diagnosed before and after neoplasia treatment. The patients were separated in three groups: Group A – (22) patients treated with radiotherapy exclusive for head and neck tumors, Group B – (14) patients treated with cisplatin chemotherapy and Group C – (34) patients treated with radiotherapy combined with cisplatin chemotherapy for head and neck tumors.

Results: According to ASHA criteria, 15,7% left ears and 26,3% right ears presented decreased hearing soon after radiotherapy treatment; 23,0% left ears and 7,6% right ears presented decreased hearing soon after chemotherapy treatment and 70,0% left ears and 60% right ears soon after combined treatment. Risk factors as age under than 60 years old were significative for decreasing hearing just for Group C. In comparative analysis between groups, cisplatin addiction for the radiotherapy treatment was responsible for the decreasing hearing.

Conclusion: The three therapeutic methods were responsible for causing auditory damage soon after treatment; the combined treatment presented more auditory alteration; the cisplatin addition to the radiotherapy treatment presented higher risk for ototoxicity

O16

A NEW ANIMAL MODEL FOR MENIERE´S DISEASE

Akinobu Kakigi[1], Naoya Egami[1], Takashi Sakamoto[1], Rie Nishioka[2], Masamitsu Hyodo[2], Taizo Takeda[2],Tatsuya Yamosoba[1]

([1]The University of Tokyo; [2]Kochi Medical School)

Meniere's disease (MD) is histologically characterized by endolymphatic hydrops (EH) in the inner ear. The mechanisms underlying over accumulation of endolymph still remain an enigma. However, there is considerable evidence that water homeostasis in the inner ear is regulated via vasopressin-aquaporin2 (VP-AQP2) system in part. If this is the case, EH, the morphological characteristics of MD, reflects the mal-regulation of VP-AQP2 system in inner ear fluid. This hypothesis requires considerable clinical and experimental verification before it may be countered as a theory. In this paper, experimental evidence to support the above-mentioned hypothesis will be presented based on our new animal model for MD.

This model is based on a combination of chronic endolymphatic sac dysfunction, induced by destruction of the endolymphatic sac and duct, and acute endolymph production by VP-AQP2 system in the stria vascularis with desmopressin (V2 antagonist). Light microscopy of the fluid compartments of cochleas and vestibules were used to examine them for the presence of endolymphatic hydrops. The combination of chronic endolymphatic sac dysfunction and acute attacks of endolymph production by desmopressin administration revealed the severe degrees of hydrops in cochlears and vestibules. This new model may represent a more physiologic and dynamic approach to MD and may explain the etiology of many symptoms in patients such as the fluctuant nature and the types of balance disorder.

O17

DIRECT ENTRY OF GD-DTPA INTO THE VESTIBULE FOLLOWING INTRATYMPANIC APPLICATION IN GUINEA PIGS

Elisha King[1], Alec N. Salt[2]; Hayden Eastwood[1]; Stephen O´Leary[1]

([1]University of Melbourne, Melbourne, Australia; [2]Washington University School of Medicine, St Louis, United States of America)

OBJECTIVE: While intratympanic (IT) administration of drugs has gained wide clinical acceptance, the distribution of drugs in the ear following IT administration is not yet well understood, particularly during and after cochlear implantation. The purpose of this study was to monitor the distribution of drug in the inner ear after cochlear implantation.
METHODS: Gadolinium (Gd) has been previously used as a marker in conjunction with Magnetic Resonance Imaging (MRI) to demonstrate qualitative distribution in inner ear fluids. In the present study we applied Gd to the round window niche of 12 guinea pigs in Seprapak (carboxlmethylcellulose-hyaluronic acid) pledgets, used to stabilize the fluid volume in the round window niche area. Gd distribution was monitored sequentially with time following application. Distribution in normal, unperforated ears was compared with ears that had undergone a cochleostomy in the basal turn of scala tympani and implanted with a silastic electrode. Results were quantified using image analysis software.

RESULTS: In all animals, Gd was seen in scala tympani, scala vestibuli, and the vestibule. Although Gd levels in ST were higher than those in the vestibule in some ears, this was in a minority of cases. The majority of ears showed higher Gd levels in the vestibule than ST at both early and later time points.

CONCLUSIONS: Quantitative computer simulations of the experiment, taking into account the larger volume of the vestibule compared to scala tympani, suggest a major proportion (up to 90%) of Gd entering the inner ear did not enter through the round window membrane, but entered the vestibule by another route, probably via the annular ligament of the stapes. Gd levels were minimally affected by the implantation procedure after 1hr.

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O18

MYOSIN VIIA AND SANS LOCALIZATION AT STEREOCILIA UPPER TIP-LINK DENSITY IMPLICATES THESE USHER SYNDROME PROTEINS IN MECHANOTRANSDUCTION

M´hamed Grati, Bechara Kachar[1]

(NIDCD, NIH)

In the most accepted model for hair cell mechanotransduction (MET), a cluster of myosin motors located at the stereocilia upper tip-link density (UTLD) keeps the tip-link under tension at rest. Both myosin VIIa (MYO7A) and myosin 1c (MYO1C) have been implicated in MET based on functional studies. However, localization studies are conflicting, leaving open the question of which myosin localizes at the UTLD and generates the tip-link resting tension. Using immunofluorescence we now show that MYO7A and sans, a MYO7A interacting protein cluster at the UTLD. Analysis of the immunofluorescence intensity indicates that 8 or more MYO7A molecules are present at each UTLD, consistent with a direct role for MYO7A in maintaining tip-link tension. MYO7A and sans localization at the UTLD is confirmed by transfection of hair cells with GFP-tagged constructs for these proteins. Co-transfection studies in a heterologous system show that MYO7A, sans, and the UTLD protein harmonin-b form a tripartite complex and that each protein is capable of interacting with one another independently. We propose that MYO7A, sans, and harmonin-b form the core components of the UTLD molecular complex. In this complex, MYO7A is likely the motor element that pulls on CDH23 to exert tension on the tip-link.

O19

ACOUSTIC OSCILLATION OF GUINEA PIG STAPES VISUALIZED THROUGH HIGH-SPEED VIDEO CAMERA ANALYSIS

Mitsuru Ohashi, Nozomu Matsumoto, Takashi Kimitsuki, Shizuo Komune

(Department of Otorhinolaryngology, Graduate School of Medical Science, Kyushu University)

Objective
Fixation of the ossicles with chronic inflammation or sclerotic degeneration leads to hearing loss. In order to improve the level of hearing, fixated part must be repaired surgically. In such cases, it is most important to evaluate the stapes mobility. However, it has not cleared the dynamic behavior of the stapes to date. The aim of this study is quantitative visualization of the stapes motion during acoustic oscillation using the new type of high speed video camera system.

Methods
Adult albino guinea pigs were used in this study.
The high speed video camera system (VW-5000, Keyence, Osaka, Japan) was used for recording and analysis.
The tympanic bulla was exposed and then opened through submandibular approach. The microscope of the system was placed just above the tympanic bulla, then the incudo-stapedial joint and stapes head were clearly viewed under the monitor of the system.
The tone burst sound waves were used as acoustic stimuli and delivered at a distance 5 to 10mm from tympanic membrane. The stimulus sound of 125, 250, 500 and 1 kHz at different levels of magnitude were generated by the EP/EMG measuring system (Nihon Kohden, Tokyo, Japan).

Result
We succeeded to record the fine acoustic oscillation of the stapes in absolute value. Images were recorded at the rate of 4000fps (frame per second). Vibration profiles were measured with propriety analysis software. The period of oscillation was in synchronism with the frequency of stimulus sound. The amplitude of oscillation was proportional to the acoustic pressure level.

Conclusion
Our new technique is valuable for evaluation of stapes mobility and may provide important information concerning the surgical procedures.

O20

OPEN FOR DISSECTION: HEARING IN THE FRUIT FLY DROSOPHILA

Joerg T. Albert,

(UCL Ear Institute, WC1X 8EE, London, UK)

During the past decade, the antennal ears of *Drosophila melanogaster* have provided a powerful scientific model for the molecular and mechanistic analysis of auditory transduction [1]. Despite vast differences of auditory anatomy, the fundamental mechanisms of transducer activation, and adaptation, were found to shared striking similarities between vertebrates and fruit flies [2]. In part at least, these discoveries were owed to the different morphology of the fly's ear. The ear of the fruit fly, just as in other insects, lacks an equivalent of the vertebrate 'middle ear': The external (antennal) sound receiver is directly connected to the auditory neurons of Johnston Organ (the 'inner ear' equivalent). As a result of this construction, the mechanical gating of auditory transducer channels produces characteristic signatures in the receiver's mechanics and thus allows for using the experimentally accessible, external sound receiver as a tool to directly probe transducer function *in vivo [3]*. The comparative study of 7 different *Drosophila* species has recently provided insights into how auditory transducer channels can facilitate frequency-specific acoustic signalling by establishing species-specific communication channels [4]. Here, I will present the latest findings on the molecular requirements of sensitive hearing in *Drosophila*, its possible contributions to the evolution of Drosophilid flies and finally, the wider implications of the model system *Drosophila* for the study of inner ear function and homoeostasis.

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O21

DEVELOPMENT OF A POLYMERIC COATING FOR COCHLEAR IMPLANT ELECTRODES TO DELIVER DEXAMETHASONE INTO THE INNER EAR

Piera Ceschi[1], Anne Roock[2], Katrin Sternberg[2], Klaus-Peter Schmitz[2], Thomas Lenarz[1], Manfred Kietzmann[3], Timo Stöver[4], Gerrit Paasche[1]

([1]ENT, Hannover Medical School, Germany; [2]IBMT, Rostock University, Germany; [3]Pharmacology and Toxicology, School of Veterinary Medicine Hannover, Germany; [4]ENT, KGU Frankfurt, Germany)

Objective: Therapeutic strategies to treat inner ear diseases are currently object of debate. For instance, glucocorticosteroids are widely used for the treatment of several auditory diseases; however, their application and efficacy depend strictly on the route of delivery. For this reason we tried to better understand if a safe and sustained intracochlear delivery of dexamethasone can be developed on the basis of a modified cochlear implant (CI) electrode coated with poly(4-hydroxybutyrate) (P(4HB)) (kindly supplied by Tepha, Inc., Lexington, MA, USA).

Methods: Freshly isolated murine spiral ganglion cells were chosen as a model to verify the effects of dexamethasone *in vitro*. Hence, prototypes consisting of a silicone carrier coated respectively with P(4HB) or P(4HB) incorporated with different concentrations of dexamethasone (weight ratio of polymer to drug of 85/15 and 70/30) were tested *in vivo* in guinea pigs. Two additional control groups were also included in this study: subjects who received a silicone prototype and normal hearing (non-treated) animals. After an experimental period of twenty-eight days subjects were sacrificed. Consequently, cochleae were embedded, sectioned, stained and then analysed for morphology, dimensions and number of surviving spiral ganglion cells.

Results: At the concentrations and conditions we tested, neither P(4HB) nor dexamethasone exerted toxic effects on spiral ganglion cells *in vitro* and *in vivo*. However, hearing thresholds were shifted at high frequencies for all groups.

Conclusions: According to our data, we can assume that dexamethasone can be considered as a safe drug for the inner ear and P(4HB) can represent an innovative biomaterial able to deliver this drug inside the cochlea. These results open the doors to the concept of a sustained intracochlear glucocorticosteroid delivery by biodegradable polymers combined with a modified CI.

O22

SIOP PLATINIUM END OF TREATMENT OTOTOXXICITY SCALE

Dr Kaukab Rajput

([1]Great Ormond Street Hospital, London)

Cisplatin ototoxicity typically leads to bilateral, symmetrical, sensorineural, permanent. The otoxicity appears first in the high frequencies but can progress to low frequencies with continued treatment, higher cumulative dose. Hearing loss is gradual onset, progressive and cumulative, or sudden. There is evidence that hearing loss can progress years after cisplatin is stopped. I will discuss the impact of such hearing loss on children.

Risk factors for Cisplatin ototoxicity includes younger age, Cisplatin dose, combination of cisplatin and radiation therapy, genetic predisposition, use of other ototoxic drugs such as Aminoglycosides.

In the past two years of published clinical ototoxicity research, at least 7 different ototoxicity criteria/grading scales were used to analise and reprot results. A new grading system will be proposed which has been agreed and will discuss the need of developing otoprotective agents with minimal side effects.

O23

COMBINING CELL-BASED THERAPIES AND A COCHLEAR IMPLANT TO PROMOTE NEURAL SURVIVAL

Andrew Wise[1], James Fallon[1], Alison Neil[1], Lisa Pettingill[1], Marilyn Geaney[2], Robert Shepherd[1]

([1]Bionics Institute, Melbourne, Australia; [2]Living Cell Technologies, Auckland, NZ)

Spiral ganglion neurons (SGNs) in the deafened cochlea undergo continual degeneration ultimately resulting in cell death. The exogenous application of neurotrophins (NTs) can prevent SGN degeneration, with the survival effects enhanced by chronic intracochlear electrical stimulation (ES) from a cochlear implant. However, previously described techniques to administer NTs to the cochlea have limited clinical applicability, thus restricting the use of NTs in treating neurodegenerative diseases.

We have used a cell-based technique to provide NTs in a clinically viable manner that can be combined with cochlear implant use. Neonatal cats were ototoxically deafened systemically and at two months of age were unilaterally implanted with encapsulated NT-producing cells and a scalar tympani electrode array to deliver environmentally-derived ES. Animals received chronic ES only (n=5), NTs without chronic ES (n=6) or NTs in combination with chronic ES (n=6) for up to 7 months. In all cases the contralateral ear served as a deafened, un-implanted control.

Chronic ES alone did not result in greater SGN survival when compared to the contralateral cochlea. NT treatment alone resulted in significant SGN survival in the upper basal cochlear region ($p<0.05$). Importantly, chronic ES in combination with NT provided significant SGN survival throughout the basal and lower middle regions ($p<0.05$). NT treatment, with or without chronic ES, resulted in a significantly greater density of peripheral fibers within the osseous spiral lamina, compared to the contralateral cochlea ($p<0.01$). Furthermore, resprouting peripheral fibers were observed in the scala tympani and the scala media compartments of cochlea irrespective of treatment condition. There was not difference in the extent of fibrous tissue response to cochlear implantation between the experimental groups supporting the clinical viability of this approach.

We conclude that cell-based NT delivery is clinically viable and effective in preventing SGN degeneration and preserving peripheral fibers over extended durations of deafness. These findings have important implications for therapies that deliver therapeutic drugs safely to the cochlea.

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O24

EFFCTS OF DEXAMETHASONE ELUTING IMPLANTS ON POSTOPERATIVE HEALING - A HISTOLOGICAL STUDY

Anne Jakob[1], Katharina Niedermeier[1]; Susanne Braun[2]; Henning Bier[1]; Fred Sinowatz[3]; Thomas Stark[1]

([1]Hals-Nasen-Ohrenklinik des Klinikums rechts der Isar; [2]MED-EL Deutschland GmbH; [3]Lehrstuhl für Anatomie, Histologie und Embryologie der LMU München)

Objective: Dexamethasone is able to preserve residual hearing after cochlear implantation, which is especially important for patients that are implanted with devices for Electric acoustic stimulation (EAS). A dexamethasone-eluting electrode-dummy has been shown to preserve residual hearing by means of sustained drug delivery directly into the cochlea However, aside from its beneficial effects dexamethasone is also known to delay wound healing. Hence, a delayed closure of cochleostomy might lead to an enhanced infection risk and has to be taken into consideration.
The aim of the study was to look whether dexamethasone eluted from implants influences cochleostomy healing and tissue growth.

Methods: Silicone electrode-dummies either loaded with 10% dexamethasone or without dexamethasone were implanted bilaterally in guinea pigs (9 per group). On the left ear the cochleostomy was covered with a tissue flap around the rod to investigate the effect of an additional tissue flap on sealing of insertion site. The implants were allowed to heal for various time spans (8, 12, 35 days) to identify a point of time when the healing of the cochleostomy results in a full closure. Animals were sacrificed under deep anaesthesia and bullae were filled with a suspension of ink particles in the size of bacteria. The assessment of the full closure was performed by histological staining and identification of intracochlear ink particles. Un-implanted control cochleae did not contain ink particles.

Results: Preliminary results demonstrated that ink particles may still be found within the cochlea 12 days and in some cases even 35 days after implantation. The results of a quantitative comparison between dexamethasone eluting and non-eluting implants will be presented.

Conclusions: Results suggest that a thorough antibiotic treatment is advised for implanted patients as long as one month post surgery or even longer.

O25

NEURONAL SUPPORTING AND SURVIVAL MECHANISM IN HUMAN COCHLEA

Wei Liu, Helge Rask-Andersen, Marja Boström, Anders Kinnefors

(Dept Otology, Uppsala University Hospital)

OBJECTIVE

Human inner ear cells share in many aspects with other species patterns of degeneration caused by a variety of etiologies such as ototoxic agents, acoustic trauma, aging, etc.. However human spiral neurons have been found to possess unique survival capacity after loss of organ of Corti. The nerve cell bodies and their central axons could survive for many years after deafferentation. Unknown properties around human neuronal degeneration and preservation/regeneration need to elucidate in order to find ways of improving the well-being and number of the neurons.

METHODS

Human cochlear specimens which had to be destroyed during operation, via trans-cochlear approach, on patients suffering from giant posterior cranial fossa meningioma were carefully collected, fixed, decalcified and cryo-sectioned. And the sections were subjected to immunohistochemistry with antibodies against neurotrophic factors and their receptors as well as other molecules relevant to neuroprotection.

RESULTS

TrkB receptor expression was found in spiral neurons' perikarya and nerve fibers, neurturin and c-Ret receptor in the neurons´ perikarya , persephin in the satellite glial cells and p75NTR in both satellite glial cells and cochlear Schwann cells. BDNF, GDNF and TrkA were not found in the cochleae. Satellite glial cells expressed Cx43 and lacked myelin basic protein (MBP), mimicking astrocytes in CNS and satellite glial cells in nociceptive ganglia in rodents.

CONCLUSIONS

Lack of report on distribution of trophic molecules in adult human inner ear has been due to difficulty in obtaining well fixed specimen. Human spiral neurons seem to have multiple protection mechanisms which need further exploration. The findings, based on studies of well preserved human cochlear specimens, of molecules relevant to auditory neuroprotection can lead to better strategies for hearing restoration and rehabilitation.

O26

IN VITRO PROTECTION OF THE AUDITORY HAIR CELLS BY SALICYLATE FROM GENTAMICIN-INDUCED BUT NOT NEOMYCIN-INDUCED-LOSS

Agnieszka J. Szczepek[1], Xiangxin Lou, Heidi Olze, Heidemarie Haupt, Birgit Mazurek

(Charité University Hospital)

Salicylate has been shown to protect *in vivo* the inner ear from gentamicin-induced ototoxicity. The mechanism of protection is generally attributed to the ability of salicylate to scavenge free radicals, generation of which is induced by gentamicin. Other aminoglycoside antibiotics are also ototoxic. The objective of our study was to test the *in vitro* ability of salicylate to protect the auditory hair cells from aminoglycoside ototoxicity. As an experimental model, we used the membranous tissues dissected from the cochleas of p3-p5 Wistar pups. These tissues containing the organ of Corti, spiral limbus and spiral ganglion neurons were divided into apical, medial and basal parts, explanted in the tissue culture dish and cultured for 24 h in tissue culture medium, followed by 48-h incubation in presence or absence of 100 µM gentamicin, 100 µM neomycin and 5 mM salicylate. Following tissue fixation and visualization of filamentous actin with phalloidin-TRITC, the number of inner and outer hair cells (IHCs, OHCs) was scored under the fluorescent microscope. We found that cochlear explants cultured in presence of 100 µM gentamicin and 5 mM salicylate had notably reduced loss of IHCs and OHCs, as compared to explants exposed to gentamicin alone. In contrast, neomycin-induced auditory hair cell loss remained unaffected by the presence of salicylate. Our results validate the use of cochlear explants for the study of ototoxicity and its prevention. Moreover, our findings point at possible differences in the mechanisms of auditory hair cell loss induced by two different aminoglycoside antibiotics.

O27

HEMATOPOIETIC STEM CELLS PREVENT HAIR CELL DEATH AFTER TRANSIENT COCHLEAR ISCHEMIA THROUGH PARACRINE EFFECTS

Nobuhiro Hakuba, Tadashi Yoshida, Kiyofumi Gyo

(Ehime University School of Medicine)

Transplantation of hematopoietic stem cells (iHSCs) is regarded to be a potential approach for promoting repair of damaged organs. Here, we investigated the influence of hematopoietic stem cells on progressive hair cell degeneration after cochlear ischemia in gerbils. Transient cochlear ischemia was produced by extracranial occlusion of the bilateral vertebral arteries just before their entry into the transverse foramen of the cervical vertebra. Intrascalar injection of HSCs prevented ischemia-induced hair cell degeneration and ameliorated hearing impairment. We also showed that the protein level of glial cell line-derived neurotrophic factor (GDNF) in the organ of Corti was upregulated after cochlear ischemia and that treated with HSCs augmented this ischemia-induced upregulation of GDNF. A tracking study revealed that HSCs injected into the cochlea were retained in the perilymphatic space of the cochlea, although they neither transdifferentiated into cochlear cell types nor fused with the injured hair cells after ischemia, suggesting that HSCs had therapeutic potential possibly through paracrine effects. Thus, we propose HSCs as a potential new therapeutic strategy for hearing loss.

O28

NOVEL AMINOGLYCOSIDE DERIVATIVES WITH REDUCED OTOTOXICITY AND ENHANCED SUPPRESSION OF DISEASE-CAUSING PREMATURE STOP MUTATIONS

Baasov T[1], Belakhov V[1], Kandasamy J[1], Cherniavsky M[1], Hainrichson M[1], Xie J[2], Schacht J[2]

([1]Technion-Israel Institute of Technology, Haifa, Israel; [2]Kresge Hearing Research Institute, University of Michigan)

OBJECTIVE: Numerous human genetic diseases and types of cancer are caused by single-point alterations in DNA, creating stop codons in mRNA coding regions and leading to premature termination of translation and to non-functional proteins. Such non-sense mutations are present in about 12% of all mutations, including cystic fibrosis, Duchenne muscular dystrophy, Usher syndrome, and Hurler syndrome. An emerging therapy is to promote the selective translational read-through of premature but not of normal stop codons, restoring the (partial) expression of a full-length protein. Gentamicin has successfully been used in this fashion in several disease models as well as in patients harboring mutations in CFTR or Dystrophin genes. However, ototoxicity and reduced suppression efficacy at sub-ototoxic doses limit the use of gentamicin.

METHODS: We describe here the systematic development of novel aminoglycoside derivatives exhibiting superior in-vitro stop codon suppression DNA fragments derived from mutant genes representing Usher syndrome, cystic fibrosis, Duchenne muscular dystrophy, and Hurler syndrome. In addition to optimizing read-through efficacy, the major concerns associated with a long-term therapy were also addressed. Ideally, the compounds should be free of ototoxic side effects and lack antibacterial activity so as not to cause emergence of resistant bacterial strains.

CONCLUSIONS: The effective compounds discussed here show much reduced general inhibition of prokaryotic translation and essentially no antibacterial activity. Potential ototoxicity was screened as toxicity to hair cells in cochlear explants of the postnatal mouse and found to be significantly lower than that of gentamicin.

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O29

ADENOSINE AMINE CONGENER AMELIORATES CISPLATIN-INDUCED HEARING LOSS

Srdjan Vlajkovic[1], Niliksha Gunewardene[1], Cindy Guo[1], Ann Wong[2], Gary Housley[3], Peter Thorne[1]

([1]The University of Auckland; [2]The University of Auckland; University of New South Wales; [3]University of New South Wales)

Cisplatin is one of the most commonly used chemotherapeutic agents. The principal side effects of cisplatin include ototoxicity, neurotoxicity and nephrotoxicity. Cisplatin ototoxicity is manifested in most patients as tinnitus and bilateral high-frequency hearing loss.

OBJECTIVE: Here we present a novel pharmacological intervention to mitigate cisplatin ototoxicity using systemic administration of a selective adenosine A_1 receptor agonist adenosine amine congener (ADAC).

METHODS: Wistar rats were exposed to a two-cycle cisplatin treatment similar to clinical course of cancer chemotherapy. Each cycle comprised 4 days of intraperitoneal cisplatin injections (1 mg/kg twice daily) separated by 10 days of rest. ADAC (100 mg/kg) was administered intraperitoneally for 5 days at 24 hour intervals during the second cisplatin cycle, or immediately upon completion of the cisplatin treatment. Hearing thresholds were measured using auditory brainstem responses (ABR).

RESULTS: In control cisplatin-treated animals (n=8), ABR threshold shifts ranged from 12-28 dB across the frequency range used in this study (4-28 kHz). ADAC treatment during the second cisplatin cycle reduced cisplatin-induced threshold shifts by 8-14 dB ($p<0.05$) at higher frequencies (16-28 kHz), but the treatment was ineffective if ADAC administration was delayed until after the completion of the cisplatin regime. Functional recovery was supported by increased survival of hair cells and reduced apoptotic activity.

CONCLUSION: These findings indicate that systemic administration of ADAC may partially protect the cochlea from the cisplatin-induced hearing loss, however its potential interference with antineoplastic effects of cisplatin is yet to be established. This study was approved by the University of Auckland Animal Ethics Committee.

O30

MRI MACROPHAGE DETECTION IN A GUINEA PIG MODEL OF INNER EAR INFLAMMATION

Le Floc´h J[1] , Tan W[1], Telang RS[1], Vlajkovic SM[1], Pontre B[2], Thorne Pr[3]

([1]Department of Physiology, The University of Auckland; [2]Centre for Advanced MRI, The University of Auckland; [3]Discipline of Audiology, The University of Auckland)

Inner ear inflammation is considered a major contributor to the development of hearing loss. Yet most information is derived from acute animal studies and little is known about the progression of inflammatory disease in the living inner ear.
To chronically study the inner ear we have developed acquisition methods for magnetic resonance imaging (MRI) of the cochlea and have reported dramatic changes in cochlear vascular permeability with inflammation[1,2].

Purpose: To better understand the etiology of inflammation we have studied the extent and time course of macrophage infiltration in the inflamed cochlea using ultrasmall superparamagnetic iron oxide particles (USPIOs).

Methods: To induce cochlear inflammation guinea pigs (GPs, n=6) were sensitised by bacterial lipopolysaccharide (LPS,0.8mg/kg) followed 24 hours later by bilateral LPS intra-tympanic injection (30µl) and intravenous injection of USPIOs (50mg/kg, P904,Guerbet research). One control animal was treated with saline. Anaesthetised GPs were scanned before LPS sensitization (baseline, n=5) and then at 2 days (n=4), 3 days (n=3), 4 days (n=1) and 7 days (n=2) after LPS sensitisation using a 4.7T MRI system. T2-weighted MR sequences were acquired to determine the signal intensity changes and spatial locations, and to calculate the transverse relaxation time(T2). Some animals were euthanized at 2 days for histology.

Results: The signal intensity in perilymphatic spaces dropped (up to 8-fold) in some or all cochlear turns at 2 days, and then progressively increased the following days. T2 decreased at these locations, and the iron staining was observed in the perilymphatic spaces, modiolus and spiral ligament.

Conclusion: These results reveal the recruitment of macrophages at the onset of cochlear inflammation, and suggest that MRI can characterize this process in the living cochlea.

1. Le Floc'h J et al. Characterization of inner ear inflammation in rodents using in vivo DCE-MRI and ex vivo light microscopy. ISMRM2010;18:2380.
2. Le Floc'h J et al. Understanding cochlear inflammation processes: longitudinal changes in vascular permeability. ANS2011:44.

O32

MORPHOLOGICAL CHANGES OF SPIRAL GANGLION CELL DENDRITES AFTER INTRACOCHLEAR APPLICATION OF BRAIN-DERIVED NEUROTROPHIC FACTOR IN DEAFENED GUINEA PIGS

Waaijer L[1], Klis SFL[2], Van Deurzen MHW[2], Hendriksen EGJ[2], Grolman W[2]

([1]University Medical Center Utrecht; [2]Department of Otorhinolaryngology and Head & Neck Surgery, University Medical Center Utrecht, The Netherlands)

Objective. To characterize effects of deafening and subsequent treatment with brain-derived neurotrophic factor (BDNF) on spiral ganglion cell dendrites in guinea pigs. BDNF may be a compound that can prevent degeneration of neural structures after loss of hair cells, with possible relevance for cochlear implant candidates.

Methods. We used cochleae from animals which were previously described by Agterberg et al. (2008; 2009). Briefly, the animals were deafened with a combination of kanamycin and furosemide. Two weeks after deafening, BDNF treatment was started locally in the basal turn of the cochlea with osmotic pumps. The cochleae were cut after 4 weeks of BDNF treatment. We acquired transverse sections of the osseous spiral lamina, cutting the fibers perpendicularly. Fibers were counted and morphologically characterized with respect to myelinisation and size.

Results. Deafening dramatically reduced the number of fibers. Packing densities were reduced by a factor of more than 2 in the basal turn. Apically, this reduction was less, but still very significant. BDNF treatment significantly reduced the degenerative effect of deafening. The remaining fibers showed an altered morphology: the size of the axoplasm was reduced in deafened animals, but increased with respect to controls in animals treated with BDNF after deafening. The myelin sheath appeared reduced in size, especially in animals which were sacrificed more than two weeks after treatment with BDNF.

Conclusions. Deafening with kanamycin and furosemide evokes degeneration of peripheral dendrites which seems to precede degeneration of the cells themselves. BDNF treatment not only reduces this degeneration but also induces morphological alterations in the fibers. The physiological consequences of these alterations remain to be determined.

References:
Agterberg MJH Versnel H De Groot JCMJ Smoorenburg GF Albers FWJ Klis SFL (2009) Hear Res 244: 25-34.
Agterberg MJH Versnel H Van Dijk LM De Groot JCMJ Klis SFL (2010) JARO 10: 355-367.

O33

A GUINEA PIG MODEL OF PARTIAL DEAFNESS

Havenith S[1], Klis SFL[2]; Versnel H[3]; Grolman W[2]

([1]University Medical Center Utrecht; [2]Department of Otorhinolaryngology and Head & Neck Surgery, University Medical Center Utrecht, The Netherlands; [3]Department of Otorhinolaryngology and Head & Neck Surgery, University Medical Center Utrecht,The Netherlands)

Our goal was to develop a stable guinea pig model for selective high-frequency hearing loss. This model can subsequently be used in experiments aimed at optimizing cochlear implant surgery to reduce (post)surgical damage, especially relevant for patients with residual low-frequency hearing. It is known that the single administration of an aminoglycoside antibiotic in combination with a loop diuretic results in severely deafened animals. Based on dose-response curves presented by Brummett et al. (1979), Stronks et al. (2011) lowered the kanamycin dose which preserved low-frequency hearing in guinea pigs. In this study we implanted guinea pigs with a permanent round window electrode in the right ear, which was used to monitor the cochlear function by means of acoustically evoked compound action potential recordings over time. Kanamycin (200 mg/kg) was administered subcutaneously and furosemide (100 mg/kg) was infused into the external jugular vein, at least 2 weeks after electrode placement. The animals were sacrificed for histological analysis at 2, 4 and 7 weeks after this treatment. Individual tone audiograms showed large high-frequency hearing losses, with threshold shifts greater than 50 dB for 8, 11.3 and 16 kHz in 15 out of 17 animals. We found that greatest threshold shifts occurred the second day after deafening and thresholds were stable 1 week after deafening. The sloping high-frequency hearing loss corresponded to the observed increase in hair cell loss towards the basal region. Also, hair cell loss was comparable at 2, 4 and 7 weeks after deafening. However, inner hair cell loss increased from 50% at week 2 to 90% at week 4 after deafening only in the most basal region. We conclude that this is an appropriate animal model for selective high-frequency hearing loss, which is stable at 4 weeks after deafening.

O34

STRUCTURAL AND MOLECULAR CHANGES IN THE LATERAL WALL OF THE COCHLEA OF MICE WITH AGE-RELATED HEARING LOSS

Paramanthasivam V[1], Vlajkovic SM[1], Housley GD[2], Donaldson PJ[1], Thorne Pr[3]

([1]Department of Physiology, The University of Auckland; [2]Department of Physiology, University of New South Wales; [3]Section of Audiology and Department of Physiology, The University of Auckland)

Degeneration of fibrocytes in the lateral wall, along with loss of sensory cells and spiral ganglion neurons, are all pathological changes observed in the C57/BL6 mouse cochlea during ageing. The lateral wall fibrocytes connected by gap junctions play an important role in intercellular communication in the cochlea and buffering extracellular potassium. The impact of the progressive loss of fibrocytes on intercellular communication pathways with age and the development of hearing loss is unknown.

Objective: This study investigated the structural changes in the lateral wall tissues and correlated these with the expression and localisation of connexins (Cx26, Cx29, Cx30, Cx43), NaK-ATPase (a1) in C57BL/6 mice.

Methods: Tissue was collected at 1 (n=10), 3 (n=9), 6 (n=8) and 12 months (n=8). Real time qPCR and immunohistochemistry were used to quantitate gene expression levels and to localise expression of these proteins in the cochlea. Light and transmission electron microscopy was used to examine the structure of the lateral wall.

Results: Histology showed progressive degenerative changes and loss of fibrocytes in the area of the spiral ligament occupied by Type 3 and Type 4 fibrocytes and these changes were more pronounced in the basal turn. The different connexins and NaK-ATPase were found to have a distinct expression pattern within the cochlea, as described previously, with high levels of expression of Cx26, Cx30 and NaK-ATPase(a1) in the fibrocytes of the spiral ligament. No significant changes were observed in the expression of Cx26, Cx30 and NaK-ATPase in the Type 1 and Type 2 fibrocyte regions with age.

Conclusion: These data confirm the structural changes in the lateral wall of the C57BL/6 with age and show that remaining fibrocytes retain gap junction pathways and NaK-ATPase important for intercellular communication and potassium homeostasis. Approved by the University of Auckland Animal Ethics Committee.

O35

TNF-ALPHA COMPROMISES THE INNER EAR MICROCIRCULATION VIA ACTIVATION OF ENDOGENOUS S1P SIGNALLING: A TRANSLATIONAL STUDY FOR THE TREATMENT OF SSHL

Scherer EQ[1], Yang J[2], Reimann K[3], Canis M[4], Ivanov K[2], Diehl CD[2], Strieth S[4], Wangemann P[3], Lidington D[2], Bolz Ss[2]

([1]Hals-Nasen-Ohrenklinik und Poliklinik, Klinikum rechts der Isar der Technischen Universität München, Munich, Germany; [2]Department of Physiology, Faculty of Medicine, University of Toronto, Canada; [3]Department of Anatomy and Physiology, Kansas State University, USA; [4]Ludwig-Maximilians-Universität München, Munich, Germany)

This study indicates a causal link between inflammation and inner ear vascular dysfunction. Recovery profiles of auditory function in a sudden sensorineural hearing loss (SSHL) patient group treated with a TNFα inhibitor were consistent with a vascular origin. We investigated the inner ear microcirculation using (1) an in vitro model of the spiral modiolar artery (SMA), the end artery feeding the inner ear, (2) intra vital microscopy of stria vascularis perfusion, and (3) in vitro measurement of cochlear lateral wall capillary constriction that control the blood supply to the stria vascularis. We demonstrate that TNFα induces a pro-constrictive state via activation of sphingosine-1-phosphate (S1P) signalling in the SMA, lateral wall capillaries and stria vascularis. Detailed analysis of the molecular signalling pathway identified the phosphorylation of sphingosine kinase 1 (the S1P-generating enzyme activated by TNFα) as a potential new therapeutic target for SSHL. We conclude that any pathology linked to the release of TNFα has the potential to reduce cochlear blood flow and cause SSHL. The present study integrates SSHL into the family of cardiovascular pathologies, with immediate implications related to risk stratification, diagnosis and treatment.

O36

CONTRIBUTION OF GJB2 MUTATIONS FOR NON-SYNDROMIC SENSORINEURAL HEARING LOSS IN PORTUGAL

Matos T.D.[1], Simões-Teixeira H.[1], Caria H.[2], Chora J.[1], Rosa H.[3], Monteiro L.[4], O'Neill A.[5], Dias O.[6], Andrea M.[6], Fialho G.[7]

([1]Centre for Biodiversity, Functional, and Integrative Genomics (BioFIG), Faculty of Science, University of Lisbon, Lisboa; [2]Centre for Biodiversity, Functional, and Integrative Genomics (BioFIG), Faculty of Science, University of Lisbon, Lisboa; Higher School of Health, Polytechnic Institute of Setúbal, Setúbal; [3]ORL Service, Hospital Garcia de Orta, E.P.E., Almada; [4]ORL Service, Hospital Dona Estefânia (CHLC, E.P.E.); [5]Department of Head and Neck, Hospital Egas Moniz (CHLC, E.P.E.), Lisboa; [6]ORL Service, Hospital Santa Maria (CHLN, E.P.E.)), Lisboa; [7]1Centre for Biodiversity, Functional, and Integrative Genomics (BioFIG), Faculty of Science, University of Lisbon, Lisboa)

Objective: A variable proportion of NSSHL cases are due to mutations in the *GJB2* gene in several populations, which present distinct spectra and prevalence of such mutations. In order to assess the contribution of *GJB2* mutations to NSSHL and determine whether a few of these explain the majority of *GJB2*-related cases, in a given population, *GJB2* screening is necessary in NSSHL patients.

In this study we have investigated the prevalence of GJB2 mutations in 301 Portuguese unrelated individuals (sporadic and familial cases), presenting with mild to profound NSSHL. A recessive mode of inheritance was predominantly observed in the familial cases.

Methods: Allele-specific PCR, SSCP, multiplex PCR, and sequencing were used in this screening.

Results: At least one *GJB2* coding mutation was found in 82 (27.2%) of the 301 patients. Biallelic mutations were found in 50 (16.6%) of the probands, of which 84% harboured at least one c.35delG allele. The most prevalent mutated genotype was c.35delG/c.35delG, accounting for 54.0% of the biallelic individuals. Next to this genotype, each of the c.35delG/p.Glu47X and c.35delG/p.Trp172X genotypes represented 6% of the biallelic patients. Thirty-two out of 301 (10.6%) probands were monoallelic, harbouring only one coding mutation. Subsequent analysis, comprising the *GJB2* basal promoter and donor splice site, as well as the two common *GJB6* deletions, revealed that at least 18.8% of these monoallelic patients presented an additional DFNB1 mutation, c.-259C>T, -23+1G>A or del(GJB6-D13S1854).

Conclusion: The present study demonstrates that mutations in the *GJB2* gene are an important cause of hearing impairment in Portugal. With basis solely on coding mutations, a diagnosis of *GJB2*-associated hearing loss was confirmed for 49/301 (16.3%) of the cases. Six other cases, with only one *GJB2* coding mutation, were elucidated by the identification of an additional DFNB1 mutation. Overall, 55/301 (18.3%) of the patients were found to have DFNB1-related NSSHL.

O37

PREVALENCE OF GJB2 MUTATIONS IN THE PORTUGUESE MAINLAND POPULATION

Chora J.R.[1] , Rodrigues R.[1], Trincão C.[1], Simões-Teixeira H.[1], Matos T. D.[1], Fialho G.[1], Caria H.[2]

([1]Center for Biodiversity, Functional and Integrative Genomics (BioFIG), Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal; [2]Center for Biodiversity, Functional and Integrative Genomics (BioFIG), Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal; Escola Superior de Saúde, Instituto Politécnico de Setúbal, Setúbal, Portugal)

Objective: A high heterogeneity of *GJB2* variants has been observed around the world in different populations. Moreover, the prevalence of some variants was shown to be population specific. Therefore, in order to facilitate molecular diagnosis of congenital deafness and improve genetic counselling in each country/region, it is important to determine the prevalence of the different *GJB2* alleles among the population.
In this study, aiming at determining the spectrum and prevalence of *GJB2* variants in the Portuguese population, we extended our previous studies on the carrier frequency of *GJB2* mutations by screening neonates, born in different Portuguese regions and randomly selected from the general population.

Methods: We have analysed a total of about 470 samples of neonates representing the major regions of Portugal mainland. Screening of *GJB2* gene was performed by direct sequencing of the entire coding region.

Results and Discussion: The analysis of the 470 samples included in the present study led to the identification of different carriers of common *GJB2* mutations and polymorphisms, some of them found for the first time in our population. These results, together with those obtained in the previous study involving 300 hearing individuals, were jointly analyzed (n=770) and compared.

Conclusion: A total of 22 different variants was found, including a novel variant (T5M), and their geographic distribution is discussed. Allelic frequency of most variants is similar to the frequency observed in other European populations. Once more, frequency of 35delG was shown to be lower than in other Mediterranean countries.

O38

GENETIC CHARACTERIZATION OF CHILDREN WITH CONGENITAL HEARING LOSS ATTENDING AN AUDITORY REHABILITATION CLINIC IN LISBON: CASE-REVIEW

Araújo-Martins J[1], Correia I[2], Ferreira R[2], Santos PB[2], Gonçalves R[3], Nunes L[3], Monteiro L[2]

([1]Serviço de Otorrinolaringologia, Hospital de São José, CHLC, EPE; Departamento de Fisiopatologia, CEDOC, Faculdade de Ciências Médicas, Universidade Nova de Lisboa; [2]Serviço de Otorrinolaringologia, Hospital Dona Estefânia, CHLC, EPE; [3]Serviço de Genética, Hospital Dona Estefânia, CHLC, EPE)

Background: Congenital hearing loss seriously compromises children's self knowledge, social interaction and learning skills. It's therefore advisable an early intervention from a specialized multidisciplinary team in order to promote auditory rehabilitation and optimize children's development.
In Portugal, since the implementation of RANU (neonatal universal auditory screening), many children are promptly referred for evaluation. Hospital Dona Estefânia is a specialized pediatric reference centre for the southern half of Portugal. In average, 120 children are referred to our clinic every year. Our congenital hearing loss diagnosis protocol follows a well established workflow involving a multidisciplinary approach that includes detailed prenatal and familial clinical register. After careful clinical assessment, the patients' DNA is analyzed by reference portuguese laboratories with international accreditation. Relevant genetic mutations are sought, using single gene mutational approach or array-CGH diagnostic methods.
Objectives: Our study aims to: assess the epidemiological background of children with congenital hearing loss attending our clinic; identify the prevalence of single gene disorders in this population; establish genotype/phenotype correlation with the patients' clinical presentation and long term manifestations; determine whether specific findings in audiologic assessment correlate with genetic diagnosis.
Methods: A retrospective study cohort is ongoing, using clinical records of all the children with congenital hearing loss attending our clinic. The study has been submitted to the Ethics Committee for approval. Informed consent was obtained.
Results: A total of 613 children are currently attending our clinic and about 40% have documented proof of hearing loss. We expect to describe and discuss data concerning about 300 children when finished.
Conclusions: Our protocol has allowed us to determine the etiological genetic diagnosis in many children with congenital hearing loss. This study is intended as a first step for a better understanding of this disorder and to improve prevention and children's care and auditory rehabilitation.

O39

MICRORNA REGULATION IN THE INNER EAR: IMPLICATIONS FOR DEAFNESS

Anya Rudnicki, Lilach Friedman, Tal Elkan-Miller, Karen B. Avraham

(Department of Human Molecular Genetics and Biochemistry, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel)

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression through the RNA interference (RNAi) pathway or inhibition of mRNA translation. The central approach to understand the biological roles of miRNAs is to identify and study their targets. In the mammalian inner ear, miRNAs have been shown to be crucial for its development and function, with miRNA target identification as an important goal. The relevance of miRNAs in the inner ear was recently exemplified by the discovery that mutations in a miRNA lead to hereditary hearing loss both in humans and mice. Most compelling, this is the first case where a Mendelian disease is caused by a miRNA mutation. Our group has shown that loss of Dicer in the inner ear hair cells of mice leads to complete deafness. We further examined the differential expression profile of miRNAs in mouse postnatal inner ear cochlear and vestibular sensory epithelia by microarray and deep sequencing analysis, providing a comprehensive miRNA expression profile of the sensory epithelia in the inner ear. Functionally relevant miRNA targets were identified by integration of mRNA and protein expression data with *in silico* target prediction. One of these miRNA-target pairs is mmu-miR-224 and pentraxin 3 (Ptx3). This miRNA is conserved between mouse and human. Its high expression pattern in the cochlear sensory epithelium and its low expression pattern in the vestibular sensory epithelium was demonstrated in the mouse inner ear by *in situ* hybridization. Ptx3 is known to be involved in regulation of the immune system and inflammation, but its role in the human or mouse inner ear is unknown. Immunohistochemistry revealed expression of Ptx3 in several components of the mouse inner ear. We speculate that miRNAs differentially expressed between the cochlea or vestibule participate in regulating these tissue identities and maintaining their distinct function.

O40

CHARACTERISATION OF MOUSE MUTANTS WITH NOVEL MUTATIONS IN THE TMC1 GENE

Shehnaaz SM Manji, Kerry A Miller, Louise H Williams, Henrik M Dahl

(Murdoch Childrens Research Institute)

OBJECTIVE:
Mouse N-ethyl-N-nitrosourea (ENU) mutagenesis screens have proven to be an invaluable tool for the functional identification of novel disease genes. To identify mouse mutants with defects in auditory function we conducted phenotypic screen of the ENU mutant mouse libraries at the Australian Phenomics Facility (APF, Canberra, Australia).

METHODS:
Heritability of the deafness phenotype was established by performing ABR hearing test. To identify the gene causing hearing loss in these ENU mutants, we used classical mapping studies to localise the deafness locus. Identification of the causative deafness gene involved using DNA sequencing and database mining. Molecular analysis was conducted to characterise the deafness causing gene including developmental, spatial and temporal gene expression studies using immunohistochemistry, as well as anatomical and SEM analysis.

RESULTS:
We present detailed characterisation of three ENU mouse mutants with novel mutations in the Tmc1 gene. DNA sequence analysis revealed A708G, T1508C and G1824T changes leading to Y182C, Y449H and W554L amino acid substitutions in *baringo*, *nice* and *stitch* mutants, respectively. The strains exhibit profound sensorineural deafness, with no vestibular dysfunction. Scanning electron microscopy revealed degeneration of outer hair cells in the basal region of *baringo, nice* and *stitch* mutants. Immunolocalisation studies revealed expression of Tmc1 protein in the hair cells, spiral ganglion neurons, supporting cells and stria ligament in the inner ear. Reduced levels of Tmc1 protein were observed in the spiral ligament of the mutants compared to wild type.

CONCLUSIONS:
These ENU strains are a valuable resource that will provide important insights into the molecular mechanisms involved in non-syndromic DFNB7/11 human hereditary hearing loss. Furthermore, these strains will enable the study of how genetic and environmental factors, including noise and cytotoxic drugs, interact to bring about hearing loss. Future studies will identify new target molecules and/or activities, for development of disease-modifying hearing loss therapies.

O41

MOLECULAR DISSECTION OF TMPRSS3 COCHLEAR HAIR CELL SIGNALING PATHWAY

Lydie Fasquelle[1], Laurence Molina[2], Régis Nouvian[1], Nicolas Salvetat[2], Michel Guipponi[3], Jean-Luc Puel[1], Franck Molina[2]

([1]Inserm U1051; [2]Sysdiag; [3]University of Geneva)

Tmprss3 is a type II serine protease mutated in human DFNB8/10 deafness. In order to determine the role of the protein in the cochlear physiology, we generated a mutant mouse and phenotyped it. Like human patients, homozygous mutant mice are profoundly deaf. The deafness is due to a rapid and drastic degeneration of the hair cells at the onset of hearing. This degeneration follows the well know base to apex maturation gradient. In order to decipher the molecular mechanism leading to hair cells degeneration, we compared the cochlear proteome of wild type vs homozygous mutant using 2D gel. Then, we analyzed the variants spot by mass spectrophotometry. This screen allows us to identify 133 variant proteins. Using bioinformatics, we clustered the protein in signaling pathways. One of this pathway is involved in the modulation of BK potassium channel. Using immunohistochemistry and patch-clamp techniques, we were able to show that in the absence of a functional Tmprss3, BK channels are no more expressed in cluster in the neck of the inner hair cell and that the biophysical properties of the remaining channels are altered. Altogether, our data show that Tmprss3 is a novel modulator of the cochlear hair cell BK channel.

O42

POLYMORPHISMS IN GENES INVOLVED IN INFLAMMATORY PATHWAYS IN PATIENTS WITH SUDDEN SENSORINEURAL HEARING LOSS

Masaaki Teranishi[1], Mariko Hiramatsu[1], Yasue Uchida[2], Naoki Nishio[1], Hidenori Suzuki[3], Ken Kato[1], Hironao Otake[1], Tadao Yoshida[1], Mitsuhiko Tagaya[4], Hirokazu Suzuki[1], Michihiko Sone[1], Saiko Sugiura[5], Fujiko Ando[6], Hiroshi Shimokata[7], Tsutomu Nakashima[1]

([1]Department of Otorhinolaryngology, Nagoya University, Graduate School of Medicine, Nagoya, Japan; [2]Dep. of Otolaryngology, Aichi Medical University, Nagakute, Aichi, Japan; [3]Dep. of Head and Neck Surgery, Aichi Cancer Center, Nagoya, Japan; [4]Dep. of Otorhinolaryngology, Tosei General Hospital, Seto, Aichi, Japan; [5]Dep. of Otorhinolaryngology, National Center for Geriatrics and Gerontology,; [6]Dep. of Health and Medical Sciences, Aichi Shukutoku University, Aichi, Japan; [7]Dep. for Development of Preventive Medicine, Center for Development of Advanced Medicine for Dementia (CAMD), National Center for Geriatrics and Gerontology, Aichi, Japan)

Objective: Although the etiology of idiopathic sudden sensorineural hearing loss (SSNHL) remains unclear. an involvement of inflammation in the etiology of SSNHL was implied through pathologically increased permeability of blood vessels elucidated by gadolinium-enhanced MRI. We investigated the associations of polymorphisms of inflammatory mediator genes with the susceptibility to SSNHL in the present study.

Methods: We compared 72 patients affected by SSNHL and 2010 adults (1010 men and 1000 women; mean age 59.2 years; range 40–79) who participated in the National Institute for Longevity Sciences - Longitudinal Study of Aging (NILS-LSA). Multiple logistic regression was used to obtain odds ratios (ORs) for SSNHL in subjects with polymorphisms in the genes *IL-6* C-572G, *IL-4R* G1902A, *IL-10* A-592C, *TNFα* C-863A, *TNFRSF1B* G593A, *VEGF* C936T, *VEGF* C-2578A, *VEGF* G-1154A, with adjustment for age, gender and any history of hypertension, diabetes or dyslipidemia.

Results: The per-allele OR for the risk of SSNHL in subjects bearing *IL-6* C-572G was 1.480 (95% confidence intervals [CI], 1.037–2.111) in model 1 (no adjustment); 1.463 (CI, 1.022–2.094) in model 2 (with adjustment for age and gender) and 1.460 (CI, 1.016–2.097) in model 3 (with adjustment for age, gender and history of hypertension, diabetes and dyslipidemia). Under the dominant model of inheritance, the ORs were 1.734 (CI, 1.080–2.783) in model 1, 1.690 (CI, 1.050–2.721) in model 2 and 1.669 (CI, 1.035–2.692) in model 3. The remaining seven polymorphisms failed to show any associations with the risk of SSNHL.

Conclusion: The *IL-6* C-572G polymorphism is associated with the risk of SSNHL.

Reference: Association of interleukin-1 gene polymorphism with sudden sensorineural hearing loss and Ménière's disease (Furuta T et al). Int J Immunogenet. 2011

O43

GENERATION OF MICE WITH HEARING IMPAIRMENT INDUCED BY GENE TRANSFER IN THE EMBRYONIC INNER EAR UTILIZING A CONNEXIN30-TARGETED SHRNA EXPRESSION VECTOR

Toru Miwa, Ryosei Minoda, Eiji Yumoto

(Department of Otolaryngology-Head and Neck Surgery, Graduate School of Medicine, Kumamoto University, Kumamoto, Japan)

OBJECTIVE: Mutation in gap junction beta-6 (GJB6), the gene that codes for connexin30 (Cx30), causes congenital hearing loss in humans and mice. Short hairpin RNAs (shRNA), that are used for gene silencing, are processed into short interfering RNAs (siRNA) that bind to the RNA-induced silencing complex. This complex cause interruption or suppression of the expression of a gene..
We investigated whether hearing impairment was induced by RNAi utilizing a Cx30-targeted shRNA expression vector (shRNA-Cx30-EGFP), which was transferred into the mouse otocyst using electroporation.

METHODS: At embryonic day 11.5 (E11.5), shRNA-Cx30-EGFP was microinjected into the otocysts of CD-1 normal mice and electroporated. The electroporated embryos were delivered by caesarean section at E18.5. Some delivered pups which underwent shRNA-Cx30-EGFP inoculation were used for immunohistological analyses. Others were left with surrogate mothers until functional and/or morphological assessments at postnatal day 30 (P30): auditory brainstem response (ABR) testing; and immunostaining. As a control, a random sense shRNA expression vector (shRNA-scramble-EGFP) was used instead of shRNA-Cx30-EGFP.

RESULTS: At E18.5, both shRNA-Cx30-EGFP and shRNA-scramble-EGFP were transferred to the prosensory lesion and lateral wall. At P30, both shRNA-Cx30-EGFP and shRNA-scramble-EGFP were transferred to the inner hair cells, outer hair cells, stria vascularis, and supporting cells, as well as to the spiral ligament and spiral limbus. Additionally, Cx30 expressions were detected in a part of the spiral ligament and supporting cells in the shRNA-Cx30-EGFP inoculated mice, and in the spiral ligament, spiral limbus and supporting cells in the shRNA-scramble-EGFP inoculated mice.
The mice which underwent the shRNA-Cx30-EGFP inoculation showed significant hearing deterioration compared with the shRNA-scramble-EGFP inoculation mice.

CONCLUSIONS: We showed that electroporation of shRNA-Cx30-EGFP plasmid efficiently knockdowned the expression of Cx30 and induced hearing deterioration.

O44

CORTICAL REPRESENTATION AT THE PERCEPTION AND PRODUCTION OF SIGN LANGUAGE BY FUNCTIONAL MAGNETIC RESONANCE IMAGING

Valadão MN

(Department of Neuroscience and Behavior, Ribeirão Preto School Medicine (FMRP), University of São Paulo (USP), Ribeirão Preto, SP, Brazil)

The language signs are natural languages that share the properties of spoken languages. Several studies have demonstrated cortical activation in classical language areas in the left cerebral hemisphere in the processing of sign languages. More recently, the processing of sign languages has also been described in homologous areas in the right hemisphere. Objective: was to determine the cortical areas activated in through tasks that involve sign language. Method: fMRI exams have been done in 12 deaf signers and 13 hearing non signers using a 1.5T equipment (Siemens, Magneton Vision, Erlangen, Germany) operating in the hospital HCRP-USP. EPI sequence was used for BOLD contrast associated with a whole brain high resolution imaging for co-register. The statistic maps were obtained using General Linear Model with *Brain Voyager™* software. Block paradigm was used with two tasks: perception and production sign language. Results: We found that the right inferior frontal cortex, bilaterally regions in the inferior temporal and parietal cortex was strongly activated in the brains of deaf signers compared with hearing participants for a task involving perception of sign language. When analyzing selective areas to the hearing, we observed activation in the left frontal and superior temporal gyrus. For tasks involving sign production, the deaf signers showed activations in the left cerebral hemisphere for regions of the pre-central gyrus, supramarginal gyrus and inferior parietal lobule, while in the middle and superior temporal gyrus, medial frontal gyrus and cingulate gyrus were bilaterally. For this task, the hearing had no significant activations. Conclusion: These data suggest that sign languages are processed in classical language regions such as the languages spoken.

O45

BEST PREDICTIVE FACTOR FOR COCHLEAR IMPLANT PERFORMANCE IN ADULTS: AGE OR DURATION OF DEAFNESS?

Susana Andrade , José Oliveira, Jorge Humberto Martins, Marisa Alves, Luís Silva, Jorge Quadros, Carlos Ribeiro

(Serviço de Otorrinolaringologia, Centro Hospitalar de Coimbra)

Introduction
Prevision of cochlear implantation outcomes has been mainly focused on patient, ear and device variables. Age-related physiological effects on both peripheral and central auditory systems might have a considerable impact on adult cochlear implant rehabilitation. However, its negative effects might be soften by the presence of a shorter preoperative auditory privation.

Objectives
This study aimed to investigate the predictive value of preoperative duration of deafness and age at time of implantation in the discrimination performance after cochlear implantation, in an adult implanted population.

Methods
A retrospective chart review was conducted. The cohort consisted of 230 adult patients that underwent cochlear implantation at the Otorhinolaryngology Department of Centro Hospitalar de Coimbra, from 1985 to 2010, and that completed a minimum of 6 months of listening experience with the cochlear implant.
Data concerning postoperative auditory performance was obtained; multiple regression analyses were performed using duration of deafness, postoperative discrimination scores and age at implantation as independent variables.

Results and conclusion
The duration of deafness prior to cochlear implant surgery was a more relevant factor in the discrimination performance than age at the time of implantation.

O46

IMPACT OF COCHLEAR IMPLANTATION ON QUALITY OF LIFE IN THE ELDERLY: BENEFITS OUTWEIGH RISKS

Susana Andrade[1], Conceição Peixoto, Jorge Humberto Martins, Luís Silva, Jorge Quadros, Carlos Ribeiro

(Serviço de Otorrinolaringologia, Centro Hospitalar de Coimbra)

Introduction: Recently, a growing number of elderly patients with severe to profound deafness have been part of hearing rehabilitation programs with cochlear implant. Studies have demonstrated that this rehabilitation method leads to very satisfying results in the geriatric population, particularly improving quality-of-life, independence and social interaction. The risk of general anesthesia or postoperative complications in this population still concerns both healthcare professionals and consumers.

Objectives: This study intended to evaluate the impact of cochlear implantation in the quality of life of in adults with more than 65 years of age at time of surgery. The authors also aimed to investigate whether advanced age is a risk factor when undergoing general anesthesia for cochlear implantation.

Methods: A retrospective chart review was conducted. 32 patients were selected from the adult population that underwent cochlear implantation at the Otorhinolaryngology Department of Centro Hospitalar de Coimbra, from 1985 to 2010, ageing more than 65 years of age at time of surgery and that completed a minimum of 6 months of listening experience with the cochlear implant.
Quality-of-life assessment of the elderly group was performed based on the questionnaire P-HHIE. Medical and surgical records were reviewed to identify intra-operative and postoperative complications.

Results: The results obtained at the questionnaires answered by the elderly group revealed a significant reduction of hearing handicap after cochlear implantation. There were no anesthesia-related complications as well as long-term morbility or mortality.

Conclusion: Cochlear implant in the elderly provides a significant improvement in quality of life. General anesthesia is well tolerated by elderly patients undergoing cochlear implantation.

O47

CONGENITAL DEAFNESS - DIFFICULTIES IN THE ETIOLOGY

Rosa MH, MD[1], Silva MI, MD[1], Nunes R[1], Ferreia JC, PhD[2], Fialho G, PhD[3], Tavares P, PhD[4], Antunes L, Md[1]

([1]Hospital Garcia de Orta, Almada, Portugal; [2]Human Genetics Laboratory, Jacob Medical Center, NYC, USA; [3]BioFig Research Center, Faculdade Ciências da Universidade de Lisboa, Portugal; [4]Centro de Genética Clínica, Portugal)

The authors present a case report of a 21 months child who was diagnosed with severe bilateral hearing loss at 6 months by auditory brain-stem response (PEATC). The child had been flagged as positive by our newborn hearing screening program (RAUN), based on otoacoustic emissions.

He was later recognized to have prior risk factors for hearing loss: the mother had rubella in the first trimester of pregnancy and there is family history of hearing loss. Genetic assessment by targeted screening of most common gene mutations known to be responsible for hearing loss identified heterozigosity for 35delG.

The possible underlying causes of the hearing loss of this child, the diagnostic strategy and the results of further testing will be presented.

We believe this case raises several interesting discussion points. Besides the etiologic diagnostic challenges, the good results of the early intervention can be used as support for the universal newborn screening program (RAUN). On the other hand, the known risk factors can make a case for selective screening as an alternative to universal screening.

O48

AUDITORY BRAINSTEM RESPONSE DISTURBANCES IN YOUNGER ADULTS WITH INSULIN DEPENDENT DIABETES MELLITUS TYPE 1

Cavalcante JMS[1], Lima MS, Bernardez-Braga GRA, Martins JG, Jorge R, Anastasio ART

(Department of Ophthalmology, Otorhinolaryngology and Head and Neck Surgery, Faculty of Medicine of Ribeirão Preto, University of São Paulo)

OBJECTIVE
To evaluate the auditory brainstem response (ABR) in young adults with insulin dependent diabetes mellitus (DM).

METHODS
The clinical analyses included 56 patients at 18-82 years of age. The patients were divided into three groups: Group A included 13 patients (X=24,62 years) with insulin dependent DM type 1 with normal hearing; Group B included eight patients (X= 53 years) with no insulin dependent DM type 2; and Group C included 35 patients (X=71 years) with no DM. Groups B and C with and without DM, respectively and no more than moderate hearing loss. Audiologic test included ABR (80dBNHL, click, 27.7c/s at least one replication). Data were analyzed statistically using the Graph Pad Instat software, version 3.0 for Windows 95. One-Way Analysis of Variance (ANOVA) was used to compare the ABR latencies waves (I, III and V) and intervals (I-III, III-V and I-V) variations among groups. RESULTS: The latencies time (ms) mean values for groups were: group A (I=1.84; III= 4.05; V=5.97; I-III=2.22; III-V=1.92; I-V=4.13); group B (I=1.80; III= 4.08; V=6.05; I-III=2.28; III-V=1.97; I-V=4.25); and group C (I=1.90; III= 4.09; V=6.06; I-III=2.19; III-V=1.97; I-V=4.16). One-way ANOVA comparing the mean latencies waves and intervals among three groups showed no significant differences. No difference inter aural was founded between ears in all groups. The analysis of the results showed that patients with DM type I had mean latencies similar to other groups, regardless of age. It would be assumed that the reason for this phenomenon is the presence of DM, mainly insulin dependent type I.

CONCLUSIONS
Central auditory dysfunction was present in young adults with insulin dependent diabetes mellitus.

O49

BELL'S PALSY – IS SURGICAL TREATMENT AN OPTION?

Oliveira F., Santos R., Oliveira V., Sousa P., Escada P., Madeira da Silva J.

(HOSPITAL EGAS MONIZ - CHLO)

Periferical Facial Palsy can have several etiologies (infectious, traumatic, ischemic and idiopathic) and regardless the cause, always has a wide repercussion in patient life, in functional aspects as well as psychosocial. The therapeutic approach for this pathology should be prompt and appropriate in each case, in order to accomplish a better recovery and prevent possible facial sequelae.

The purpose of this case report is to demonstrate that surgical decompression is a valuable treatment in some cases of Bell's palsy.

A 26-year old woman with the diagnosis of Bell 's palsy lasting 6 months was referred to our hospital for treatment. The patient was first submitted to medical treatment and physiotherapy, without complete resolution of the paralysis. After imaging tests that showed inflammation in the first, second and third portions of the facial nerve we decided to do surgical decompression. 48 hours after surgery the patient was clinically improved and almost completely recovered from the facial palsy. She was discharged eight days after surgery.

Based on literature, and in our patient, we conclude that Bell´s palsy should have an early and appropriate treatment, in order to improve the prognosis. In selected cases, the choice of surgical facial nerve decompression may provide a clinical improvement, even against all odds, as has happened in our patient.

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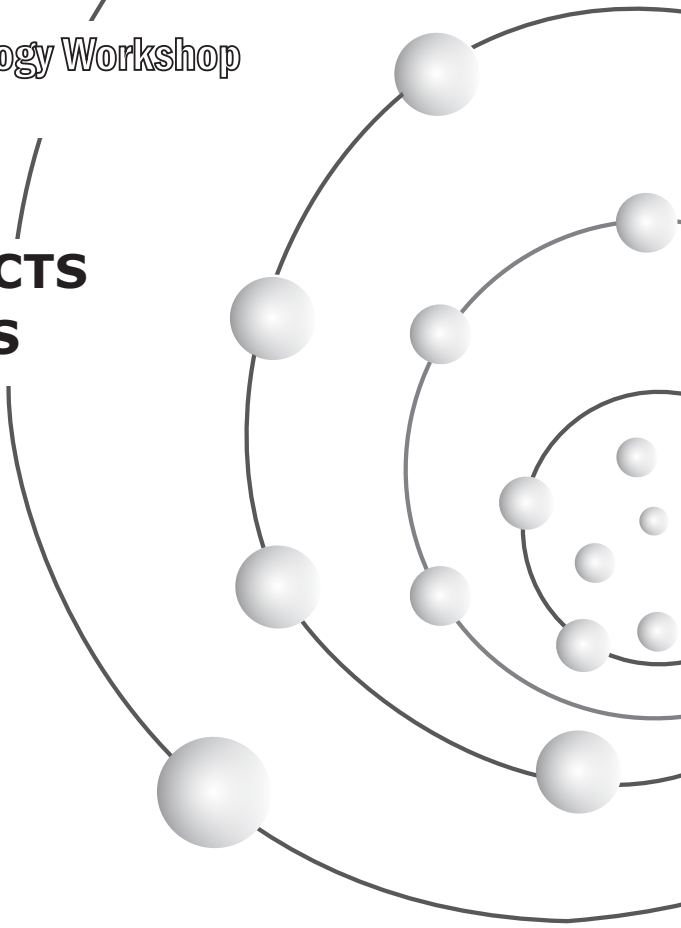
ABSTRACTS
POSTERS



DEVELOPMENTAL BIOLOGY

PO1

THE ROLE OF ACTOMYOSIN CONTRACTILITY IN SHAPING THE APICAL CIRCUNFERENCE OF HAIR CELLS

Tomoki FUJITA[1] ,Hirofumi Sakaguchi[1]; Toshihiro Suzuki[1]; Shigenobu Yonemura[2]; Yasuo Hisa[1]

([1]Kyoto Prefectural University of Medicine; [2]Riken Center for Developmental Biology, Kobe)

Objective: Organ of Corti is composed of hair cells and supporting cells properly arranged in mosaic pattern. For achieving the high sensitivity and the structual integrity of the organ, each type of cell must establish its specific shape. In order to elucidate the mechanism underlying the formation of the specific cell shape, we focused on the development of the apical cell circumference of the outer hair cells(OHC). We first demonstrate that the morphology of the outer hair cells during perinatal period is classified into three developmental stages. We then studied the involvement of the actomyosin contractility in shpaping the apical OHC circumference during development.

Method: We observerd the morphological development of the OHC in murin organ of Corti. Cochler sensory epithelia were dissected from E16 to P14 mice and stained by anti-ZO-1 antibody for visualizing the apical cell circumference. The samples were observed under confocal laser microscope and the obtained images were analyzed for quantifying the shape of the apical cell circumference in OHC and the surrounding supporting cells. Next we used primary culture system for elucidating the role of actomyosin contractility in the formation of the apical OHC circumference. We cultured cochlear sensory epithelia dissected from P2 mice and observed the effect of several inhibitors of actomyosin.

Result: The shape of OHC changes from polygonal to circular,and finally to the matured "heart" shape during E16 to P8. Myosin II is localized beneath the apical cell junction during the observed period. Inhibition of myosin II alterd the shape of the OHC apical circumference.

Conclusions: The circular shape of the apical OHC circumference seen in early postnatal stage is especially unique among all types of epithelial cells in every organ. Our data suggest thar this circular form is achieved and maintained by actomyosin contractility.

BASIC HEARING AND VESTIBULAR RESEARCH

PO2

NEURAL CREST STEM CELLS FROM ADULT HUMAN HAIR FOLLICLES AND THEIR POTENTIAL APPLICATION IN THE DEVELOPMENT OF A THERAPY FOR DEAFNESS.

Huisman MA[1], Rivolta Mn[2]

([1]Leiden University Medical Center, Leiden, The Netherlands.; [2]Centre for Stem Cell Biology, The University of Sheffield, Sheffield, United Kingdom)

Objective: Recent advances in inner ear stem cell research offer hope for many deaf patients. However, the identification of the optimal stem cell population for humans remains difficult. In this report, we call the attention to neural crest stem cells (NCSC), harvested from adult human hair follicles, as potential candidates for the treatment of deafness. This population, descendants from the embryonal cranial neural crest, shares a related developmental origin with the cells of the otic placode, their maturation is comparable and they can produce neurons and glia. Moreover, the easily accessible source facilitates autologous transplantation. Significant in the development of a clinically-viable cell therapy for deafness is the generation of sufficient cell numbers under serum-free conditions. Towards this aim, using defined culture conditions, we were able to expand *in vitro* human hair follicle stem cells expressing pertinent NCSC characteristics.

Methods: Human hair follicles were cultured in serum-free medium, OSCFM, designed in the Rivolta laboratory. The stem cells were characterized based on their outgrowth rate, morphology, proliferation, yield, migration and molecular profile using immunohistochemical and reverse transcriptase-PCR.

Results: 2-3 days after the start of the culture the cells grew out of the follicle and doubled in number per day, resulting in ~10^5 cells/well after 7 days. The cells were round and showed hardly any arborisation; concomitant with NCSC. Cells were highly motile, migrating about ~1 cm/day. IHC showed high yields of nestin-positive cells, >85%, RT-PCR, showed positivity for the most important NCSC markers: Nestin/SOX9/ SNAIL/SLUG/ and TWIST.

Conclusions: Hair follicle stem cells -grown in OSCFM- can be expanded into numbers sufficient for transplantation. Based on our results, we consider hair follicle stem cells promising candidates for autologous cell-based inner ear therapy in humans. Our next goal is to differentiate these stem cells into either inner ear hair cells or spiral ganglion neurons.

PO3

OBJECTIVE AUDIOMETRICAL PROFILE OF DIFFERENT ANIMAL MODELS BY COCHLEAR MICROPHONIC AUDIOMETRY

Francisco Carricondo, Mar Sanjuan, Julio Sanjuan, Pablo Gil-Loyzaga

(Lab. of Neurobiology of Hearing. Dept Ophtalmology and ORL, School of Medicine, University Complutense of Madrid)

Objective audiological tests constitute a valuable tool to know data about hearing status without needing the collaboration of the patient. Some of these tests are especially designed to work with children (universal newborn hearing screening) and non-collaborative patients, who are especially difficult candidates for classic audiometry. The same problem exists when objective audiological profiles are required from animal models commonly used in the laboratory. The objective of present work is to test if the objective audiological profile obtained by using cochlear microphonic audiometry (CMA) can be useful to know the hearing status of animal models. To test it, two different animal models with interest in audiology research have been used, rat (Wistar strain) and chicken (*Gallus gallus*). CMA of each animal was based in the electrophysiological recording of the cochlear microphonic potential. From that, the auditory threshold to continuous pure tones at five audiometric frequencies: 0.25, 0.5, 1, 2 and 4 KHz, was determined. For each animal model, mammalian or avian, CMAs were grouped, analyzed and the descriptive statistics obtained and plotted in audiometry-like plots. The results showed that the audiometrical profiles of both animal models are different. While chickens maintained the auditory thresholds near 10 dB(SPL) at all the frequencies, rats showed a fall in the most bass frequencies. Since cochlear outer hair cells have a very important role in the cochlear frequency discrimination, the study of their activity should be determinant to know the hearing status. For that reason, CMA could be a very relevant audiological test to know the hearing status of animals and the normal hearing patterns of the animal models used in audiology research.

PO4

DEVELOPMENTAL CHANGES IN SHORT TERM PLASTICITY PROPERTIES AT THE TRANSIENT MEDIAL OLIVOCOCHLEAR-INNER HAIR CELL (MOC-IHC) SYNAPSE

Javier Zorrilla de San Martín, Jimena Ballestero, Ana Belén Elgoyhen, Eleonora Katz

(Instituto de Investigaciones en Ingeniería Genética y Biología Molecular (INGEBI-CONICET), Buenos Aires, Argentina)

From birth until the onset of hearing (postnatal day (P)12), IHCs are transiently innervated by cholinergic medial olivocochlear (MOC) fibers. Evoked transmitter release at P9-11 has a quantal content (m) of 2, is supported by N- and P/Q-type voltage-gated calcium channels (VGCCs) and negatively modulated by BK-type K^+ channels (Zorrilla de San Martín et al., J. Neurosci 2010). The fast formation and retraction of the MOC-IHC synapse suggests there may be associated changes in synaptic transmission. Short term plasticity (STP) is a dynamic process that depends on the balance between facilitation and depression of synaptic responses caused by preceding activity. To determine whether there are changes in the STP properties at the MOC-IHC synapse during development synaptic activity was recorded in voltage-clamped IHCs from excised apical turns of the mouse cochlea during electrical stimulation of the MOC fibers at two postnatal ages (P5-7 and P9-11). In P5-7 mice, omega-AgatoxinIVA (200 nM), a P/Q-type VGCC antagonist, reduced m to 37±6% while iberiotoxin (100 nM), a BK antagonist, increased this parameter to 193±11% (control=0.75±0.33). Therefore, transmitter release is also partially supported by P/Q-type VGCCs and negatively modulated by BK channels at this stage. Ten-pulse trains at 40 and 100 Hz applied at P5-7 synapses caused a 1.8±0.3 and 2±0.4-fold increase in synaptic efficacy, respectively. At P9-11, this caused a progressive decrease in synaptic efficacy (0.6±0.1; 0.4±0.1 for the 40 and 100 Hz trains, respectively). Depression upon high frequency stimulation at P9-11 was reversed to facilitation when reducing m by decreasing [Ca2+]o or by blocking P/Q-type VGCCs and intensified by blocking BK channels. Moreover, facilitation at P5-7 was prevented by increasing [Ca2+]o or by blocking BK channels. Our results show there is a developmental switch from facilitation to depression upon high frequency stimulation consistent with the increment in the efficacy of the synapse.

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PO5

GABA REGULATES THE RELEASE OF ACH AT THE TRANSIENT OLIVOCOCHLEAR EFFERENT-INNER HAIR CELL SYNAPSE THROUGH PRESYNAPTIC GABAB RECEPTORS

Carolina Wedemeyer[1], Javier Zorrilla de San Martín, Ana Vanesa Torbidoni, Bernhard Bettler, Ana Belén Elgoyhen, Eleonora Katz

(Instituto de Investigaciones en Ingeniería Genética y Biología Molecular (INGEBI-CONICET), Buenos Aires, Argentina; [2]Department of Clinical-Biological Sciences, Institute of Physiology, University of Basel, Switzerland)

Before the onset of hearing, inner hair cells (IHCs) of the mammalian cochlea are transiently innervated by medial olivocohlear (MOC) efferent fibers. Although acetylcholine (ACh) is the main neurotransmitter, γ-aminobutiric acid (GABA) is also present at MOC synaptic terminals. To study the role of the Gabaergic system at the MOC-IHC synapse, we evaluated the effects of compounds selective for GABAB receptors on the quantal content of transmitter release. Postsynaptic currents evoked by electrically stimulating the efferent fibers were recorded in voltage-clamped IHCs from isolated mouse organs of Corti at postnatal days 9 to 11. The quantal content of evoked release was increased by the GABAB antagonist CGP35348 (65±19%, n=6 $p<0.05$) and decreased by the agonist baclofen (43.07±8%, n=8, $p<0.001$). These results, together with the lack of effect of these two drugs on spontaneous synaptic current amplitude, suggest that GABA exerts a negative control on synaptic transmission through presynaptic GABAB receptors. To confirm this hypothesis, we studied the effects of baclofen on GABAB knock-out (KO) mice. Functional GABAB receptors are formed by the GABAB2 subunit with either the GABAB1a or the GABAB1b subunit. We compared the effects of baclofen in GABAB1a-1b, GABAB1a and GABAB1b KO mice. Application of 1uM baclofen, caused a significant reduction in the quantal content of evoked release in both wild-type (32.3±6.9 %, n=5, $p<0.05$) and GABAB1b KO mice (26.7±7.7 %, n=4, $p<0.05$). However, baclofen did not affect this parameter in GABAB1a-1b ($p>0.05$, n=3) or GABAB1a KO mice ($p>0.05$, n=3). Our results show that ACh release at the MOC-IHC synapse is negatively regulated by GABA acting on presynaptic GABAB1a receptors.

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PO6

CONGENITAL HEARING LOSS IN PORTUGUESE CHILDREN – HOW MUCH IS CAUSED BY CMV?

Araújo-Martins J[1], Correia I[2], Monteiro L[2], Santos PB[2], Paixão P[3], Campos O[3], Vilarinho L[4], Almeida S[5], Marques T[3]

([1]Serv. de Otorrinolaringologia, Hospital de São José, CHLC, EPE; Dep. de Fisiopatologia, CEDOC, Fac. de Ciências Médicas, Univ. Nova de Lisboa; [2]Serviço de Otorrinolaringologia, Hospital Dona Estefânia, CHLC, EPE; [3]Dep. de Microbiologia, CEDOC, Fac. de Ciências Médicas, Univ. Nova de Lisboa; [4]Inst. de Genética Jacinto de Magalhães; [5]Centro Hospitalar da Cova da Beira)

Background: Congenital cytomegalovirus (CMV) infection has been implicated as one of the main causes of congenital sensorioneural hearing loss in children. A previous study conducted by some of the authors used Dried Blood Spots (DBS), also known as Guthrie cards, to estimate congenital CMV infection in the Portuguese population. Prevalence was determined to be 1,05% of all neonates. Other European studies have been conducted and one estimated that up to 20% of congenitally deaf children had congenital CMV infection.

Objectives: The aim of the present study was to determine the prevalence of congenital CMV infection in children with congenital hearing loss followed in an auditory rehabilitation clinic.

Methods: A retrospective study was conducted involving two main tasks. First, we reviewed our clinical files to select for children born from 2003 and onward with permanent sensorioneural hearing loss. Children were selected if they had bilateral sensorioneural hearing loss and pure-tone thresholds at least 40dB and the parents provided informed consent. Children with an established genetic diagnosis were excluded. Then the DBS stored in a national reference laboratory were sent to Faculdade de Ciências Médicas for analysis. They were submitted to a heat-induced extraction method and nested polymerase chain reaction to amplify CMV deoxyribonucleic acid (DNA).

Results: So far, 83 DBS have been tested, with 8 (9,6%) positive for congenital CMV infection.

Conclusions: The prevalence of congenital CMV infection in our sample is higher than the estimate for the general population, but lower than in other studies. Nevertheless, it would appear CMV may play a role in congenital hearing loss even if the infection wasn't apparent during pregnancy. This is an ongoing study and more definitive results are to be expected while sample size is growing.

PO7

A MODEL OF PRECISE SOUND LEVEL CODING IN THE AUDITORY NERVE USING COOPERATING NERVE FIBERS

Zbynek Bures

(College of Polytechnics, Tolsteho 16, 58601 Jihlava, Czech Republic; Institute of Experimental Medicine, ASCR, Videnska 1083, 14220 Prague 4, Czech Republic)

In the auditory nerve (AN), sound intensity is believed to be represented by the cumulative discharge rate of AN fibers. However, if a given stimulus is presented repeatedly, the spike counts are generally different for each repetition of the stimulus and the precise assessment of sound level depends on the statistic properties of the spike count, particularly on its mean and variance. To distinguish between two different sound intensities, the respective spike counts must differ by a detectable degree; however, due to the randomness of neuronal firing, the difference in mean firing rate may be masked by the variance of the spike count. The published experimental data indicate that the spike count variances in individual AN fibers may be rather large and the derived neuronal just-noticeable differences (JND) of sound level are markedly higher compared with the psychophysical values. Employing a computational model of inner hair cell and its synapse with the auditory nerve, the current work shows that it is possible to surpass the discrepancy between neuronal and psychophysical data on condition that the AN fibers do not behave as independent units, but instead cooperate in small sets on sound level coding. Using this approach, the derived JNDs of sound level are approximately two to three times lower than using totally independent AN fibers. At the same time, the statistical independence of the cooperating fibers, tested in accordance with published methods of analysis of AN data, is retained. The results indicate that cooperation rather than parallel independent operation may be a viable way of information coding in the auditory nerve. Supported by the project ”Podpora a individualni rozvoj perspektivnich akademickych pracovniku na VSPJ” at the College Of Polytechnics Jihlava.

PO8

ISOLATION OF FIBROBLASTS FROM THE SPIRAL GANGLION

Annett Anacker[1], Alice Burghard[1], Thomas Lenarz[1], Karl-Heinz Esser[2], Gerrit Paasche[1]

([1]ENT, Hannover Medical School, Germany; [2]School of Veterinary Medicine Hannover, Germany)

Objective
To investigate the effects of different drugs on spiral ganglion cells (SGC), a cell culture of freshly isolated spiral ganglion cells is well established. This culture typically contains more cell types than just SGC. In the present study we try to characterize the other cell types and purify fibroblasts from this culture.

Materials and Methods
Cells were isolated from newborn rats (P3) and cultured for 48 hours. SGC were stained for the 200 kD neurofilament. Staining of fibroblasts was done for vimentin and glial cells were stained for the S100 protein. For purification of fibroblasts (fibrocytes) the culture medium for cell isolation and also cultivation was changed from SGC specific medium to fibroblast specific medium. The influence of passaging of the cells was investigated.

Results
Using immunofluorescence neuronal cells, glial cells and fibroblasts could be detected. Medium optimized for fibroblasts in combination with dropping the laminin/ ornithine coating as typically used for SGC prevented growth of SGC but not glial cells. Fibroblasts and glial cells are more spread out when using fibroblast specific medium. Furthermore, experiments indicate that the number of glial cells is reduced when using this medium. Additionally, there are still some cells which were not stained indicating that also other cell types are present in the cell preparation from the spiral ganglion.

Conclusions
It is possible to isolate fibroblasts from the spiral ganglion, but to get purified fibroblasts there is still a long way to go.

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PO9

DIRECT SPIRAL GANGLION CELL INTERACTIONS IN THE HUMAN SPIRAL GANGLION

Glueckert R[1], Rieger G[1], Pritz Ch[1], Schrott-Fischer A[1], Rask-Andersen H[2]

([1]Department of Otolaryngology, Inner Ear Research Laboratory, Medical University of Innsbruck, Anichstr. 35, A-6020 Innsbruck, Austria; [2]Deptartment of Surgical Sciences, Section of Otolaryngology, Uppsala University Hospital, Uppsala, Sweden)

Objective: A characteristic feature of spiral ganglion neurons (SGN) in human is the absence of compact myelin of the somatic region. SGNs are often clustered with perikarya ensheathed by common satellite cells. Here, there are intimate contacts between cell somata. Aim of this study was to evaluate ultrastructurally distribution of membranous junctions between satellite glial cells as well as between SGCs and SGNs that could indicate that these cell types are physiologically coupled. This may have great influence for both their physiological function as well as at various pathology.

Methods: Transmission electron microscopy (TEM) of well preserved human cochleae from five different subjects were evaluated for cellular contact specializations in the spiral ganglion throughout Rosenthal's canal focusing on the basal and apical part.

Results: TEM demonstrated typical gap junctions only between cell processes of satellite cells but there were various membrane specializations at some contact regions between satellite cells and SGNs. Direct interaction of membranes of adjacent SGNs was found along the entire cochlea bur were most abundant in the apical region..

Conclusions: This TEM study shows that the satellite cells surrounding human type I neuron perikarya are physiologically coupled through gap junctions (GJ). We believe that these GJs consist of connexin 43 (Liu et al. 2009). Such GJ do not seem to occur when cells are surrounded by compact myelin such as in most mammals investigated so far. Since several neural cell somata are frequently surrounded by “sharing SGCs”, the situation reminds about the architecture of CNS astroglia and sensory ganglia where SGCs are believed to play a significant role for regulation of nerve excitation and synchronized firing. They could also be essential for nerve preservation after hair cell loss. Unspecific membrane junction are noted between type I cells at regions allowing for cross excitation. These structures are still under investigation.

PO10

CALCIUM HANDLING AROUND THE RIBBON SYNAPSES OF ADULT MOUSE INNER HAIR CELLS

J.Ashmore[1] , S.Culley[1], A.M.Garcia de Diego[2], M.Tobin[2], J.Boutet de Monvel[3], S.Saffieddine[3]

([1]UCL; [2]UCL Ear Institute; [3]Institut Pasteur)

Inner hair cells (IHCs) of the mammalian cochlea provide sensory input to the auditory nerve such that each cell relays information to multiple afferent boutons. There are numerous unsolved issues surrounding the operation of IHC ribbon synapses. For example evidence suggests that sound intensity information is segregated between different fibres: synapses on one cell appear to be heterogeneous, producing postsynaptic firing rates and thresholds differing from one fibre to the next.

We addressed the cellular mechanisms underlying this 'dynamic range problem' by imaging IHCs of the cochlea in the isolated temporal bone of the adult mouse using 2 photon laser scanning microscopy to achieve high resolution images. The preparation preserves cells in good condition for 2-3 hours with or without superfusion, and in the correct orientation: cells from the 10-20 kHz region were imaged from C57BL/6 P23-P50 mice. IHCs were whole cell voltage-clamped using Cs in the pipette to reduce large outward K currents, and OGB1 or OGB5N dye to measure calcium entry into the cell. On depolarization from -70 to -10 mV, IHCs revealed a small (<100pA) inward Ca current.

Calcium entry 'hotspots' were identified at the base of the cells during 100 ms depolarizing steps. Several of these 'hotspots' could often be simultaneous localised within 5 µm of the IHC basal pole. The amplitude of the response was 35% larger at modiolar side as compared to the pillar side of the cell, possibly reflecting the larger ribbon structures reported there (Liberman et al, 2011). The voltage dependence of Ca entry was not significantly different between the two sides of the cell, suggesting that further ultrastructural synapse differences are implicated.

PO11

AUDITORY BRAINSTEM RESPONSE CHANGES IN TINNITUS

Lukas Rüttiger, Sze Chim Lee, Wibke Singer, Mirko Jaumann, Annalisa Zuccotti,
Rama Panford-Walsh, Iris Köpschall, Karin Rohbock, Ulrike Zimmermann, Marlies Knipper

(Hearing Research Centre, University of Tübingen)

Tinnitus is often accompanied by hearing loss, hyperacusis, and aberrant neuronal activity known to lead to changes in neuronal plasticity and central reorganisation. Since human and rat ABR functions are generated by the activity within distinct auditory brainstem-, midbrain-, and interbrain-structures, we focused on the ABR signal waveform to reveal information about the brain areas being involved in tinnitus.

In a rat animal model we exposed rats to mildly traumatizing noise, resulting in slight hearing loss but, nevertheless, leading to tinnitus in only a subgroup of animals. Animals were categorized for experiencing tinnitus or no-tinnitus by our behavior model (Rüttiger et al., Knipper, 2003). In addition, the expression of trauma and activity related genes was analysed in the cochlea and in the central auditory system. ABR responses were analysed for amplitude and latency changes in relation to stimulus sound pressure level and hearing threshold by selecting characteristic signal deflections (wave-I to wave V) and correlation measures that delineate the functional loss by noise and tinnitus.

Here, we present a summary of recent analyses correlating expression of different genes with functional and physiological data.

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PO12

MOLECULAR ASPECTS OF TINNITUS

Hao Xiong, Lukas Rüttiger, Wibke Singer, Marlies Knipper

(University of Tübingen, Department of Otorhinolaryngology, Tübingen Hearing Research center, Molecular Physiology of Hearing, Tübingen, Germany)

OBJECTIVE : To compare different aspects on molecular level between equally hearing impaired animals with tinnitus and without tinnitus induced by acoustic trauma.

METHODS: Well-trained adult female Wistar rats that served as a behavior model were exposed to different acoustic trauma paradigms and sacrificed at different time points post-trauma. Then immunohistochemistry for CtBP2, as a measure for inner hair cell ribbon structure and a combined protein and mRNA assay for Arc/Arg3.1 expression in the different regions of the brain were performed.

RESULTS: Changes were found for inner hair cell ribbon numbers in tinnitus animals as compared to no-tinnitus animals. These changes could be correlated to Arc/Arg3.1 expression in hippocampal CA1 and the basolateral amygdale.

CONCLUSIONS: Arc/Arg3.1 could be used as a tinnitus-specific marker and modulating Arc/Arg3.1 expression may be an attractive approach for tinnitus therapy.

PO13

THE EFFECT OF SYSTEMIC KETAMINE ON CHRONIC TINNITUS

Elmar Oestreicher, Jasmin Sasse

(ENT department Ludmillenstift Meppen, GERMANY)

Introduction: Tinnitus is a disturbing symptom without any pharmacological treatment so far. Both animal studies and clinical observations suggest that glutamate antagonists might have potential for the treatment of tinnitus. We investigated the NMDA receptor antagonist ketamine for the treatment of chronic tinnitus.

Methode: Patients recieved up to 25 mg ketamine slowly i.v. over 3 hours and over 5 consecutive days. Treatment effects were assessed on day 0, 5, 30 and 90 by using a numerical estimation of tinnitus loudness/annoyance and the Tinnitus Handicap Inventory (TBF-12). A total of 44 patients completed the trial so far. 18 patients had acute tinnitus (less than 120 days) and 26 patients had chronic tinnitus (more than 120 days).

Results: In the acute tinnitus group we detected a reduction for loudness, annoyence and the TBF score at day 30 and 90. In contrast the chronic tinnitus group showed no changes at all.

Conclusion: This study does not provide evidence to recommend ketamine for the treatment of chronic tinnitus with the treatment protocol used. A possible effect could be seen in the acute tinnitus group.

PO14

LOW-FREQUENCY MODULATED CUBIC AND QUADRATIC DPOAES IN HUMAN SUBJECTS

Drexl M, Gürkov R, Krause E

(Integrated Centre for Research and Treatment of Vertigo, Balance and Ocular Motor Disorders, University of Munich, Munich, Germany)

OBJECTIVE: Previous studies have used low-frequency modulation of cubic distortion product otoacoustic emissions (DPOAEs), 2f1-f2, to assess the effect of endolymphatic hydrops on cochlear mechanics. The cubic DPOAE is mostly chosen because amplitudes sufficient for modulation can be evoked fairly easy with moderate sound pressure levels. Quadratic DPOAEs, f2-f1, are more sensitive to minute changes of the cochlear operating point (OP) and are therefore potentially better suited to assess changes of the cochlear OP. To the best of our knowledge, no data concerned with low frequency modulated quadratic DPOAEs in humans have been published.

METHODS: In this study, we compared the properties of low-frequency (30 Hz, 80-120 dB SPL) modulated cubic and quadratic DPOAEs evoked at F2= 2 and 5 kHz in healthy human subjects. The modulation depth was quantified with the modulation index (MI).

RESULTS: Modulated cubic DPOAEs showed amplitude maxima at the zero crossings of the bias tone and consequently amplitude minima at the extremes of the bias tone. Modulated quadratic DPOAEs showed the opposite behaviour: amplitude minima correlated roughly with zero crossings of the bias tone, amplitude maxima appear at extremes of the bias. Significantly lower bias tone amplitudes were needed to produce the same MI with quadratic DPOAEs as compared to cubic DPOAEs evoked with the same primary tone frequencies.

CONCLUSIONS: The modulation behaviour of low-frequency-modulated quadratic DPOAEs in humans is similar to modulation patterns seen in animal studies and as predicted by mathematical models. Human Low-frequency modulated quadratic DPOAEs are ideally suited to estimate cochlear operating point shifts because of their higher OP shift sensitivity and the lower biasing levels needed.

PO15

CALCIUM ACTIVATED POTASSIUM CURRENTS IN TYPE I AND TYPE II HAIR CELLS OF THE RAT

Enrique Soto, Jonathan Melchor, Angélica Almanza, Rosario Vega

(Instituto de Fisiología, Universidad Autónoma de Puebla, México)

The characteristics of the receptor potential in type I and II hair cells depends on the voltage change induced by the transducer current and its interaction with the voltage dependent conductance of the basolateral membrane. That is why it is relevant to determine the properties of voltage dependent ionic current in hair cells.

Amphibians express a functional IK_{Ca} that participates in the electrical resonance. However in mammalians there are no explicit characterization of the IK_{Ca}.

Vestibular hair cells from the semicircular canals were dissociated from P 20-23Long-Evans rats and its ionic currents were recorded using the whole cell voltage clamp. In both cell types it was evident a reduction of the outward current when using a Ca^{2+} free external solution, indicating that both cell types express outward currents activated by the inflow of Ca^{2+}. Three types of K_{Ca} currents have been described on the basis of their single channel conductance (BK > 250 pS, IK » 20-80 pS, SK » 6-14 pS). To define the subtype of the IK_{Ca} in hair cells specific toxins were used. The iberitoxin (100 nM, BK channel blocker) reduced the outward current 26 % in type I hair cells and 22 % in type II cells. Charibdotoxin (100 nM, BK and IK blocker) produced a 40% reduction of outward current in both hair cell types. In contrast apamine (100 nM, specific SK blocker) did not produce any significant effect in both hair cell types. In current clamp iberitoxin produced a significant change in voltage response waveform in type II hair cells.

Our results indicate that both type I and II hair cells express the IK_{Ca}, the pharmacology shows that they express BK and IK channels. No evidence of the SK current was found. The IK_{Ca} seems to have a significant role in shaping the receptor potential in type II hair cells and only a marginal role in type I hair cells.

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PO16

HCN SUBUNITS OF THE HYPERPOLARIZATION-ACTIVATED CURRENT (Ih) EXPRESSION IN THE RAT VESTIBULAR EPITHELIA, GANGLION AND CULTURED AFFERENT NEURONS.

Rosario Vega[1], Blanca Cervantes[2], Francisco Mercado[3], Angélica Almanza[1], Enrique Soto[1]

([1]Instituto de Fisiología, Universidad Autónoma de Puebla, México.; [2]Escuela Nacional de Ciencias Biológicas IPN.; [3]Instituto Mexicano de Psiquiatría, México)

Previous electrophysiological experiments indicate that in rat the afferent vestibular neurons express the hyperpolarization-activated current (I_h) carried by HCN channels. The activation of I_h started at a potential closed to -60 mV showing a half-maximal activation around -102 mV with a slope factor of 8 mV. The I_h activation range can be modulated by cAMP shifting it to more functional membrane potential. RT-PCR experiments have shown that the mRNA coding for the 4 isoforms of HCN channels are found in the vestibular epithelia and ganglia (Almanza et al, 2010).

To determine the tissue location of HCN subunits, immunohistochemical experiments were done in young and adult Long-Evans rats (P7-10 and P-28). Specific monoclonal antibodies against HCN1, HCN2, HCN3 and HCN4 were purchased from UC Davis/NIH Neuromab Facility. The immunorreactivity was studied in the vestibular epithelia (semicircular canal crista, utricle, sacule and ganglia) and in isolated afferent neurons placed in primary culture (cells maintained in a similar condition to those used for electrophysiological experiments).

Immunostaining for the four subunits was identified at the hair cells in the vestibular epithelia in neurons of the Scarpa´s ganglion and in cultured afferent neurons of both young (P7-10) and adult (P-28) rats. Immunorreactivity was clearly higher in all the cases for HCN1. Although the staining for the four subunits were clearly identifiable (HCN1>HCN2>HCN3³HCN4).

In conclusion, the four subunits of HCN were detected in the vestibular ganglia, vestibular epithelia and afferent neurons in culture. These results are in agreement with previous results using RT-PCR and show that most probably the I_h in rat vestibular afferent neurons and hair cells is formed by heteromers of the four HCN subunits.
Supported by grant PIFI-2010 and VIEP-BUAP 2011 to RV.

COCHLEAR MECHANICS

PO17

THE ´SCISSORS´ PARADIGM (L2 < L1) MAXIMIZES BASAL DPOAE COMPONENTS

Glen K Martin[1], Barden B Stagner[2], Bora Lee[3], Brenda L Lonsbury-Martin[1]

([1]VA Loma Linda Healthcare System and Dept of Otolaryngology, Loma Linda University Medical Center, Loma Linda, CA; [2]VA Loma Linda Healthcare System; [3]Dept of Otolaryngology, Loma Linda University Medical Center, Loma Linda, CA)

Recent studies in humans (Martin et al 2009), rabbits (Martin et al 2010), and a variety of laboratory species (Martin et al 2011) demonstrate that significant DPOAE components are generated basal to f_2. If sizeable basal DPOAE components are generated in the tails of the overlapping f_1 and f_2 traveling waves (TWs), then these components should be maximized by the commonly used 'optimal' $L_2<L_1$ or 'scissors' paradigm to the extent that this manipulation aligns the tails of the TW envelopes. To examine this hypothesis, DPOAE level/phase (L/P) maps, onset latencies, and interference response areas were obtained from three rabbits with L_1,L_2=50,65 dB SPL, L_1,L_2=50,50 dB SPL, L_1,L_2=65,50 dB SPL and L_1,L_2=65,65 dB SPL. The results indicated that DPOAEs collected with $L_2<L_1$ or the higher-level $L_1=L_2$ paradigms were dominated by basal-source DPOAE components originating above f_2 as compared to the $L_2>L_1$ or lower-level $L_1=L_2$ conditions. DPOAE L/P maps for the $L_2<L_1$ and higher-level $L_1=L_2$ conditions exhibited complex phase patterns and wider phase banding resulting in shorter group delays. DPOAE onset latencies were also shorter and interference response area residuals showed more components above f_2 when the $L_2<L_1$ or higher-level $L_1=L_2$ primary-tones were used. Taken together, these results strongly suggest that the optimal $L_2<L_1$ paradigm, at least, in part, achieves large DPOAE levels by both maximizing the overall region of emission generation basal to f_2 and aligning the phases of these basal DPOAE components. Practically, these findings imply that lowering L_2 may increase DPOAE levels at the expense of decreasing the ability of the DP-gram to detect small lesions and to accurately reflect the high-frequency extent of larger lesions.

PO18

COMPARATIVE STUDY OF HIGH FREQUENCY AUDIOMETRY IN PATIENTS WITH TINNITUS AND DIABETES

Cavalcante JMS, Lupoli LM, Cantarella PC, Martins JG, Rezende IA, Anastasio ART

(Department of Ophthalmology, Otorhinolaryngology and Head and Neck Surgery. School of Medicine at University of São Paulo, Brazil)

OBJECTIVE: Compare hearing thresholds in High Frequency Audiometry (HFA) in patients with tinnitus and patients with diabetes mellitus type II.

METHODS: The sample consisted of two groups, tinnitus group (TG): 11 patients of both genders (39-66 years of age, X= 55,4 years) with tinnitus and Diabetes group (DG): nine patients of both genders (44-69 years of age, X= 54.9 years). Inclusion criteria: average hearing thresholds for frequencies (Hz) of 500, 1.000, 2.000, 3.000 and 4.000 up to 25 dBHL in both ears. Audiologic tests were perfomed out using a model Unity, Siemens, with the earphones HDA 200 Sennheiser. In HFA had been investigated the frequencies from 9.000 to 16.000Hz, which are available in equipment. In the absence of answers, it was added 10dB for statistical analysis.

RESULTS: The average hearing thresholds (dBHL) for HFA in TG was 46.59 (9.000Hz), 46.36 (10.000Hz), 59.09 (11.200Hz), 68.40 (12.500Hz), 66.36 (14.000 Hz) and 60.22 (16.000Hz), and the DG was 35.27 (9.000Hz), 36.38 (10.000Hz), 42.22 (11.200Hz), 50.55 (12.500Hz), 57.77 (14.000Hz) and 54.44 (16.000Hz). No difference inter aural was founded between ears in all groups. For frequencies from 9.000 to 12.500Hz, the TG had presented worse hearing thresholds than the DG group, with statistically significant difference. There was no statistical difference in the frequencies of 14.000 and 16.000Hz, however the TG showed worse hearing thresholds. For both groups, we could see a worsening in hearing sensitivity with increasing frequency tested. The analysis of the audiogram showed that results should be analyzed with caution even though the audiometric classification is considered normal.

CONCLUSIONS: Hearing thresholds for the HFA TG were worse than for the DG.

PO19

CHANGES IN EXTENDED HIGH FREQUENCY AUDIOMETRY AND TRANSIENT OTOACOUSTIC EMISSION IN PATIENTS WITH TINNITUS

Cavalcante JMS[1], Lupoli LM[2], Rodrigues PC[2], Anastasio ART[2]

([1]School of Medicine of University of São Paulo; [2]Department of Ophthalmology, Otorhinolaryngology and Head and Neck Surgery, Faculty of Medicine of Ribeirão Preto, University of São Paulo)

OBJECTIVE: To compare hearing thresholds of extended high frequency and signal-noise ratio of the transient otoacoustic emission in patients with tinnitus. METHODS: The sample consisted of 28 patients (X=49.5 years of age) and subdivided in two groups- Group A (GA): 20 ears (hearing thresholds from 250Hz to 8.000Hz $\leq$ 25dBHL) and group B (GB): 27 ears (average from 500Hz to 4.000Hz $\leq$ 25dBHL and > 25dBHL at least one frequency from 250Hz to 8.000Hz). Audiologic tests were performed with Unity/Siemens and Echoport 292/Otodynamics equipments (frequency bands 1000, 1414, 2000, 2828 and 4000Hz). Data were analyzed statistically using the Graph Pad Instat software, version 3.0 for Windows 95. Unpaired t Test was used to compare hearing thresholds and the signal/noise ratio.

RESULTS: The mean hearing thresholds of extended high frequency audiometry (dBHL) for the GA and GB were 15.75 and 50.37 (9.000Hz); 15.25 and 50 (10.000Hz); 22.75 and 62.59 (11.200Hz); 28.25 dB and 68.14 (12.500Hz); 28 and 63.7 (14.000Hz); 29 and 58.88 (16.000Hz), respectively. There was statistically significant for all frequencies. The average signal/noise ratio (dBNPS) for the transient otoacoustic emission for the GA and GB were 10.91 and 6.85 (1.000Hz); 16.53 and 13.15 (1.414Hz); 13.32 and 13.64 (2.000Hz); 12.98 and 7.31 (2.828Hz); 8.29 and 2.20 (4.000Hz), respectively. There was a statistically significant difference for the bands at 3.000Hz and 4.000 Hz. The analysis of the results showed those audiologic tests have clinical relevance in the cochlear dysfunction diagnose prior to pure tone threshold test.

CONCLUSIONS: Hearing thresholds of extended high frequency and signal-noise ratio of the transient otoacoustic emissions for the GB were worse than for GA.

PO20

PERIVASCULAR RESIDENT MACROPHAGES CONTROL BLOOD-LABYRINTH-BARRIER PERMEABILITY

Shi X, Nuttall AL

(Oregon Health & Science University)

The blood-labyrinth barrier (BLB) in the stria vascularis of the cochlea maintains the endocochlear potential, ion transport, and fluid balance in the inner ear. Disruption of the BLB has long been considered a major etiologic factor in a variety of hearing disorders. Despite the importance of the BLB, the mechanisms that control BLB permeability remain largely unknown. Previously, we found a large number of perivascular resident macrophages (PVMs) at the BLB. In this study, with transmission electron microscopy, we found that a rich network of PVMs' processes contacted the capillary wall by an electron dense basal lamina. Their end-feet of the processes were strikingly rich in mitochondria and also contained numerous vesicles. Using both *in vitro* (cell culture-based) and *in vivo* (genetically PVMs ablation) models, the functions of PVMs in the BLB was assessed. We found that PVMs are a critical functional component for blood-labyrinth integrity. The *in vitro* study showed that endothelial cell monolayer permeability was significantly increased in the absence of PVMs. Consistent with results from the *in vitro* study, ablation of PVMs *in vivo* caused marked leakage from vasculature. We also determined that PVMs released a pigment epithelial-derived factor directly affected BLB permeability through an effect on the expression of tight junction proteins ZO-1, occludin, and ve-cadhenrin. Supported by NIDCD DC 000105, NIDCD DC 010844 and NIDCD DC 005983

PO21

DOPAMINERGIC REGULATION OF ENAC FUNCTION IN THE REISSNER'S MEMBRANE OF THE COCHLEA

Chang-Hee Kim, Hye-Young Kim, Ho Sun Lee, Sun O Chang, Seung-Ha Oh, Jun Ho Lee

(Seoul National University College of Medicine)

Dopaminergic neurons have been thought to exist in the lateral olivoocchlear efferent system and modulate the afferent nerve activity. Several studies have identified diverse dopamine receptor subtypes in the cochlea such as spiral ganglion neurons and inner hair cell regions. Dopamine is also known to increase lung fluid clearance mainly due to activation of amiloride-sensitive sodium channels (ENaC) in the alveolar cell by dopaminergic stimulation. . The Reissner's membrane (RM) form much of the boundary between endolymphatic and perilymphatic space in the cochlea and are capable of transporting Na^+ out of cochlear endolymph via ENaC. However, much remains to be known as dopaminergic regulation of ENaC function in RM. In the present study, the authors investigated the effect and mechanism of dopaminergic signaling on ENaC in the RM of gerbils at the age of postnatal day 21. We investigated dopaminergic signaling as a possible regulatory mechanism of ENaC in gerbil RM using voltage-sensitive vibrating probe technique and immunohistochemistry. Results showed that dopamine induced partial activation of the amiloride-sensitive short-circuit current, but did not change short-circuit current when applied in the presence of amiloride. The response to dopamine was inhibited by SCH-23390 (D1 receptor antagonist). D1 receptors were weakly immunopositive on RM. These results suggest that the physiological role of dopaminergic receptor in RM is likely to regulate Na^+ homeostasis in the endolymph by increasing ENaC activity.

PO22

A TRIGEMINAL INNERVATION OF THE COCHLEA

Vass Z[1], Nuttall Al[2]

([1]Studiomed Plusz; [2]Oregon Health & Science University)

Innervation patterns of neurons from the trigeminal ganglion to cochlea were studied using retrograde transport of wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP). Guinea pigs were unilaterally implanted with an osmotic pump for cochlear delivery of 2% and 20% WGA-HRP (Group 1), 2% WGA-HRP followed by 100 mmol capsaicin (Group 2), or vehicle alone (Group 3). Histological sections were obtained after 48h of infusion. In Group 1, a large number of labeled nerve cell bodies were observed in the anteromedial portion of the trigeminal ganglion and at the origin of the ophthalmic nerve. Some labeled cells were also found on the lateral side of the ophthalmic nerve and on the medial side of the maxillary nerve root. A few labeled neurons were also found in the trigeminal brain stem nucleus complex and in olivocochlear neurons. No WGA-HRP-positive cells were observed in the spinal C1 or C2 cervical ganglia or in the superior or inferior glossopharyngeal ganglia. In contrast, WGA-HRP application to the middle ear resulted in labeled cells in the middle-posterolateral portion of the trigeminal ganglia and in the superior ganglion of the glossopharyngeal nerve. Capsaicin pretreatment, which damages sensory nerves, significantly reduced the density of labeled neurons in the trigeminal ganglion.

These results provide the direct evidence that the trigeminal ganglion projects to the cochlea. We propose the possibility that vascular sudden hearing loss may reflect a disturbance in the interaction between the neurons in the the trigeminal ganglion and the cochlear blood vessels. We also propose that the vestibular and auditory deficits associated with migraine are mediated by the excitatory effects of the trigeminal ganglion. In addition, some neurotrophic viruses such as herpes zoster,which is localized in the trigeminal ganglion, are also capable of causing sensorineural hearing loss.

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PO23

THE DEVELOPMENT, DISTRIBUTION AND DENSITY OF THE PMCA2 CALCIUM PUMP IN RAT OUTER HAIR CELLS

Carole Hackney[1] , Shanthini Mahendrasingam[2], David Furness[2], Qingguo Chen[3], Robert Fettiplace[3]

([1]Sheffield University, UK; [2]Keele University, UK; [3]University of Wisconsin-Madison, USA)

Calcium plays a crucial role in cochlear outer hair cells (OHCs). It enters mainly via mechano-transducer (MT) channels and is extruded by the plasma membrane calcium pump isoform PMCA2, mutations in which cause progressive hearing loss. To assess how pump expression matches the differing demands of hair cell Ca^{2+} balance, immuno-fluorescence and post-embedding immunogold labeling were used to quantify the distribution of PMCA isoforms at different cochlear locations during development. The PMCA2 isoform was confined to the mechano-sensory stereociliary bundle, first appearing at the base of the cochlea around post-natal day 0 (P0) followed by the middle and then the apex by P3, and was unchanged after P8. The developmental time course closely matches maturation of the MT channels in rat OHCs (Waguespack et al. 2007). High resolution immunogold labeling in adult rats showed PMCA2 was distributed along the lengths of all three rows of OHC stereocilia at similar densities and at about a quarter the density in IHC stereocilia. The difference between OHCs and IHCs is similar to the ratio of their MT channel resting open probabilities. Gold particle counts revealed no significant difference in PMCA2 density between apical and basal OHC bundles despite basal OHCs having five-fold larger MT currents. By calibrating the immunogold particle counts against gels containing the antigen, the absolute PMCA2 density in OHC stereocilia was determined at both apex and base as about $9000 \cdot \mu m^{-2}$, implying an extrusion rate for a single pump of about $70 \text{ ions} \cdot s^{-1}$. Absolute PMCA2 density was also inferred from quantitative Western blots. We suggest that the limited ability of PMCA2 to extrude the large Ca^{2+} load through MT channels open at rest constitutes a major cause of OHC vulnerability and early onset high-frequency hearing loss. Work supported by NIDCD (RO1 DC01362) & UW Steenbock Foundation (RF) and RNID (DNF).

OTOTOXICITY, PROTECTION AND REGENERATION

PO24

ANTIPROLIFERATIVE EFFECT OF DEXAMETHASONE IN VITRO

Wrzeszcz A[1], Dittrich B[2], Haamann D[2], Nolte I[3], Lenarz T[1], Reuter G[1]

([1]Department of Otolaryngology, Hannover Medical School, Hannover, Germany; [2]Institute of Technical and Macromolecular Chemistry, Chair of Textile and Macromolecular Chemistry, RWTH Aachen University, Aachen, Germany; [3]Small Animal Clinic, University of Veterinary Medicine Hannover, Hannover, Germany)

The insertion of cochlear implants into the inner ear often causes inflammation and fibrosis inside the scala tympani and thus growth of connective tissue on the implant. This leads to the loss of function in both electrical and laser-based implants with optical fibers. In the former by increasing the impedance and in the latter by reduction of penetration of laser light through the connective tissue, respectively.

The approach is growth inhibition of fibroblasts through the release of dexamethasone (Dex) from the base material of the implant (polydimethylsiloxane, PDMS) and a cell adherence reducing NCO-sP (EO-stat-PO) hydrogel coating. Dex-loaded, unloaded, coated and uncoated PDMS filaments were co-cultivated *in vitro* with cultures of fluorescent fibroblasts, analysed qualitatively by light and fluorescent microscopy and quantified by cell counting.

In wells with Dex-loaded samples, the total number of fibroblasts in the wells decreased by 70% compared to the unloaded. The hydrogel showed no effect on the total number of cells in the well, yet about 95% fewer cells grew directly on the samples.

The diffusion of the released substance into the medium achieves a reduction of the fibroblast growth on the sample as well as inside the whole well. The hydrogel prevents direct cell seeding on the sample surface. The combination of Dex-releasing PDMS and protein-repellent hydrogel is suitable for the reduction of fibrosis and the prevention of function loss of cochlear implants.

PO25

HYDROGEL BASED APPLICATION OF DEXAMETHASONE FOR INNER EAR THERAPY

Hütten M[1], Möller M[2], Hessler R[3], Stöver T[4], Esser K-H[5], Lenarz T[6], Scheper V[6]

([1]Department of Otolaryngology, Hannover Medical School, Germany; [2]Institute for Textile and Molecular Chemistry, RWTH Aachen, Germany; [3]MED-EL, Innsbruck, Austria; [4]Department of Otolaryngology, KGU Frankfurt am Main, Germany; [5]Institute of Zoology, University of Veterinary Medicine Hannover, Foundation, Germany; [6]Department of Otolaryngology, Hannover Medical School, Hannover, Germany)

Objective: Patients suffering from sensorineural hearing loss can be provided with a cochlear implant (CI) to regain a hearing perception. Postoperative connective tissue growth affects the CI efficiency by increasing the impedances and reducing residual hearing. CIs functionalized with glucocorticoides like dexamethasone could suppress the fibroblast growth and may protect residual sensory cells and thus improve the outcome of the implant.

Methods: The hydrogel STAR-PEG-stat-PO, consisting of hyaluronic acid, can incorporate active ingredients and its chemistry and consistency allows the filling of the hydrogel into a silicone reservoir. The hydrogel was saturated with a dexamethasone solution (50mg/ml), injected into the reservoir and implanted into the inner ear of guinea pigs. In order to assess the effects of the delivered dexamethasone on the animals' hearing, the hearing threshold was weekly measured via acoustically evoked auditory brainstem response over a period of 28 days. On day 28 the inner ears were explanted, embedded in epoxy casting resin, and grinded. Subsequently fibrous tissue growth was examined histologically.

Results: The interim results suggest no difference between the hydrogel reservoir and a positive control - represented by an animal group treated with a conventional osmotic pump - regarding the effects on hearing ability and fibroblast growth. Compared to a negative control the growth of connective tissue seems to be reduced.

Conclusion: Up to now it can be stated that the flexibly deployable hydrogel is a promising application aid for releasing water-soluble drugs in therapeutically relevant doses into the inner ear for a sustained treatment period.

PO26

ACUTE NOISE INDUCED PERMANENT THRESHOLD LOSS IS REDUCED BY TIMELY LOCAL APPLICATION OF DEXAMETHASON

Marcus Müller[1], Heinz Maier[2], Matthias Tisch[2], Hubert Löwenheim[1]

([1]University Tübingen, Germany; [2]Bundeswehrkrankenhaus Ulm, Germany)

Objective: Excess noise exposure leads to an acute threshold shift and quite frequently to a permanent threshold shift (PTS). Treatments to limit the PTS show that corticosteroids may provide a potent medication. Here we investigated the use of corticosteroids in a guinea pig model of acute noise trauma.

Methods: Exposure to impact noise led to a permanent hearing loss in the entire frequency range. Hair cell loss was observed in the middle and apical region of the cochlea. For treatment osmotic pumps were subcutaneously implanted immediately, 1, 3, or 7 days after the noise exposure. A catheter was used to apply Dexamethason (4 mg/ml) to the round window of the cochlea, the pumps (0.5 µl/h) lasted for 14 days, and then PTS was determined. *Results:* The success of treatment with corticosteroids showed a dependence on the initiation of treatment. When treatment started immediately or one day after noise exposure the PTS was the lowest. When treatment started 3 days after the exposure a reduced effectiveness of rescue in the high frequency range was observed. When treatment started 7 days after noise exposure, effectiveness was reduced further, at most frequencies in the range of untreated controls.

Conclusion: Permanent hearing loss and hair cell loss was reduced using 4 mg/ml Dexamethason applied at the round window of the cochlea. This treatment had a statistically significant protective effect when applied within 3 days after the noise insult.
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PO27

DEVELOPMENT OF AN IMPROVED COCHLEAR ELECTRODE ARRAY FOR USE IN EXPERIMENTAL STUDIES

Andrew Wise[1], James Fallon[1], Kristien Verhoeven[2], Jin Xu[1], Frank Risi[2], Robert Shepherd[1]

([1]Bionics Institute; [2]Cochlear Ltd)

Animal studies play an important role in establishing the safety and efficacy of cochlear implants and the development of new electrical stimulation strategies. In the present study we evaluate the safety and efficacy of a new electrode array designed to more accurately simulate the electrode insertion depths achieved clinically.

The insertion depth and trauma associated with the insertion of a new generation electrode array (Hybrid-L) was compared with a standard experimental electrode array. Each array was inserted into a cat cadaver cochlea (n=6) and a micro-focus X-ray imaged their anatomical location within the scala tympani. The implanted cochleae were then serially sectioned and at every 300µm they were photographed to determine the position of the array and to examine for insertion trauma.

Mean insertion depth for the Hybrid-L array was 334.8° (SD=21°; n=4) versus 175.5° (SD =6°; n=2) for the standard electrode array. This relates to an insertion depth of approximately 10.5 mm and 6 mm respectively. Each electrode array was located in the scala tympani and showed no evidence of electrode insertion trauma.

Two cats were chronically implanted with Hybrid-L arrays and electrically-evoked potentials recorded over a six month period. A similar insertion depth was measured in a chronically implanted animal with a Hybrid-L array. Evoked potential data from the chronically implanted animals exhibited significantly lower thresholds compared with animals implanted with a standard 8 ring array, with electrical thresholds remaining stable over the six month chronic stimulation period.

Cochlear´s Hybrid-L electrode array can be safely inserted ~50% of the length of the cat scala tympani, placing the tip of the array at approximately the 4 kHz place. This insertion depth is considerably greater than is routinely achieved using a standard array (~12 kHz place). The Hybrid-L array has application in research associated with bilateral cochlear implantation, electric-acoustic stimulation and plasticity studies.

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PO28

MULTIFUNCTIONAL NANOPARTICLE DELIVERY TO THE HUMAN INNER EAR

Anneliese Schrott-Fischer[1], Soumen Roy[2], Alex H.Johnston[3], Thomas Perrier[4], Tracy A. Newmann[5], Mario Bitsche[6], Patrik Saunier[7], Rudolf Glueckert[8]

([1]Inner Ear research laboratory; [2]Inner Ear Research laboratory; [3]School of Biological Scienes Southhampton; [4]Universte d Anger Inserm U646; [5]School of Biological Scienes; [6]Inner ear Research Laboratory; [7]Universite D Anger Inserm U646; [8]Inner Ear Laboratory Medical University, Austria)

Objective: Hearing loss is a very significant health problem. The methods currently available for inner ear drug delivery are limited and a non-invasive cell specific drug delivery strategy needs to be found. In this study we investigated the ability of polymersomes, lipid core nanocapsules and hyperbranched poly-L-lysine to cross the round window membrane (RWM). Nanoparticles (NPs) used in this study have different size and chemical compositions.

Methods: Freshly frozen human temporal bones were used for this investigation. Nps were placed on the intact human RWM niche and incubated for 24 h. Permeabilisation and distribution within the cochlea were evaluated.

Results: In this investigation we could visualize the NPs crossing the RWM. The NPs were subsequently found to be distributed in the sensory hair cells, nerve fibers and to other cells of the cochlea. Both small (~10nm) and bigger sized particles (~100nm) were found overcome the RWM barrier.

Conclusion: This finding raises hope in terms of future multifunctional NPs based drug delivery strategy to the human inner ear.

PO29

PEPTIDE CONJUGATED MULTIFUNCTIONAL NANOPARTICLE BASED TrkB ACTIVATION IN G7 CELL LINES

Roy S[1], Johnston AH[2], Syed TM[3], Dudas J[1], Newman TA[4], Hausott B[5], Glueckert R[1], Schrott-Fischer A[1]

([1]Department of Otolaryngology, Inner Ear Research Laboratory, Medical University of Innsbruck, Anichstr. 35, A-6020 Innsbruck, Austria; [2]School of Biological Sciences, University of Southampton, Southampton, SO17 1BJ, United Kingdom; [3]Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan; [4]School of Medicine, University of Southampton, Southampton, SO17 1BJ, United Kingdom; [5]Division of Neuroanatomy, Innsbruck Medical University, Muellerstrasse 59, A-6020 Innsbruck, Austria)

Objectives: Nanotechnology is currently providing a promising approach for multidisciplinary breakthrough solutions to the problems with hearing impairments. Multifunctional nanoparticles (MFNPs) enable receptor specific targeted interactions to activate specific signaling pathways. Our recent work has demonstrated that peptide conjugated nanoparticles can specifically target the spiral ganglion neurons, Schwann cells, nerve fibers and some population of hair cells of the mice inner ear. We hypothesized that this peptide-nanoparticle conjugates may activate TrkB receptors, which are involved in survival signaling of neurons. In this study we have conjugated hNgf_EE peptide (mimetic of NGFβ) to the surface of polymersome nanoparticles (NPs) to see whether TrkB is activated in SHSY-G7 cells.

Methods: The PEG-*b*-PCL polymer was functionalized with hNgf_EE peptide and rhodamine prior to formation. SHSY-G7 cells express high amount of TrkB and thus served as an efficient model for TrkB receptor specific targeting. The hNgf_EE peptide and TrkB receptors were manually docked using chimera software. Immunofluorescence for TrkB phosphorylation was studied by confocal microscopy.

Results: The hNgf_EE conjugated NPs were shown to activating the TrkB receptors *in vitro* in the SHSY-G7 cell line and were confirmed by immunofluorescence. We suggest that polymersomes could be used as a support for the delivery of TrkB activating moieties as the polymersome have been shown to increase *in vivo* retention time. In this present study we showed specific chemical interaction between hNgf_EE peptide and TrkB by manual docking.

Conclusion: We conclude that these multifunctional NPs would have a great potential for targeted delivery as well as for activating survival signal in the neurons. Our finding may help to develop TrkB target based cellular therapy in the inner ear.

PO30

FETAL THYMUS GRAFT PREVENTS PRESBYCUSIS AND AGE-RELATED UP REGULATION OF THE IL-1 RECEPTOR TYPE II GENE IN HELPER T CELLS

Hiroshi Iwai[1], Muneo Inaba[2], Susumu Baba[1], Koichi Tomoda[1]

([1]Department of Otolaryngology, Kansai Medical University; [2]First Department of Medicine, Kansai Medical University)

OBJECTIVE: There has been no strategy for the prevention and treatment of age-related hearing impairment (AHI), presbycusis, which is seen concomitantly with physical signs of aging, including immunosenescence mainly caused by thymic involution. The aim of this study is to demonstrate that SAMP1 (senescence-associated mouse type 1) mice, a murine model of human senescence, show prevention of age-related T cell dysfunction, auditory impairment, and degeneration of spiral ganglion cells (SGCs) by syngeneic transplantation of the fetal thymus.

METHODS AND RESULTS: These mice can overcome the progress of AMI by adoptive transfer of helper T (Th) cells, but not cytotoxic T (Tc) or B cells, collected from young syngeneic donors (2 months old). Furthermore, DNA microarray and flow cytometric analyses indicate that thymus grafts prevent age-related up regulation of the IL-1 receptor type II (Il1r2) gene of Th cells and surface expression of Il1r2 on the cells, whereas aging promotes them.

CONCLUSIONS: The findings from the current study suggest that up regulation of Il1r2 genes in Th cells is associated with age-related dysfunctions of T cells and the spiral ganglion in the cochlea, and that rejuvenation of the recipient immunity by inoculation of young Th cells or a fetal thymus graft leads to down regulation of the gene and prevents those senescence features. Our studies on the relationship between age-related systemic immune dysfunctions and neurodegeneration mechanisms open up new avenues of treatment of neurosenescence, including presbycusis, for which there is no effective therapy.

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PO31

INTRACOCHLEAR INJECTION OF ADENO-ASSOCIATE VIRUS VECTOR CARRYING THE GJB2 GENE TO A MOUSE MODEL CREATED BY A CONDITIONAL KNOCKOUT OF GJB2 GENE

Katsuhisa Ikeda[1], Takashi Iizuka[2], Hideki Mochizuki[3], Tomoko Nihira[3], Ayako Inoshita[2], Misato Kasai[2], Hiroko Okada[2], Hiromi Kasagi[2], Kazusaku Kamiya[2], Osamu Minowa[4], Tetsuo Noda[5]

([1]Department of Otorhinolaryngology, Juntendo University School of Medicine; [2]Department of Otorhinolaryngology, Juntendo University School of Medicine, Tokyo; [3]Department of Neurology, School of Alied Health Sciences, Kitasato University, Sagamihara; [4]Mouse Functional Genomics Research Group, Riken, Yokohama; [5]Department of Molecular Biology, Cancer Institute, Tokyo)

OBJECTIVE: Hereditary deafness affects about 1 in 2,000 children and mutations in the *GJB2* gene, cording the gap junction, are the major causes in various ethnic groups, which require normal gene transfer in the early developmental stage to prevent deafness. Mice present an ideal model for inner ear gene therapy because their genome is being rapidly sequenced and their generation time is short. In order to establish the fundamental therapy of congenital deafness, we generated targeted disruption of mouse *Gjb2* gene using Cre recombinase controlled by P0.

METHODS: Using this animal model, we examined the potential of gene therapy in the inner ear, using the homozygous mutant mice and the heterozygous mutant mice.

RESULTS: Adeno-associated virus vectors (AAV) carrying *Gjb2* gene were injected into the scala tympani through the round window of the cochlea of the homozygous mutant adult mice. The expression of Cx26 was observed in the fibrocytes of the spiral ligament and spiral limbus, but was not seen in the supporting cells and failed to improve the hearing ability. However, we succeed in gene introduction to the supporting cells of neonatal mice without hearing loss using AAV (Iizuka T, et al. 2008). The present paper will present the preliminary data regarding introduction of the virus vector into the *Gjb2* knockout mouse at the neonatal stage.

PO32

RETENTION OF STEM CELL PHENOTYPES IN LONG-TERM CULTURE NEONATAL MOUSE OTOSPHERES

Xiangxin Lou, Takayuki Nakagawa, Koji Nishimura, Juichi Ito

(Kyoto University)

In mammalian, loss of hair cells in the cochlea leads to permanent hearing loss. Stem cells offer a potential cell based therapy for the treatment of sensori-neural hearing loss. Otospheres, which were derived from mammalian cochleae, exhibit proliferation potential, self-renewal and multipotent differentiation ability.

Objectives: This study was to determine the expression of potent stem markers, Naong, Sox2, Klf4, Oct3/4, Jagged1 and Nestin in the early postnatal cochlear specimens *in vivo*, and to evaluate the potential stem cell phenotypes in long term development of cultured otospheres from dissociated organ of Corti *in vitro*.

Methods: Organ of Corti were surgically isolated from postnatal day-1 mice, dissociated and cultivated under suspension conditions. Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) and Immunofluorescence assays were conducted for phenotype characterization.

Results: In postnatal day-1 organ of Corti, we show stem markers Nanog, Nestin, Sox2, and Jagged1 displayed overlapping expression pattern in the supporting cell subtypes. RT-PCR data show cultivated otospheres from propagation 1 (P1) to propagation 4 (P4) expressed Nanog, Sox2, Klf4, Jagged1 and Nestin as well as other early otic makers, but no expression of mature otic makers. However, mature otic makers Espin and Phalloidin displayed expression in the full differentiated long term cultivated otospheres cells.

Conclusion: The results indicate that the neonatal organ of Corti supporting cells express several stem markers related to their capabilities to act as inner ear stem/progenitor cells under conditional culture. *In vitro,* Long-term cultivated otospheres dissociated from organ of Corti retain a latent stem cells phenotype.

PO33

EFFECT OF NEUROTROPHIC FACTORS ON HEARING RESTORATION AND SPIRAL GANGLION REGENERATION IN DEAFENED ANIMAL MODEL

Jeong-Hoon Oh , Shi-Nae Park, Kyoung Ho Park, Sang W. Yeo

(Department of Otolaryngology–HNS, The Catholic University of Korea)

Background and objectives: Multiple neurotrophic factors have been shown to have important roles in the survival of auditory neurons and protecting spiral ganglion cells. The purpose of this study is evaluating the efficacy of combination of multiple neurotrophic factors including brain-derived neurotrophic factor(BDNF), glial-cell derived neurotrophic factor(GDNF), neurotrophin-3(NT-3), insulin growth factor(IGF) and epidermal growth factor(EGF) with fibroblast growth factor(FGF) in a deafened animal model.

Materials and method: Healthy 30 guinea pigs were deafened and 2 weeks later the combination of neurotrophic factors soaked in gelfoam were applied on the round window membrane on one ear with saline application to opposite ear. On 1 week after drug application, hearing test was performed and the cochleae were collected in every 2 weeks. And the number of spiral ganglion cells was quantitatively analyzed.

Results: Significant hearing restoration was observed in group 1(BDNF, GDNF, NT-3 treated group) & group 2(IGF treated group). The number of spiral ganglion cells of group 1 on 5 week after the treatment, and group 3(EGF, FGF treated group) on 3 week after the treatment were significantly increased them compared with control group. However, no statistically significant regeneration was observed in group 2.

Conclusion: These findings suggest that BDNF+GDNF+NT-3 neurotrophic factor application through round window membrane might have the potential for regeneration of spiral ganglion cells & hearing restoration.

COCHLEAR IMPLANTS

PO34

CHILDREN WITH NEUROSENSORIAL DEAFNESS CANDIDATES OR SUBMITTED TO COCHLEAR IMPLANT.

Carla Elsa Marques

(Hospital Pediatrico de Coimbra- Unidade de Neurodesenvolvimento e Autismo-Centro Desenvolvimento Luis Borges)

The number of children receiving cochlear implants has been increase in the latest years in Portugal, and consequently the number of the children who have significant disabilities in addition of their deafness such other commorbility deficits (cognitive deficit, autism spectrum disorders). For all this group is essential to promote an evaluation of the neurodevelopment and behavior to identifying sources of variability useful in determining implant candidacy, designing new educational programs and addressing benefits and limitations of cochlear implantation.

In Coimbra, children with neurossensorial deafness (NSD) are followed in the outpatient clinic of Biological Risk that is integrated in the Centro Desenvolvimento Luis Borges (CDCLB) of the Hospital Pediátrico de Coimbra (HP).

This outpatient clinic was created with the main purpose of realize neurodevelopment, psychological and behavioral assessment children who had been affected by biological incidents for the central nervous system (SNC) and sensorial system . To give reply to this specific group, we have, an interdisciplinary team focused on hearing impairments patients evaluation and rehabilitation.

In this specific group of children with NSD, the main idea it is to promote an evaluation of the neurodevelopment and behavior of the child with deafness, in the children followed in the scope of the program of Cochlear Implantations of the Unit of Cochlear Implantations of the Hospital of the Covões - Coimbra, if necessary to guide and to promote a harmonious development and learning development.

Our methodology is to follow children, with regularity, through predefined protocols, organized for ages and neurocognitive and emotional and behavioral milestones

Have been observed children with NSD diagnosis, for medical referencing of the Unidade de Implantes Cocleares of the Hospital of the Covões –Coimbra.

We will present some clinical data of our group that have implication in the rehabilitation process such as: neurocognitive, behavior and emotional profiles

PO35

COCHLEAR IMPLANT – ARE THERE ANY RELATION BETWEEN COMPLICATIONS AND SPEECH PERCEPTION?

Peixoto M, Alves M, Martins J, Bastos J, Ribeiro C

(Centro Hospitalar de Coimbra)

Introduction: Complications of cochlear implant may result in removal of the cochlear implant and subsequent re-implantation. The trauma is an important complication of cochlear implantation in children. Whether the surgical procedure or the absence of auditory stimulation may arise associated with changes in auditory perception and speech.

Material and methods: The authors present a case report describing the occurrence of a complication related to cochlear implants, which had its base in a direct injury in the region where is located the antenna of the cochlear implant. Cochlear reimplantation was need. Audiometric and speech therapy results were compared with those from a similar survey before the re-implantation, trying to infer the consequences of this complication and / or surgery on auditory perception and speech.

Results: The re-implantation surgery was performed in two different times, taking place without complications. The authors did not identify significant differences in the impedances obtained intraoperatively and the free field audiometry of the first implant and after re-implantation. In the context of development of speech and language seems to have been, during the absence of stimulation, a stop in language development without acquiring new words, with loss of intelligibility and being heavily dependent on lip reading, however, no significant differences were found in the objective assessment.

Conclusions: After a relatively short rehabilitation process that followed the re-implantation, it is considered that the child recovered the general performance at the level of auditory perception, communication and language, continuing his natural intellectual and language development.

PO36

COCHLEAR IMPLANTATION IN CHILDREN WITH WAARDENBURG SYNDROME

Susana Andrade, Conceição Peixoto, Jorge Humberto Martins, Luis Filipe Silva, Carlos Ribeiro

(Serviço de Otorrinolaringologia - Centro Hospitalar de Coimbra)

Introduction: Waardenburg syndrome (WS) is an inherited disorder characterized by the association of skin, iris and hair pigmentation abnormalities, and varying degrees of sensorineural hearing loss. Congenitally deaf children with WS, severely or profoundly impaired with limited hearing aids benefit, have been integrating cochlear implant programs with encouraging results.

Objectives: The aim of this study was to review the outcomes of children with documented WS implanted in the Otorhinolaryngology Department of Centro Hospitalar de Coimbra, concerning postoperative speech perception and production, in comparison to the rest of non-syndromic implanted children.

Methods: A retrospective chart review was performed for children congenitally deaf who had undergone cochlear implantation with multichannel implants, diagnosed as having Waardenburg syndrome, between 1992 and 2011. Postoperative performance outcomes were assessed and confronted with results obtained by children with non-syndromic congenital deafness also implanted in our department.Open-set auditory perception skills were evaluated by using european portuguese word discrimination tests (vowels test, monosyllabic word test, number word test and words in sentence test). Meaningful auditory integration scales (MAIS) and categories of auditory performance (CAP) were also measured. Speech production was further assessed and included results on MUSS and speech intelligibility rating (SIR).

Results: To date, 7 implanted children were clinically identified as having WS type I, and one met the diagnosis of type II. All WS children received multichannel cochlear implants, with a mean age at implantation of 30,6 ± 9,7 months (ranging from 19 to 42 months).
Postoperative outcomes in WS children were similar to other non-syndromic children. In addition, in number word and vowels discrimination test WS group showed slightly better performances, as well as in MUSS and MAIS assessment.

Conclusions: A significant benefit after cochlear implantation was obtained by children with Waardenburg syndrome, comparable to that achieved by the general population of implanted children.

PO37

IS MENINGITIS RISK INCREASED BY COCHLEAR IMPLANTATION? INVESTIGATION OF TWO TYPES OF IMPLANTS IN GUINEA PIGS

Katharina Niedermeier[1], Susanne Braun[2], Claudius Fauser[3], Jan Kiefer[3], Reinhard K. Straubinger[4], Sven Hammerschmidt[5], Hennig Bier[1], Thomas Stark[1]

([1]Hals-Nasen-Ohrenklinik des Klinikums rechts der Isar, München; [2]MED-EL Deutschland GmbH, Starnberg; [3]HNO Zentrum Regensburg; [4]Institut für Infektionsmedizin und Zoonosen der Tierärztliche Fakultät der LMU München; [5]Ernst-Moritz-Arndt Universität Greifswald)

Objective: Preservation of usable residual hearing after cochlear implantation is essential for electric acoustic stimulation (EAS). In previous studies it was demonstrated that dexamethasone has a positive impact on hearing preservation. A drug-eluting electrode-dummy loaded with 10% dexamethasone was developed in order to provide a sustained local dexamethasone application.
A rare but dangerous complication of cochlear implantation is meningitis. Despite the efforts of health organizations and manufacturers to prevent post-operative infections new cases of meningitis occur each year. The question whether a cochlear implant *per se* increases the risk for meningitis is still unclear.
The aim of the present study was to evaluate the potential meningitis risk in animals without cochlear implant and in animals implanted with two different types of implants using a model of otogenic meningitis.

Methods: Guinea pigs were implanted unilaterally with dexamethasone-eluting (n=15) and unloaded silicone rods (n=15), respectively. A third group of animals (n=15) did not receive an implant. Five weeks later animals of all groups were infected with a bacterial concentration leading to meningitis in 30% of cases. This bacterial concentration was evaluated in a previous study. The development of meningitis within five days after infection was assessed by means of clinical symptoms, analysis of CSF samples and histological evaluation of brains and cochleae.

Results: The results do not demonstrate a significant difference regarding the meningitis risk among the three groups. In the group with unloaded silicone rods, 3 of 15 (20 %) animals developed meningitis, while in the group with dexamethasone-eluting rods 4 of 15 (26 %) animals acquired meningitis. 5 of 15 (30 %) unimplanted animals developed meningitis.

Conclusions: Neither implantation *per se* nor dexamethasone-eluting implants enhances the infection risk five weeks after surgery. Whether infection risk is enhanced by implantation after a shorter post-operative time has to be investigated.

INNER EAR (HISTO)PATHOLOGIES

PO38

HISTOLOGICAL AND ELECTROPHYSIOLOGICAL EVALUATION OF THE INNER EAR IN THE PROGRESSIVE MOTONEURONOPATHY MOUSE

Kristen Rak, Silke Frenz, Rudolf Hagen, Robert Mlynski

(Klinik und Poliklinik für Hals-, Nasen- und Ohrenkrankheiten, plastische und ästhetische Operationen, Universitätsklinikum Würzburg)

The progressive motorneuronopathy (*pmn/pmn*) mouse is an animal model for a developing human motor neuron disorder like the amyotrophic lateral sclerosis. The homozygous mutation in the TBCE gen leads to a dysfunctional co-factor of the tubulin-dimerization. This results in a loss of microtubules, which negatively influences the axonal transport. Affected animals are characterized by a progressive axonal degeneration of peripheral nerves, beginning in postnatal week three. Simultaneously, the animals develop a progressive hearing loss, which has been presented previously by ABR measurements.

In order to reveal the origin of this hearing loss, we investigated the animal in postnatal week three to five. Electrophysiological experiments including DPOAE for the evaluation of outer hair cell function and frequency-specific ABR analysis for the measurement of auditory thresholds were performed. For the histological examination the cochleae were dissected in transverse and longitudinal direction to the modiolus and immunhistological and electron microscopy investigations of the spiral ganglion and the auditory nerve carried out. In addition, whole mount preparations were performed and stained for visualisation of the organ of corti. Subsequently, histomorphological analyses of the organ of corti, spiral ganglia and the auditory nerve were assessed.

Electrophysiological measurements displayed elevated thresholds in ABR measurements from postnatal week three in *pmn/pmn* mice, whereas DPOAE thresholds were not affected until postnatal week four. Histological investigations revealed an age dependent loss of outer hair cells, but additional a massive degeneration of microtubules in normally myelinated auditory nerve fibres.

These results imply that *pmn/pmn* mice represent a mixed (cochlear and neuropathy) type of hearing loss and may be an interesting model to study time related interactions between the different components of the auditory pathway in the pathophysiology of hearing loss.

PO39

OTOTOXICITY OF METHYLROSANILINE CHLORIDE (GENTIAN VIOLET)(A CHANGE OF CAP WITH THE LAPSE OF TIME IN THE GUINEA PIG)

Hitomi Higuchi[1], Takafumi Yamano[1], Mayumi Sugamura[1], Takashi Nakagawa[1], Tstsuo Morizono[2]

([1]Fukuoka University; [2]Nishi-Fukuoka Hospital)

Purpose: As an empirical treatment of intractable ear discharge, Gentian Violet (GV) has been used in the external ear canal for its excellent antimicrobial and antifungal activity. When GV solution is used in the ear with perforated ear drums, the drug may come in contact with the round window membrane, thus finding a way to the inner ear.

To date, however, no report is available on the ototoxicity of GV, except our preliminary reports made in the ARO meetings in February 2008 and 2010 and Inner Ear Biology in August 2010. The purpose of this report is to present data on the ototoxic effect of GV that we applied in the middle ear cavity in the guinea pig for 5 minutes, 30 minutes, 1 hour, 2hours, 24 hours. Two different concentrations, 0.5% and 0.13% were studied.

Material and Methods: Using Hartley adult guinea pigs, ototoxicity was evaluated by measuring eighth nerve compound action potentials (CAP) from an electrode on the round window membrane. The stimulus sound consisted of click sounds and tone bursts of 4 and 8 kHz. After the initial CAP measurement, the middle ear cavities of the animals were filled with GV solution, with concentrations of 0.5% or 0.13%. After an interval of 5 minutes, 30 minutes 1 hour, 2 hours, and 24 hours, the middle ear was washed with saline, carefully wicked dry and the reduction in CAP was measured.

Results: Mild ototoxicity was detected at 30 minutes when using a 0.5% solution, and the same concentration caused more reduction in CAP at 60 minutes and a complete abolishment of CAP at 24 hours. A 0.13% solution caused reduction in CAP at 2 hours and severe reduction in CAP was seen at 6 hours. Thus, concentration dependent reduction in CAP was found. Even when a more diluted 0.13% solution was applied on the round window for only 5 minutes and washed with saline, still a severe reduction in CAP was seen at 24 hours.

Conclusions: Although GV has excellent antibacterial and antifungal activity, the use of GV should be limited to the external ear canal. The use of this drug in the middle ear cavity is not recommended.

PO40

TEMPORAL ONSET PATTERN OF CRE RECOMBINASE UNDER PROMOTOR BRN3.1 IN HAIR CELLS

Silke Frenz, Katja John, Kristen Rak, Robert Mlynski, Rudolf Hagen

(Klinik und Poliklinik für Hals-, Nasen- und Ohrenkrankheiten, plastische und ästhetische Operationen, Universitätsklinikum Würzburg)

Several mouse mutant strains serve as models of human hearing disorders. Unfortunately, germ line mutation of a gene that is expressed in many tissues and many cell types often results in embryonic lethality. Cre recombinase mediated tissue-specific gene targeting is a powerful tool for studying development and differentiation of inner ear cells. The aim of this study was to identify the temporal onset pattern of Cre recombinase activity under the promoter Brn3.1. Brn3.1 IRES Cre mice were cross bred with floxed lacZ and EYFP reporter mice, respectively.

The cochleae of postnatal mice were fixed; the organ of Corti was dissected and stained with antibodies against EYFP and lacZ, respectively. To ensure normal hearing background mice from all used strains and their recombinant offspring were tested using ABR audiometry and DPOAE mesasurement.

All mice showed normal ABR and DPOAE values, thereby confirming that neither insertion of the IRES Cre cassette into the Brn3.1 gene led to abnormal auditory development nor the reporter strains showed inherited hearing disorders.

P14 mice showed Cre recombinase activity in outer hair cells in a mosaic pattern. Younger and older mice have yet failed to show similar activity, however the irregular display points to a cell specific onset or offset pattern. Further investigation is needed to elucidate the complete timeframe for Cre recombinase activity under the Brn3.1 promotor based on these findings.

PO41

COMPARISON OF TWO EMBEDDING METHODS FOR THE EVALUATION OF SURVIVAL OF SPIRAL GANGLION CELLS

Piera Ceschi[1], Peter Erfurt[1], Thomas Lenarz[1], Manfred Kietzmann[2], Timo Stöver[3], Gerrit Paasche[1]

([1]ENT, Hannover Medical School, Germany; [2]Pharmacology and Toxicology, School of Veterinary Medicine Hannover, Germany; [3]ENT, KGU Frankfurt, Germany)

Objective: Paraffin-embedding and slicing is the technique of choice to investigate the morphology of the inner ear. However, after cochlear implantation, when the electrode has to stay *in situ* for histological analyses, this technique can not be used due to the metal parts of the electrode array. For this reason a method for embedding and grinding a surgically implanted cochlea in epoxy was developed earlier. In this study we compared both approaches in terms of spiral ganglion cells (SGC) morphology.

Methods: Six normal hearing BFA guinea pigs and six normal hearing Dunkin Hartley (DH) guinea pigs were included in this study. Transcardial perfusion was performed under general anaesthestesia. After perfusion one cochlea of each subject was embedded in paraffin while the contralateral cochlea was embedded in epoxy. Cochleae were sectioned, stained and then analysed for morphology, dimensions and number of surviving spiral ganglion cells.

Results: No differences between BFA and DH guinea pigs were found. The number of SGC was higher when the cochleae were embedded in paraffin. Also the perikarial diameter was larger for paraffin-embedded cochleae (14.3 µm vs. 10.5 µm).

Conclusions: Our results demonstrate that also epoxy-embedding can be considered as a useful technique to investigate the morphology of the cochlea. However, it has to be taken into account that the perikarial diameter of surviving SGC is smaller compared to paraffin-embedded cochleae.

PO42

PREVENTING NOISE-INDUCED HEARING LOSS BY BLOCKING PHOSPHODIESTERASE-5 AND IDENTIFICATION OF A POSSIBLE CGKI DOWNSTREAM TARGET

M. Jaumann[1], J. Dettling[1], M. Gubelt[1], U. Zimmermann[1], A. Gerling[2], F. Paquet-Durand[3], S. Feil[2], S. Wolpert[1], N. Brandt[4], S. Kuhn[5], H. Geisler[1], K. Rohbock[1], P. Ruth[6], J. Schlossmann[7], J. Hütter[8], P. Sandner[8], R. Feil[2], J. Engel[4], M. Knipper[1], L. Rüttiger[1]

([1]THRC, University of Tübingen; [2]Interfaculty Institute of Biochemistry, University of Tübingen; [3]Centre for Ophthalmology, University of Tübingen; [4]Department of Biophysics, Saarland University; [5]Department of Biomedical Science, University of Sheffield; [6]Institute of Pharmacy, Department of Pharmacology and Toxicology, University of Tübingen; [7]Department of Pharmacology and Toxicology, University of Regensburg; [8]Common Mechanism Research, Bayer Schering Pharma AG)

NOISE-INDUCED HEARING LOSS (NIHL) IS A GLOBAL HEALTH HAZARD WITH CONSIDERABLE PATHO-PHYSIOLOGICAL AND SOCIAL CONSEQUENCES, WHICH, UNTIL TODAY, HAS NO EFFECTIVE TREATMENT. IN THE HEART, LUNG, AND OTHER ORGANS, CGMP HAS BEEN DESCRIBED TO FACILITATE PROTECTIVE PROCESSES IN RESPONSE TO TRAUMATIC EVENTS. WE THEREFORE ANALYZED NIHL IN MICE WITH A GENETIC DELETION OF CGMP-DEPENDENT PROTEIN KINASE TYPE I (CGKI) AND FOUND SIGNIFICANTLY INCREASED NOISE VULNERABILITY. IN WILD-TYPE ANIMALS, IN SENSORY HAIR CELLS AND NEURONS OF THE INNER EAR, CGKI WAS EXPRESSED AND PARTLY OVERLAPPED WITH THE EXPRESSION PROFILE OF CGMP-HYDROLYZING PHOSPHODIESTERASE 5 (PDE5). TREATMENT WITH PDE5 INHIBITOR VARDENAFIL ALMOST COMPLETELY PREVENTED NIHL AND CAUSED A CGKI-DEPENDENT UPREGULATION OF POLY (ADP-RIBOSE) IN HAIR CELLS AND THE SPIRAL GANGLION, SUGGESTING AN ENDOGENOUS PROTECTIVE CGMP-CGKI SIGNALING PATHWAY CULMINATING IN ACTIVATION OF POLY (ADP-RIBOSE) POLYMERASE. THESE DATA POINT TO THE HIGH POTENTIAL OF VARDENAFIL OR RELATED DRUGS FOR THE THERAPY OF NIHL.

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PO43

DEAFNESS INDUCTION IN MICE

Klis SFL[1], Topsakal V[2], Hendriksen EGJ[2], Grolman W[2]

([1]University Medical Center Utrecht; [2]Department of Otorhinolaryngology and Head & Neck Surgery, University Medical Center Utrecht, The Netherlands)

Objective: Tissue engineering experiments are usually performed in mice. It is relatively easy to create a guinea pig model of deafness with a combination of an aminoglycoside antibiotic and a loop diuretic. However, in mice, aminoglycosides appear particularly ineffective. In this pilot experiment we aimed to explore the effects of standard deafening treatment in mice.

Methods: Wild type C57Bl/6 mice were purchased from Harlan. The animals were treated with kanamycin sulphate (stock solution 100 mg/ml in saline) subcutaneously. Some animals received 700 mg/kg and some received 1000 mg/kg. In guinea pigs we used 400 mg/kg but such a low dose is not effective in mice. Furosemide (stock solution of 10 mg/ml in saline, Centrafarm) was slowly infused in the tail vein at 100 mg/kg. The animals survived for 4 weeks after treatment after which they were anesthetized, electrophysiological measurements were performed and their inner ears were harvested.

Preliminary results: Thus far, about 10% of the animals died directly after treatment in both groups. Brainstem response audiometry in the survivors showed threshold shifts larger than 60 dB. Little difference was seen between the group receiving 700 mg/kg kanamycin and the group receiving 1000 mg/kg. Remarkably, in both groups we found a single animal with no threshold shift at all (a non responder). Preliminary histological results show complete disappearance of the organ of Corti in the basal turn in deafened animals.

Conclusions: Deafening with a combination of kanamycin and furosemide is possible in the C57Bl/6 mouse strain. There is no difference between the 700 mg/kg kanamycin group and the 1000 mg/kg group, thus, 700 mg/kg is preferable because it is further away from the LD50 value (1350 mg/kg). The lack of effect in 2 animals, thus far, indicates that we need to improve on our experimental capabilities.

PO44

IDIOPATHIC SUDDEN SENSORI-NEURAL HEARING LOSS INTRA-TYMPANIC TREATMENT

Victor Correia da Silva, Carla Guimarães Cardoso

(Hospital CUF Porto)

Sudden sensori-neural hearing loss (SSNHL) is a clinical dilemma with great diversity in presentation and poorly understood pathogenesis and hence no definitive treatment protocol as yet. A number of treatment protocols have been suggested and used over the years. Intratympanic steroids are being increasingly used in the treatment of SSNHL after the failure of systemic therapy.

We reviewed the medical records of patients who presented with SSNHL between January 2003 and December 2010 treated with intratympanic steroids. Successful recovery was defined as complete or partial recovery using Sigel´s criteria.

PO45

THE INSULIN EFFECTS IN AN EXPERIMENTAL MODEL OF TRAUMATIC PERFORATION OF TYMPANIC MEMBRANE

Araujo, MM.[1], Piaia, AR., Murashima, AAB., Rossato, M.[1], Hyppolito, MA.[1]

(Faculty of Medicine of Ribeirão Preto - University of São Paulo)

Acute perforation of the tympanic membrane is a result of infection or trauma. There is a great interest in developing non-invasive methods for your treatment. We study the use of insulin in an experimental model of traumatic tympanic membrane perforation and its ototoxic effects to allow its use in clinical studies.

Methods: The animals were randomly in to 2 groups. Group A: control of traumatic tympanic membrane perforation. Group B: perforation of tympanic membrane, instillation of 1UI of regular human insulin daily for 7 days. The tympanic membranes were removed for histopathological assessment by hematoxylin-eosin, Sirus Red, for collagen analysis, immunocytochemistry for aspects of epithelization, angiogenesis, connective tissue repair, collagen deposition and myofibroblasts. The cochleae were prepared for scanning electron microscopy study. Animals were evaluated in the pre-and post-treatment for functionality by otoacoustic emissions and brainstem auditory evoked potential.

Results: There was closure of tympanic perforation that was shortened with the use of insulin, where all the perforations were closed between 5 and 7 days ($P < 0.05$) and 14 days in control group. In the control group noticed 3 to 4 rows of disorganized epithelial cells with few fibroblasts, predominately inflammatory cells and a few sparse and thin fibers of collagen and turgid capillaries next to the hammer. In the insulin group there was the presence of total tympanic membrane repair in 07 days, with the presence of 6 to 8 rows of epithelial cells, dense and organized collagen fibers with abundant myofibroblasts and angiogenesis close to the cable of the hammer. Audiological exams and scanning electron microscopy have shown integrity of the inner ear.

Conclusion: Insulin promoted fast and organized closing of perforation of tympanic membrane without causing ototoxic effects which allows planning for pre-clinical studies.

PO46

PNEUMOVESTIBULE: CT SIGN OF TRAUMATIC PERILYMPHATIC FISTULA

Marta Canas Marques, Luís Almeida Dores, Luís Freitas, Andrea Gaspar, Marco Alveirinho Simão, Óscar Dias, Mário Andrea

(Hospital de Santa Maria, CHLN)

BACKGROUND

The perilymphatic fistula (PLF) is an abnormal communication between the inner ear and middle ear caused by rapid changes in cerebrospinal fluid and / or middle ear pressure. The variable clinical presentation and the absence of sensitive and specific tests often mimic other diseases. Definitive diagnosis of PLF has been based on the presence of a leak of perilymph during explorative tympanostomy, but even this can be intermittent and in such small quantities that direct observation may overlook its existence.

CASE REPORT

The pneumovestibule is an image sign that has been described by many authors and has been correlated with intraoperative observation of perilymphatic fistula. The authors present a clinical case of a patient admitted to the emergency with signs of severe and disabling rotational vertigo, associated with worsening rapidly progressive hearing loss and tinnitus as a result of otologic trauma. Imaging evaluation showed the presence of air in the inner ear structures and the fistula sign was positive.

DISCUSSION

The purpose of this clinical case is to review the literature and discuss the clinical aspects of the pneumovestibule.

PO47

INTRACRANIAL HYPERTENSION AS A CAUSE OF DEAFNESS

Marta Canas Marques, Luís Almeida Dores, Marco Alveirinho Simão, Ana Rita Santos, Óscar Dias, Mário Andrea

(Hospital de Santa Maria, CHLN)

BACKGROUND

Intracranial hypertension (ICH) manifestations often include headache, visual disturbances and ocular paresis. However, otologic manifestations may also occur in about 80% of cases, including tinnitus, vertigo and hearing loss. Hearing loss is usually mild and can be sensorineural (nerve/brain stem compression) or conductive (by cochlear hidropsia) and usually improves after ICH normalization.

CASE REPORT

The authors present a case of a 27 years-old woman with a 3 months history of headaches, blindness and hearing loss with bilateral progressively worsening. CT-scan was normal and Angio-MRI showed extensive cerebral venous thrombosis. Lumbar puncture revealed cerebrospinal fluid pressure greater than 50 cmH2O, with normal cerebrospinal fluid examination. Evaluation showed bilateral, moderate to severe, sensorineural hearing loss, with a decrease in low frequencies and absence of stapedial reflexes.

DISCUSSION

Cerebral venous thrombosis is rarely mentioned as cause of hearing loss. In this case sensorineural hearing loss was of gradual evolution and improved after control of the intracranial hypertension.

P47 A

DO ISCHEMIA, NOISE AND CISPLATIN CAUSE COCHLEAR VASCULAR DAMAGE?

Olivetto E[1], Simoni E[2], Giordano P[2], Cascella V[1], Guaran V[2], Astolfi L[2] and Martini A[2]

([1] Department of Morphology and Embryology, University of Ferrara, Via Fossato di Mortara 66, 44121 Ferrara, Italy; [2] Department of Medical and Surgical Specialities, University of Padua, via Giustiniani 2, 35129 Padua, Italy)

Deafness is one of the most commonly diffused disabilities because of increasing people age and environmental noise levels. Hearing damage caused by age, noise or ototoxic drugs is generally irreversible since cochlear neurons and hair cells are not able to regenerate.

Noise-induced hearing loss and ototoxic drugs are significant sources of hearing impairment among humans. The causing mechanisms can be attributed to: direct mechanical damage and damage caused by metabolic stress, mediated by an increased of the oxidative metabolism in the inner ear. It has already been observed that routine blood pressure values recorded in young adult patients complaining of sudden sensorineural hearing loss have been significantly lower when compared with those of an age-matched control group, thus indicating a possible role of hypothension in the genesis of inner ear disorders. For the study of sudden hearing loss, in literature are available several in vivo protocols for deafening noise and ototoxic drugs and few in vitro for hipoxic damage.

This research aims to develop in rat model, the techniques to induce damages to inner ear following ischemic events like hypoxia, deafening noise or cisplatin toxicity, and to investigate in deep the amount of damage.

The preliminary histological results allowed us to detect a specific protein expression profile in relation to the tissue oxidation (HIF-1α, p-JNK) and cardiovascular disease (TM and TF). This profile, in conclusion, shows a correlation among the three different hearing loss damages: they cause vascular damage.

PO48

THERAPEUTIC MANAGEMENT OF INTRALABYRINTHINE SCHWANNOMAS

Pimentel J., Santos R., Oliveira V., Escada P., Madeira da Silva J.

(Hospital Egas Moniz - CHLO)

Intralabyrinthine schwannomas are rare tumors, resulting from Schwann cells proliferation at the proximal end of the vestibulocochlear nerve, that can involve the cochlea, vestibule, semicircular canals and even the middle ear. Treatment options include: observation with serial magnetic resonance, stereotactic radiotherapy and surgical removal of the tumor. We report a case of a patient with intracochlear intralabyrinthine schwannoma.

A 57-year-old female with history of progressive, non fluctuating, hearing loss in the left ear, permanent tinnitus and discrete vestibular symptoms. Audiologic examination showed profound sensorineural hearing loss in the left ear. Computized tomography was normal for otic capsule and middle ear, while magnetic resonance has showed aspects suggestive of intracochlear intralabyrinthine schwannoma. After discussion with the patient of the advantages and disadvantages of the several treatment options and his informed consent obtained, surgical tumor removal has achieved by subtotal petrosectomy with anterior labirintectomy.

The treatment of vestibular schwannomas can be conservative with serial magnetic resonance follow-up. Nevertheless, in specific cases (non-useful hearing, vertigo refractory to medical treatment and transotic or timpanolabyrinthine intralabyrinthine schwannomas) surgical treatment with tumor removal is a valid option. In these cases the approach is otologic (extradural) and associated with low morbility.

HEARING GENETICS

PO49

PENDRED'S SYNDROME: A NEW MUTATION IN A CONSAGUINEOUS PORTUGUESE FAMILY

Marta Canas Marques[1], Helena Simões-Teixeira[2], Sandra Agostinho[1], Marco Alveirinho Simão[1], Alberto Santos[1], Helena Caria[2], Graça Fialho[2], Óscar Dias[1], Mário Andrea[1]

([1]Hospital Santa Maria; [2]Faculdade de Ciências, Universidade de Lisboa)

Pendred's Syndrome (PDS) is an autosomal recessive disorder characterized by sensorineural hearing loss and goitre. It is caused by a dysfunction of pendrin, a transmembrane protein encoded by the SLC26A4 gene, which is mapped on chromosome 7 (7q22-31.1). To date, 174 mutations of this gene are described that cause PDS or included in the PDS-Enlarged Vestibular Aqueduct (EVA) spectrum and therefore also associated with non-syndromic deafness (DFNB4). The phenotypic variability in the PDS-EVA spectrum seems to reflect different mutations with variable function levels of pendrin.

We report a case of PDS in which a new mutation of the SLC26A4 gene is identified – Intron 14, IVS14-2A>G – present in heterozygosity in the parents, who had no clinical or audiological alterations, and in homozygosity in two brothers with typical PDS. In our case, the sensorineural hearing loss was peri-lingual, floating and with progressive worsening. The installation of an euthyroid goitre occurred at puberty. Temporal Bone CT Scan showed a Mondini malformation and a diffuse enlargement of the vestibular aqueduct bilaterally.

This is the first case of PDS diagnosed by molecular analysis in the Portuguese population, which illustrates the important contribution of Genetics and Radiology in the evaluation of patients with hearing loss.

PO50

CONTRIBUTION FOR THE STUDY OF THE GENETIC ETIOLOGY OF PRESBYCUSIS IN PORTUGAL

Pereira, L.[1], Chora, J.[1], Teixeira, H.[1], Matos, T.[1], Fialho, G.[1], Caria, H.[2]

([1]BioFIG, Center for Biodiversity, Functional and Integrative Genomics University of Lisbon, Portugal; [2]BioFIG, Center for Biodiversity, Functional and Integrative Genomics University of Lisbon, Portugal; Higher College of Health, Polytechnic Institute of Setúbal, Portugal)

Introduction: Presbycusis or age-related hearing loss (ARHL) is one of the major chronic diseases affecting the elderly population, and may become a major health problem if considering the increase in life expectancy. ARHL is characterized by progressive, bilateral high-frequency hearing loss starting in the third decade, predominately in the high frequencies and affecting the speech spectrum frequencies within the fifth decade. It results from degeneration of cochlear structures within the inner ear.
Many studies on presbycusis have been developed in different populations. Mitochondrial haplogroups U and K in an Australian population, and the *NAT2*6A* haplotype in Europe, were found to be significantly associated with ARHL. The 4977bp mtDNA deletion was also shown to be associated with the process of aging and with ARHL.

Objectives: In this study we analyzed a sample of the elderly Portuguese population with presbycusis (n=100) for (i) SNPs on the *NAT2* gene, (ii) the presence of the 4977bp mitochondrial deletion and (iii) mitochondrial haplogroups.

Methods: *NAT2* and the mitochondrial hyper-variable region 1 (HVS1) were amplified by PCR and automatically sequenced in order to determine the *NAT2* genotypes and haplogroups. The presence of the 4977bp deletion was assessed by multiplex PCR.

Results and Discussion: The patterns of variants found in this sample seem to be consistent with those previously described for the general European population. We could not find any statistically significant association between the studied variants and presbycusis. The 4977bp deletion was not present in any individual.

This is the first study aiming to identify the genetic factor(s) responsible for an increased risk to develop presbycusis in the Portuguese population.

MOLECULAR BIOLOGY

PO51

NOVEL INSIGHTS INTO THE ROLE OF IGF-I SIGNALLING IN DEAFBLINDNESS

Silvia Murillo-Cuesta[1], Lourdes Rodróguez-de la Rosa[1], Raquel Martínez-Vega[1], Rafael Cediel[2], Julio Contreras[3], Luis Lassaleta[4], Isabel Varela-Nieto[1]

([1]Instituto de Investigaciones Biomédicas Alberto Sols CSIC-UAM, Madrid, SPAIN.; [2]Facultad de Veterinaria Universidad Complutense, Madrid, SPAIN.; [3]Facultad de Veterinaria, Universidad Complutense, Madrid, SPAIN.; [4]IdiPAZ, Madrid, SPAIN)

Insulin-like growth factor I (IGF-I) is a key factor for the development and function of the cochlea, and mutations in the *IGF1* gene are associated with deafness[1,2]. During the late embryonic and neonatal periods, there is a physiological peak expression of IGF-I and of its high affinity receptor IGF1R in the cochlea. IGF-I actions are mediated by signaling networks primarily activated by the phosphorylation of insulin receptor substrates (IRS) and down-regulated by the tyrosine phosphatase PTP1B. IRS2 is fundamental for glucose homeostasis and coordinates IGF-1/IGF1R signaling in the central nervous system. *Irs2*$^{-/-}$ *Ptpn1*$^{+/+}$ mice show a profound congenital deafness before the onset of diabetes that associated to morphological alterations. Simultaneous PTP1B deficiency delays the onset of deafness. IRS2 activation finally triggers two main downstream pathways: the PI3K-AKT and the RAF/ERK cascade that promote survival and cell cycle regulation. *Igf1*$^{-/-}$ mice show a down-regulation of ERK and AKT, and the activation of the p38 stress kinase in the cochlea. Otic transcriptional targets of IGF-I are FOXM1, MEF2 and AP1[3].

IGF-I serum levels are reduced in the adult and decrease with ageing, a trait that is associated with age-related hearing loss and retinal degeneration[4,5]. We found a correlation between the serum levels of IGF-I during aging and the seeing and hearing phenotype. *Igf1*$^{-/-}$ mouse presented a congenital sensorineural deafness and a progressive reduction in electroretinographic responses. *Igf1*$^{+/-}$ mice showed a progressive age-related decrease in IGF-I levels, with mean values lower than those of wild type mice. Accordingly, *Igf1*$^{+/-}$ mice exhibited an earlier increase of the auditory thresholds and a significant decrease in the ERG wave amplitudes, compared to wild type mice.

[1]Walenkamp et al., 2005; [2]Cediel et al., 2006; [3]Sanchez-Calderon et al., 2010; [4]Riquelme et al., 2010; [5]Murillo-Cuesta et al., 2011.

PO52

COCHLEAR GAP JUNCTION PLAQUE IS DISRUPTED BY CONNEXIN26 MUTATION

Kazusaku Kamiya[1], Miho Muraki[2], Kana Ogawa[1], Katsuhisa Ikeda[1]

*([1]Department of Otorhinolaryngology, Juntendo University School of Medicine;
[2]Department of Biomedical Science, University of Tokyo)*

Hereditary deafness affects about 1 in 2000 children and GJB2 gene mutation is most frequent cause for this disease in the world. GJB2 encodes connexin26 (Cx26), a channel component in cochlear gap junction. Gap junction in the cochlea provides an intercellular ion transport to maintain high level of the endocochlear potential essential for sensory hair cell excitation. We have studied the phenotype of themouse model carrying human Cx26 with R75W mutation (R75W Tg) and inner ear specific conditional Cx26 deficient mice (Cx26cKO) with partially mosaic deficiency in cochlear tissue. In this study, we analyzed the formation of gap junction in cochlear supporting cells of R75W Tg mice and Cx26CKO. Gap junction composed of Cx26 in wild type mice showed horizontal linear gap junction plaques (GJP) along the cell-cell junction site with the adjacent cells and these formed pentagonal or hexagonal outlines of normal inner sulcus cells and border cells. The GJP in R75W Tg mice did not show normal linear structure, although the round small spots were observed around the cell-cell junction site. Cx26CKO had almost same phenotype but some of the cells with Cx26 expression due to their mosaicism showed normal linear GJP with Cx30 only at the cell junction site between two Cx26 positive cells. These indicate that Cx26 is essential for the formation of the cochlear linear GJP, and it is not compensated by other cochlear Connexins such as Connexin30. In this study, we demonstrated a new molecular pathology of sensorineural deafness, and this machinery can be a new target for drag design of hereditary deafness.

PO53

AICAR INDUCED MITOCHONDRIAL BIOGENESIS IN THE INNER EAR

Wilson TM, Omelchenko I, Shi X, Nuttall AL

(Oregon Health & Science University)

Mitochondrial defects are the underlying cause for a variety of hearing-related impairments including noise-induced hearing loss, presbycusis, and several inherited forms of hearing disorders. While the role of mitochondria in hair cell death has been relatively well studied, the importance of mitochondrial biogenesis to hair cell physiology or that of the non-sensory cells in the cochlea is poorly understood. The transcriptional co-activator, peroxisome proliferator–activated receptor-g coactivator-1a (PGC-1a), is the key regulator of mitochondrial function increasing both mitochondrial energy metabolism and biogenesis. Numerous studies have demonstrated that activation of 5' AMP activated protein kinase (AMPK), either in response to exercise or to pharmacological agents such as 5-aminoimidazole-4-carboxamide-1-*β*-D-ribofuranoside (AICAR) or metformin, leads to significantly increased expression and activity of PGC-1a and, consequently, the up-regulation of factors involved in mitochondrial biogenesis and respiratory function. At the same time, AMPK activation in endothelial cells results in increased antioxidant expression and reduced reactive oxygen species generation. In this study, we examined mitochondrial biogenesis in the inner ear by treating male CBA/CaJ mice with daily injections of AICAR (0.5 mg/g) for 5 days. Four hours after the last injection, the cochleas were rapidly removed and analyzed by immunohistochemistry of dissected cochlear tissue sections and paraffin embedded cochlear sections. AICAR treatment lead to increased detection of phosphorylated AMPK in several cell types of the stria vascularis. Additionally, higher levels of the mitochondrial fission protein, Fis1, were observed in both auditory hair cells and marginal cells of the stria vascularis. Auditory brainstem response analysis following noise exposure suggested that AICAR is protective against noise-induced hearing loss. These results demonstrate that mitochondria in auditory hair cells and cells of the stria vascularis are dynamically regulated with increasing energy demands. Supported by grants 5R01DC000105 (ALN), 1R01DC010844 (XS), and P30DC005983.

PO54

STRESS SENSITIVITY OF THE AUDITORY SYSTEM IN WISTAR HAN RATS

Haupt H, Szepek AJ, Olze H, Mazurek B

(Charité - Universitätsmedizin Berlin, Dept. of Otorhinolaryngology, Berlin, Germany)

INTRODUCTION: Our recent study has shown that 24-h exposure to stress induces hypersensitivity in the auditory system of Wistar Harlan rats lasting up to one day. In our present work, the same experimental set-up was used to test a different strain of rats, namely Wistar Han rats, in order to identify possible differences in susceptibility to stress in various animal strains.

METHODS: The animals were exposed to a low-intensity and low-frequency noise (1 s, 300 Hz, 15-s interval, 61-65 dB A) for 24 h. In addition, the device induced slight vibration of the cage. Auditory brainstem response (ABR) was measured at different times post-stress. In addition, we determined the concentration of stress markers in serum and examined the expression of selected proteins in the inferior colliculus (IC) and auditory cortex (AC) using a Western blot. Non-stressed animals served as controls.

RESULTS: Immediately after stress, we found a transient hearing loss, which was followed by a decrease of the ABR thresholds. This auditory hypersensitivity was most pronounced after 24 h and was still detectable one week later. Six hours post-stress, corticosterone and TNFa were only slightly increased in serum, whereas the concentration of BDNF increased significantly. Three hours after stress, expression of BDNF in the IC and AC was higher than in the controls. AMPA2 receptor protein increased in the IC and AC after 6 h and 24 h to 1 week, respectively. The AMPA3 increased in the AC after 3 h.

CONCLUSION: Dissimilar to Wistar Harlan rats, exposure of Wistar Han rats to stress induced a transient hearing loss followed by a long-lasting hypersensitivity of the auditory system. The slow and only minor increase of corticosterone concentration in blood implies a disturbance in the HPA axis. Changes in BDNF and AMPA protein expression could be indicative of the synaptic plasticity.

PO55

PHENOTYPIC ANALYSIS OF TWO COCHLEAR HAIR CELL SPECIFIC CONDITIONAL MOUSE MODELS FOR TRA AND TRB

Christoph Franz[1], Juliane Dettling[1], Harald Winter[1], Claudia Gampe-Braig[1], Lukas Rüttiger[1], Niels Brandt[2], Jutta Engel[2], Frederic Flamant[3], Jian Zuo[4], Robert Feil[5], Marlies Knipper[1]

([1]University of Tübingen, Department of Otorhinolaryngology, Hearing Research Centre Tübingen, Molecular Physiology of Hearing, Tübingen, Germany; [2]University of Tübingen, Institute of Physiology II and Tübingen Hearing Research Centre, Tübingen, Germany and University of Saarland, Department for Biophysics, Homburg/Saar, Germany; [3]University of Lyon, Institute of Functional Genomics, National Centre for Research Sciences, Lyon, France; [4]Department of Developmental Neurobiology, St. Jude Children´s Research Hospital, Memphis, Tennessee, USA; [5]University of Tübingen, Interfaculty Institute of Biochemistry, Tübingen, Germany)

It is long known, that a lack of thyroid hormone (TH) can have a tremendous effect on embryonic development regarding not only overall body growth but also on the maturation of the brain and the hearing system.

Since more than 10 years, we focus our investigation on how TH can regulate the maturation of the cochlea from an immature pre-hearing organ into a hearing sensory organ via its receptors thyroid-hormone-receptor a1 (TRa1) and b1 (TRb1) that are present in the cochlea. We here present the phenotypic analysis of two conditional and tamoxifen inducible mouse models with prenatal knock-in mutation of TRa1 and deletion of TRb1.

The aim of this study is to obtain a better understanding of the TH regulation and its impact on maturation to bring it into common therapies in the clinic.

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PO56

INTERACTION PARTNERS OF OTOFERLIN PLAY A ROLE IN ENDOCYTOSIS

Susanne V Duncker[1], Christoph Franz[2], Uwe Schulte[3], Bernhard Hirt[4], Bernd Fakler[3], Ulrike Zimmermann[2], Niels Brandt[5], Jutta Engel[5], Peter Ruth[1], Marlies Knipper[2]

([1]University of Tübingen, Institute of Pharmacy, Department of Pharmacology and Toxicology, Tübingen, Germany; [2]University of Tübingen, Department of Otorhinolaryngology, Hearing Research Centre Tübingen, Molecular Physiology of Hearing, Tübingen, Germany; [3]University of Freiburg, Institute of Physiology, Freiburg, Germany; [4]University of Tübingen, Institute of Anatomy, Department of Clinical Anatomy, Tübingen, Germany; [5]University of Tübingen, Institute of Physiology II and Tübingen Hearing Research Centre, Tübingen, Germany and University of Saarland, Department for Biophysics, Homburg/Saar, Germany)

Otoferlin has been proposed to be the Ca^{2+}sensor in hair cell exocytosis, compensating for the classical synaptic fusion proteins synaptotagmin-1 and synaptotagmin-2 (Roux et al. Cell 2006). Yeast two-hybrid assays reveal myosin VI as an otoferlin binding partner (Heidrych et al. HMG 2009). Co-immunoprecipitation assay and co-expression suggest an interaction of otoferlin with rab8 in supranuclear parts of inner hair cells (IHC; Heidrych et al. HMG 2008), as well as a role of the interaction of otoferlin with myosin VI for a proper maturation of the IHC synapse (Heidrych et al. HMG 2009). Long-term stimulation of IHC that do not express otoferlin showed impaired synaptic vesicle pool replinishment (Johnson et al. Nat Nsci 2010), making the search for yet undiscovered interaction partners crucial to fully understand the role of otoferlin in synaptic transmission of IHCs.

In the present study, mass-spectrometry assays revealed several new proteins as putative interaction partners of otoferlin that have been further analysed by RT-PCR, immunohistochemistry and co-immunoprecipitation.

We here introduce one candidate for interaction with otoferlin that may help to link endocytosis to otoferlin's suggested role in vesicle replenishment in IHCs.

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CLINICAL STUDIES

PO57

HEARING IMPROVEMENT IN CHILDREN: THE ROLE OF SURGERY

Peixoto M., Ferreira R, Bastos J, Ribeiro C

(Centro Hospitalar de Coimbra)

Introduction: The idea of ear drainage date from 1760. Throughout all these years myringotomy and placement of a tympanostomy tube has been widely applied as a treatment of various diseases related to the middle ear. Several studies have been conducted to evaluate the indications, contraindications and complications of this surgery. Because it is a routine procedure, accessible to a resident of the first year of specialty, is seen, in daily practice, as basic. A real sense of the possible complications and its results isn´t considered.

Material and Methods: The authors selected all children who underwent to a myringotomy with or without placement of tympanostomy tubes in an established period of one year. Analyzed individual aspects of children and surgical aspects, related to long-term evaluation, after tube extrusion, with duration of 2 years.

Results: The study included 103 children. Most of the patients were male, with a mean age of 5 years old. It was performed in 42% bilateral myringotomy and 38% bilateral myringotomy with tube insertion. Additionally it was performed adenoidectomy in 47% and adenoidectomy plus tonsillectomy in 40%. After two years 78% didn't present any complains, 12% presented conductive hearing loss and 10% recurrent otitis. Air Conduction Threshold for both procedures before surgery was of 28,90 dB and after surgery of 18,05dB [p=0,02 (p< 0,05)]. With myringotomy post operative threshold was of 16 dB (p< 0,05) and with tympanostomy tube insertion was of 22 dB(p< 0,05).

Discussion/ Conclusion: Indications are set to perform myringotomy and tympanostomy tubes. Generally speaking it is considered a safe procedure with few complications.

PO58

AUDITORY PROCESSING ASSESSMENT IN PORTUGUESE CHILDREN

Cristiane Nunes[1], Liliane Desgualdo Pereira[2], Graça Simões de Carvalho[1]

([1]CIEC, Institute of Education, University of Minho, Braga, PORTUGAL; [2]Speech language hearing department, São Paulo Federal University, BRASIL)

OBJECTIVE: The central auditory processing (CAP) is related to the individual´s ability to analyze and interpret the acoustic signals received by the auditory system, which can be evaluated by behavioural and electrophysiological tests. When behavioural auditory tests are used it is important to know the normal ranges expected for each population. In Portuguese language there are many Brazilian studies but not so much in Portugal. In this country the auditory processing assessment is recent and its standards of normality are still unclear; therefore it is necessary to evaluate and compare the values found obtained in Portugal with those already established in Brazil, since the phonetics in both countries are rather different..

METHODS: In this study we applied eight tests of auditory processing assessment in Portuguese children from ages 10 to 13 years, with or without academic difficulties and speech disabilities, and compared the results with those obtained in previous Brazil studies using the same hearing tests. The sample was composed of 51 children with normal hearing. We present descriptive studies (mean, median and standard deviation), comparing the results between groups by the Turkey method and measure the sensitivity and specificity by ROC curves in the tests that discriminate the groups.

RESULTS: The average results of the children with normal hearing, i.e. no speech or academic difficulties, were similar or very close to those found in the population from Brazil. ROC curves also showed a cutoff similar to the average found in Brazil. The Gap´s in Noise Test showed the greatest sensitivity, and the Sequential Verbal Memory Test showed the highest specificity. CONCLUSIONS: The results validate the application of these tests in Portuguese spoken in Portugal with reference values found similar to those previously found in Brazil.

PO59

AUDIOLOGIC EVALUATION IN CHILDREN WITH MITOCHONDRIAL DISORDERS

Eun Jin Son[1], Seung-Ah Hong[1], Yuri Lee[1], Yong-Mock Lee[2]; Jae Young Choi[3], Won-Sang Lee[3]

([1]Department of Otorhinolaryngology, Gangnam Severance Hospital, Yonsei University College of Medicine; [2]Department of Pediatrics, Gangnam Severance Hospital, Yonsei University College of Medicine; [3]Department of Otorhinolaryngology, Yonsei University College of Medicine)

Mitochondrial disorders (MD) are a clinically heterogeneous group of progressive disorders that result from dysfunction of the mitochondrial respiratory chain. In addition to muscular and neurological deficits, hearing loss is often associated in MD. This study aims to investigate characteristics of hearing loss in children with MD. MD was diagnosed in 13 children by clinical and laboratory testings including muscle biopsy histology and enzymology. Audiologic evaluation included pure-tone audiometry, tympanometry, transient evoked otoacoustic emissions and auditory brainstem response. Some degree of hearing impairment was identified in 10 of 13 children (76.9%). Three of these children were diagnosed with Leigh syndrome, one with MELAS and 8 was nonspecific MD. Hearing loss varied in severity from mild to profound. Audiological evaluation suggested cochlear lesions as well as retrocochlear origin of hearing loss, suggested by abnormal brainstem responses with preservation of otoacoustic emissions in 4 patients. Progression of hearing loss was identified in 2 patients. Current efforts are directed toward identifying relationship between diverse genetic mechanisms and progression of hearing loss in MD.

PO60

BARTTER SYNDROME WITH SENSORINEURAL DEAFNESS- CASE REPORT

Dores L, Freitas L, Machado MP, Agostinho S, Carvalho T, Santos A, Macor C, Dias O, Andrea M

(Centro Hospitalar Lisboa Norte, Hospital de Santa Maria)

Introduction: The incidence of severe sensorineural deafness in children is approximately 1 per 1000 at birth. The causes of hearing loss are numerous, being shown that about 50% of them are attributable to genetic causes.
Bartter syndrome (BS) is a genetic disorder that comprises a variety of autosomal recessive tubulopathy , characterized by hypokalaemic metabolic alkalosis.

Methods: The authors present a case of a congenital bilateral severe sensorineural deafness, with late renal manifestations diagnosed only at age of 20, which contrasts with the typical cases of SB type IV described in the literature.

Conclusion: A neonatal variant associated with sensorineural deafness (BS type IV) is among the more than 400 malformation syndromes associated with deafness and as such called hereditary syndromic hypoacusis transmission. It results from a mutation in the gene encoding the BSND Barttin protein, an essential subunit of chloride channels expressed in the basolateral membranes of the renal tubules and stria vascularis.
This syndrome has an unknown prevalence, coursing with an aggressive phenotype in childhood.

PO61

MELAS SYNDROME & COCHLEAR IMPLANT: CASE REPORT

Luís Freitas, Luís Almeida Dores, Marta Canas Marques, Sandra Agostinho, Tatiana Carvalho, Marco Alveirinho Simão, Óscar Dias, Mário Andrea

(Hospital de Santa Maria, CHLN)

BACKGROUND

MELAS syndrome (Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke-like episodes) presents in many different ways, being more common in adolescents with normal psychomotor development in early years that later manifested various neurological disorders. Sensorineural deafness is one of the most common clinical manifestations, often associated with other neurological manifestations such as stroke-like episodes, myoclonic epilepsy and lactic acidosis.

CASE REPORT

The authors report the case of a young man with bilateral sensorineural deafness without other progressively worsening neurological symptoms. After the development of several neurological manifestations (two episodes ´´stroke-like´´ epilepsy with generalized tonic clonic seizures) and observation by Neurology, Melas syndrome was diagnosed. In the absence of gain and vocal tone with a hearing aid the patient underwent a cochlear implant.

DISCUSSION

The purpose of this clinical case is to review the literature and discuss the clinical aspects and treatment of MELAS syndrome.

PO62

MANIFESTAÇÕES OTORRINOLARINGOLÓGICAS DA TRISSOMIA 22 – A PROPÓSITO DE UM CASO CLÍNICO

Ricardo Santos, Assunção O´Neill, Madeira da Silva J.

(Hospital Egas Moniz)

Objectivo: A trissomia 22 é uma alteração cromossómica rara, classificando-se em completa e em mosaico. A trissomia 22 completa tem mau prognóstico estando associada a elevada taxa de aborto espontâneo no primeiro trimestre de gravidez. A trissomia 22 em mosaico tem um prognóstico mais favorável, com um quadro clínico muito variável, dependendo do número e tipo de células afectadas. Caracteriza-se por múltiplas manifestações, destacando-se crânio-faciais, ano-rectais, genito-urinárias, cardio-vasculares, neurológicas, osteo-articulares.

O objectivo deste poster é a apresentação de um caso clínico de trissomia 22, evidenciando as manifestações otorrinolaringológicas.

Métodos: Doente do sexo masculino, 6 anos de idade, raça negra, portador de trissomia 22. O doente é referenciado à Consulta de Surdez Infantil do Hospital Egas de Moniz por hipoacusia bilateral de predomínio esquerdo. Apresenta imperfuração do canal auditivo externo esquerdo, esboço de apêndices auriculares bilaterais e fistula Auris Congénita. Concomitante com a trissomia 22, o doente é portador de Síndrome de Duane.

Resultados: No caso clínico exposto, verificou-se que o doente em causa, portador de trissomia 21, apresentava manifestações otorrinolaringológicas características desta alteração cromossómica.

Conclusão: O quadro clínico da trissomia 22 pode revestir múltiplas apresentações. As manifestações otorrinolaringológicas são características e frequentes em doentes portadores desta alteração cromossómica, sendo os apêndices auriculares as mais evidenciadas. De destacar igualmente as fístulas auriculares, fenda palatina, micrognatismo, displasia do pavilhão auricular e baixa implantação do pavilhão auricular, como formas de apresentação otorrinolaringológicas da trissomia 22.

PO63

ENDOLYMPHATIC SAC TUMOURS – A CASE REPORT

Pimenta Machado M., Freitas L., Canas Marques M., Barros MJ., Dores L., Amaral J., Macor C., Dias Ó., Andrea M.

(Hospital de Santa Maria)

Objective: Endolymphatic sac tumours (ELSTs) are extremely rare. They originate in the petrous part of temporal bone, in the endolymphatic system. ELSTs were firstly described by Heffner in 1989[(1)]. Initially described as low-grade papillary adenocarcinomas, their histological appearance and apparent lack of metastatic potential, lead to reclassify them as papillary adenomas. Although benign, ELSTs can be locally destructive. These tumours can arise sporadically or in association with von Hippel-Lindau disease. They can present with hearing loss, tinnitus, facial nerve weakness or paralysis, vertigo and can be lethal[(2)].

Material and Methods: We present a case of a woman, 32 years old, african, with unilateral hearing loss, ipsilateral facial palsy and sporadically otorrhea. Computed tomography (CT) imaging demonstrates erosion of posterior petrous temporal bone. Magnetic resonance imaging (MRI) demonstrates gadolinium enhancement and heterogeneous signal intensity from intratumoral calcification and vascularity. Molecular genetic analysis for von Hippel-Lindau disease was performed.

Results: Molecular genetic analysis for von Hippel-Lindau disease was negative.
Surgical resection was the modality of treatment. Histologic and immunohistochemical features of the tumor made the diagnosis of ELST: papillary cystic structures lined with a columnar epithelium siderophages and calcifications; positive for cytokeratin and epithelial membrane antigen (EMA), negative for thyroglobulin and transthyretin.
One year after surgery a new CT was made and showed tumoral recidive. The next approach options were surgery revision, surgery revision followed by radiotherapy, radiotherapy followed by surgery revision or radiosurgery. Unfortunately the patient died meanwhile.

Conclusions: Despite the benign histological nature of these tumours, ELSTs are highly local aggressive lesions. Complete resection appears crucial for ensuring success. In the presence of a lytic, vascularized tumour of the posterior face of the petrous bone, clinicians should search for manifestations of ELSTs to detect it on time.

PO64

HEMORRHAGIC LABYRINTHITIS IN A PATIENT WITH RHEUMATOID ARTHRITIS

Elias CF, Araújo-Martins J, Melo P, Barros E

(Serviço de Otorrinolaringologia, Centro Hospitalar Lisboa Central, Hospital de São José, Lisboa, Portugal)

INTRODUCTION
Hemorrhagic labyrinthitis is a rare entity. It is most often associated with trauma, haematological disease and obstructive vessels disease. Patients with rheumatoid arthritis can develop autoimmune inner ear disease related to circulating immune complexes and vascular wall injury which can manifest by sensorioneural hearing loss and/or neurolabyrinthitis.

SUBJECT AND METHODS
The authors present a clinical case of hemorrhagic labyrinthitis in a patient with rheumatoid arthritis. The physiopathology and treatment are discussed along with a literature review.

RESULTS
A 61 year old female with rheumatoid arthritis on medication with etanercept, adalimumab and steroids presents with sudden right hearing loss and tinnitus followed by vertigo, nausea and vomiting one week later. Audiometric evaluation revealed right severe sensorineural hearing loss. Caloric tests showed right caloric weakness and vestibular evoked myogenic potentials were absent on the same side. T1-weighted MRI showed signal enhancement of the right labyrinth suggesting hemorrhagic labyrinthitis.

CONCLUSION
Hemorrhagic labyrinthitis is a rare disease and few cases have been described in association with rheumatoid arthritis. Circulating immune complexes are involved in the disease mechanisms and recent evidence has suggested that adalimumab is an efficient treatment for this condition significantly improving these patients outcome. However the controversy remains over the best treatment method.

PO65

SUBTOTAL PETROSECTOMY – A SURGICAL OPTION FOR A PARAGANGLIOMA

Oliveira F., Cavilhas P., Pimentel J., Escada P., Neto de Almeida G., Sousa P., Madeira da Silva J.

(Hospital Egas Moniz - CHLO)

Paragangliomas are rare tumors, particularly in head and neck region. They arise from cells of the autonomic nervous system and are more frequently found in the carotid body, jugular body, *glossopharyngeal nerve* and *vagus* nerve. The identification of this neoplasm in the middle ear is based on the clinical manifestations and *complementary exams* (audiogram, tympanogram, computed tomography and magnetic resonance). The purpose of this case report is to demonstrate that subtotal petrosectomy is a valuable surgical technique for the treatment of glomus tumors of the temporal bone.

A 45-year old man was referred to our hospital for the surgical treatment of a jugulotympanic paraganglioma on the left ear. The operation included a subtotal petrosectomy, and the complete removal of the tumor was achieved.

Subtotal petrosectomy can be performed as an isolated surgical procedure, but most commonly the technique is used as the first surgical step of different approaches for the lateral skull base. Subtotal petrosectomy includes: 1) the emptying of the temporal bone content with preservation of the profound cortical bone and of the labyrinth; 2) the permanent closure of the external auditory canal; 3) the closure of the tympanic pharyngeal orifice of the Eustachian tube; and 4) the obliteration of the cavity with autologous abdominal fat.

In the case reported, the patient was discharged at the ninth day after surgery.

As surgical consequence, inherent to the technique, the patient had definitive conductive hearing loss. Follow-up was performed annually with magnetic resonance.
The therapeutic options for the paragangliomas of the temporal bone include surveillance without therapy, radiotherapy and surgery. The only curative solution is the surgery. In our patient we have opted for the surgical treatment which included the subtotal petrosectomy. One year after surgery, the patient is healed, having returned to his normal activity.

PO66

DEAF CHILDREN AND FAMILY SUPPORT IN A MULTIDISCIPLINARY CONSULTATION OF DEAFNESS

Isabel Galhardo, Assunção O'Neill, Madeira da Silva

(Hospital Center of Deafness Lisbon West (C.H.L.O.)

Introduction: Sharing knowledge and trust between professionals and families, multidisciplinary approach is essential in diagnosis and management of deaf children throughout their lives. Early intervention with deaf children and their families in the hospital, schools and in community programs, are essential to the achievement a normal development.

Results: Multidisciplinary methodologies have been implemented considering communication at an early age, sign language courses for children and parents, and parental groups in wisdom sharing between families and professionals. Working between ENT CHLO consultation, kindergarten school, university and in the community also considering parental education, we seemed to have fantastic results.

A continuous work is performed between parents and professionals, in true and harmony, allowing a more effective evaluation. This attendance allows deaf children and families to have better benefits in terms of communication, Sign or oral, emotional development and self esteem, including when in cochlear implant programs.

Conclusion: Multidisciplinary approach is essential in deaf children development and families habilitation and their learning management throughout their lives, based on sharing knowledge and thrust between professionals and families. Early school access and parental supported programs is crucial, according to our results. Hearing aids or cochlear implants have more benefits for deaf children when integrated in family intervention programs. All kind of attendance provides deaf people inclusion in deaf and hearing communities.

PO67

MULTIDISCIPLINARY CONSULTATION OF DEAFNESS: SCREENING, DIAGNOSIS, REFERRAL AND EARLY INTERVENTION FOR DEAF CHILDREN. FAMILY AND SUPPORT GROUPS IN COMMUNITY

Assunção O'Neill (2)[1], Isabel Galhardo[1]; Ana Penha[1]; Teresa Neto Carvalho[2]; Gracinda Benedito[1]; Shaiza Jetha[1]; Madeira da Silva J.[3]

([1]C.H.L.O. Lisbon; [2]EB Quinta do Marrocos, C.H.L.O., Lisbon; [3]CHLO, Lisbon)

Introduction: Multidisciplinary approach is essential in diagnosis and management of deaf children throughout their lives, sharing knowledge and trust between professionals and families. Early intervention programs, communication and stimulation for deaf children and their families are essential to the achievement of normal development.

Results: Multidisciplinary methodologies have been implemented considering diagnosis, technical assistance and auditory factors and also sign or oral communication at an early age. Children are sent for specific educational programs, also considering the parental education in sign and oral Portuguese European language.

A continuous work is performed between parents and clinical team including, psychologist, speech therapist, social assistant, sign language speaker and special education teacher, allowing a more effective evaluation. This attendance allows young people to have better benefits in terms of emotional development and self esteem, including when in cochlear implant programs.

Conclusion: Multidisciplinary approach is essential in diagnosis and management of deaf children throughout their lives, based on sharing knowledge and thrust between professionals and families. Early school access is crucial, according to our results. Hearing aids or cochlear implants have more benefits for deaf children when integrated in family intervention programs. All kind of attendance provides deaf people inclusion in deaf and hearing communities.

VARIA

PO68

THE TREPONEMA SURPRISE: A RARE PRESENTATION FOR OTOSYPHILIS

Araújo-Martins J[1], Elias CF[2], Melo P[2], Barros E[2]

([1]Serviço de Otorrinolaringologia, Hospital de São José, CHLC, EPE; Departamento de Fisiopatologia, CEDOC, Faculdade de Ciências Médicas, Universidade Nova de Lisboa; [2]Serviço de Otorrinolaringologia, Hospital de São José)

Background: Syphilis is considered a re-emergent disease. The rise in its prevalence has gone beyond the HIV positive patients to include the population as a whole. Oto- and neurosyphilis are rare complications of *Treponema pallidum* infection that may present during several stages of the disease and often require a high clinical suspicion to be diagnosed. Serological tests are the mainstay for diagnosis, especially when no primary lesion is found.

Objectives: The aim of the present work is to present a clinical case of a patient with otosyphilis and an uncommon serology pattern.

Methods: The clinical file and diagnostic tests of the patient were reviewed.The pathophysiology of otosyphilis is discussed along with a literature review on this subject.

Results: A 39 year old eastern european male with a past history of unilateral sensorioneural hearing loss and a Ménière-like syndrome presents for evaluation for worsening of baseline hearing, tinnitus and vertigo. In the ensuing months, severe persistent headaches develop and are attributed to transverse and sigmoid sinus thrombosis of undetermined etiology. Six months later the patient is referred to our balance disorders clinic for evaluation of rapidly progressive bilateral hearing loss and vertigo. Treponemal serology tests are repeated and show the typical pattern for primary syphilis.

Conclusions: Treponemal infections are known to remain dormant and reactivate several years after the first presentation. Up to 15% of patients may become seronegative for treponemal tests (FTA-ABS or TPHA) 3 years after the primary infection. Otosyphilis presents a diagnostic challenge and should be considered in all cases of sudden sensorioneural hearing loss and Ménière-like syndromes.

PO69

SEX HORMONES IN THE INNER EAR - A SUMMARY

Malou Hultcrantz, Rusana Simonoska

(Dept of Otorhinolaryngology, Clintec, Karolinska Univ Hospital, Sweden)

Background: Hearing loss appears to be more profound in men then women. It is known that estrogen receptors (alpha and beta) are present in the inner ear in humans and that loss of estrogen levels during menopaus triggers the hearing loss in women. In rodents the content of these receptors vary over time and with different estrogen dependent situations, (fetal and adolescent stages, pregnancy etc). Women who have a loss of estrogen production (Turner syndrome (45,X)), will have an early rapid age-related hearing loss. Variation in estrogen receptor content may spring from the fact that there are 2 estrogen receptors with interaction, but could also be due to the actions of other sex-hormones, such as progesterone and testosterone.

Aim: To show the content of sex hormones and their localization in the inner ear of rodents.

Methods: Immunohistochemical staining with antibodies against sex-hormones (estrogen alfa and beta, progetsterone A and B, and androgens) and their localization in the inner ear.

Results: Nuclear staining of estrogen alfa and beta are found in the areas of the inner ear, where sound is conducted, but no receptors could be visualized in the inner ear of progesterone or androgens.

Conclusion: In rodents, of the sex-hormones , only estrogen receptors could be detected. The effect of other sex-hormones do not seem to be a direct action, but they might still have an effect on the regulation on hearing but then through their systemic levels and interaction.

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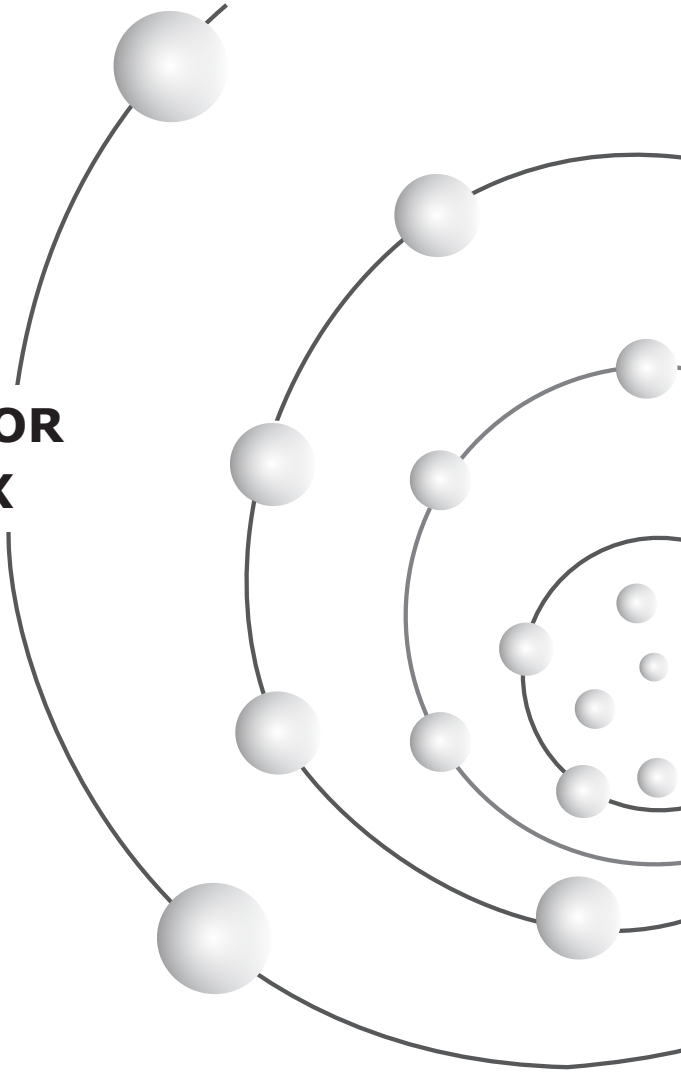

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